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INDUSTRIAL AND ENGINEERING CHEMISTRY

ANALYTICAL EDITION

PUBLISHED BY THE AMERICAN CHEMICAL SOCIETY • HARRISON E. HOWE, EDITOR

Determination of Total and Inorganic Bromide in Foods Fumigated with Methyl Bromide

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The direct determination of methyl bromide present in a food after fumigation is difficult, owing to the ease with which the fumigant is converted to inorganic bromide. In the method proposed, methyl bromide is removed under conditions which lead to the minimum decomposition and is determined as the difference between total bromide and inorganic bromide. Application to several food products indicates that no methyl bromide remains as such for more than 4 days after fumigation.

METHODS for the determination of total bromide in foodstuffs fumigated with methyl bromide have been published by Dudley (*2*) and by the authors (*7*). Uncertainty concerning the nature of this retained bromide has led to the fear that free methyl bromide might be present; in fact, some investigators have considered the total bromide to be methyl bromide, though without experimental justification. Aside from a few qualitative observations, there seems to be no published information on the state in which bromide occurs in fumigated products.

Since the experiments described in this paper were completed, a paper on "Bromide Residues in Foodstuffs" by Laug has appeared (*4*). Laug determined volatile and nonvolatile bromide by a method proposed by F. L. Hahn. The authors do not feel that Hahn's procedure is well suited for the determination of methyl bromide in foods, because it involves conditions under which some of the methyl bromide may react during analysis and thus be converted to inorganic bromide. In a comparison of the Laug-Hahn method with theirs on freshly fumigated ground walnut meats, the nonvolatile bromide was found to be from 5 to 10 per cent higher by the former method. Laug fumigated with eight times the usual dosages of methyl bromide in a deliberate attempt to secure high bromide residues, so that most of his data are not representative of commercial conditions and hence are not directly comparable with the authors'. Nevertheless they believe that his values for volatile bromide during the first 48 hours of airing tend to be too low, while those for nonvolatile bromide are too high. Furthermore, his results in some cases show erratic variations which he attributes to nonuniformity of the small samples analyzed. It seems more likely that they are due to losses during ashing, since the authors had the same difficulty until the conditions recommended in this paper were employed. Although Laug's conclusion that methyl bromide escapes fairly quickly from a fumigated food is in accord with their experience, the authors do not feel that his data alone are sufficient to establish this finding conclusively.

McLaine and Munro (*5*) demonstrated that water-soluble bromide is formed during the fumigation of plants. Dudley and co-workers (*3*) showed that most of the retained bromide is nonvolatile when a fumigated food is boiled with water. These observations indicate that little methyl bromide is retained as such.

According to various authors, bromide conceivably may be retained by a fumigated product in the following ways:

1. As inorganic bromide produced either by hydrolysis of methyl bromide to methyl alcohol and hydrobromic acid or by direct reaction of methyl bromide with chemically active constituents of the food, such as sulfur or nitrogen compounds. The former process has usually been suggested as a cause of bromide retention, but in the authors' opinion the latter mechanism is a more important factor. In either case, the liberated hydrobromic acid is likely to be fixed as alkali or alkaline earth bromides and should therefore be soluble in water, insoluble in nonpolar organic solvents, and nonvolatile at moderate temperatures.
2. As methyl bromide adsorbed on the material or dissolved in oily or fatty constituents. This should be soluble in organic solvents and volatile at moderate temperatures, provided it is not allowed to decompose into inorganic bromide.
3. As organic bromide produced by addition of methyl bromide or hydrogen bromide to an unsaturated compound or other attackable group. This type of bromide should be soluble in organic solvents, probably insoluble in water, and probably nonvolatile at moderate temperatures. No evidence of the formation of such compounds in foods has been encountered in the authors' work.

Martinek and Marti (*6*) employed a method for the determination of methyl chloride in foods, which consists in passing air through the sample at 100° C. and analyzing the vapors. Preliminary experiments showed that this method cannot be applied to samples containing methyl bromide, for when the temperature is raised methyl bromide reacts more rapidly with some substance in the food and produces inorganic bromide. The same type of reaction takes place if the food sample is boiled with water, although the rate of hydrolysis of methyl bromide in water alone is so low that practically all the halide may be boiled out. This reaction of methyl bromide with food constituents prevents one from preparing known mixtures for testing proposed analytical methods.

Attempts were made to extract methyl bromide from foods by means of organic solvents, as a result of which solvents with small molecules were found to be more effective than

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those with larger molecules. Methylene chloride was the most promising compound tested, but even successive treatments with it failed to extract the last small amounts of methyl bromide from some materials. Therefore volatilization by boiling with this solvent was resorted to after removal of most of the methyl bromide by extraction. The boiling point of methylene chloride (40° C.) is low enough so that not much reaction takes place. The problem becomes one of treating the sample under conditions as mild as possible at first, then more and more drastic, until most of the methyl bromide is removed before it has a chance to decompose. Since the methyl bromide (or other organic bromide) thus comes off in several fractions, it may be determined most easily as the difference between the total bromide originally present and the inorganic bromide remaining after treatment. However, to show that the bromide left is actually inorganic, the sample is finally extracted with water and the solution is analyzed for bromide.

Reagents

Methylene chloride. Commercial material usually contains a trace of hydrolyzable bromide which may be removed by shaking 2 liters of the solvent with 15 grams of potassium hydroxide dissolved in 300 ml. of 95 per cent ethyl alcohol and allowing the mixture to stand for several days. The alcoholic potassium hydroxide is washed out with water and the methylene chloride is filtered, dried over anhydrous calcium sulfate, and distilled.

Alcoholic potassium hydroxide, 2.5 grams of potassium hydroxide per 100 ml. of 95 per cent ethyl alcohol.

Sodium hydroxide, analytical reagent grade.

Sodium peroxide, analytical reagent.

Hydrochloric acid, about 6 *N*. This should be as free from bromide as possible. C. P. acid may be diluted to 6 *N* and distilled, the first and last fractions (each about 10 per cent of the total) being discarded to eliminate most of any free bromine or hydrobromic acid.

The other reagents are as described previously (*7*).

Procedure

Total Bromide. For most samples the procedure previously described (*7*) is satisfactory, but in certain cases of foods high in bromide and also in oil or protein, losses during ignition have since been noted. The same probably applies, in these cases, to the somewhat similar ashing method described by Dudley (*2*). A procedure modified from that of Brodie and Friedman (*1*) has been found preferable.

A sample of 5 to 10 grams is treated in a 100-ml. nickel crucible with 40 ml. of alcoholic potassium hydroxide, allowed to stand for an hour, and evaporated to dryness on a steam bath. It is then dried for a short time at 110° C. and is covered with 10 grams of sodium hydroxide pellets. The crucible is kept for an hour or two on a hot plate until the bubbling or smoking diminishes, after which it is placed in a muffle at 600° C. Fusion should be carried out without excessive burning or foaming; if the charge becomes ignited, the crucible should be removed from the muffle until the flame is extinguished. It is then returned to the muffle and this process repeated until the volatile gases have been removed. Sodium peroxide is added to the melt, a few milligrams at a time, to complete the oxidation of the remaining carbon or organic matter. The peroxide must be added cautiously while the crucible is removed from the furnace; bromide is lost if the charge burns with a flare when too much peroxide is added.

Complete combustion of the organic matter can be effected best by returning any organic matter that has raised above the sodium hydroxide to the bottom of the crucible, where it mixes with the melt and is easily destroyed by addition of the peroxide. This is accomplished by carefully rotating the hot crucible to wash down the organic matter and adding 0.5 gram more of peroxide. If no burning or bubbling takes place, the oxidation is complete. A few carbon particles which may remain after the final addition of sodium peroxide do not affect the accuracy of the results.

The crucible is rotated to allow the melt to solidify on the sides, and cooled, and the contents are dissolved in 75 ml. of water. Solution of the sodium compounds is hastened by placing the crucible on a hot plate for several minutes. The solution is transferred to a 400-ml. beaker and partially neutralized with about 50 ml. of 6 *N* hydrochloric acid. The solution is boiled to destroy peroxides and to reduce the volume to 100 to 125 ml. Nickel hydroxide and other insoluble hydroxides are removed by filtering through a No. 2 Whatman paper, collecting the filtrate and washings in a 500-ml. wide-mouthed Erlenmeyer flask. The filtrate is made slightly acid with 6 *N* hydrochloric acid, then neutralized with sodium hydroxide solution, adjusting to the color change of methyl red. The volume at this point should be approximately 150 ml.

About 2 grams of sodium acid phosphate and 5 ml. of hypochlorite solution are added and the mixture is heated to boiling. After a minute or so 5 ml. of sodium formate solution (50 grams per 100 ml.) are introduced and boiling is continued for 2 minutes. The sample is cooled and treated with a few drops of 1 per cent sodium molybdate solution, 0.5 gram of potassium iodide, and 25 ml. of 6 *N* sulfuric acid. Titration should be made immediately with standard 0.01 *N* sodium thiosulfate solution, starch indicator being added just before the end point. A blank on all the reagents should be carried through the entire procedure and subtracted. One milliliter of 0.01 *N* thiosulfate is equivalent to 0.1332 mg. of bromide ion.

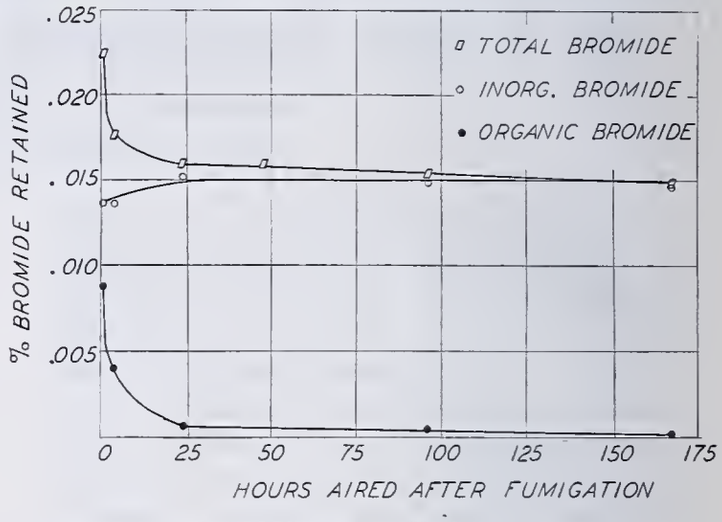

Figure 1. Retention of Bromide by White Flour

Separation of Organic Bromide. A sample of 5 to 10 grams in a 100-ml. beaker is treated with 15 ml. of methylene chloride and filtered immediately on a Gooch crucible with a dry asbestos pad, rinsing with three 5-ml. portions of solvent. In filtering by suction, the sample should not be allowed to become so cold from evaporation that moisture condenses on it. Most of the sample is transferred back to the beaker without disturbing the asbestos pad and is allowed to stand for 5 minutes with 15 ml. of methylene chloride. If the sample is lumpy it should be ground with the solvent in a mortar at this stage, then filtered and rinsed as before. The solid is again returned to the beaker and treated with 15 ml. of methylene chloride, this time for a 15-minute period, followed by a third filtration and rinsing in the same crucible.

The filtrate, which is ordinarily discarded, contains most of any methyl bromide or other soluble organic bromide, but not all, since the extraction may have been incomplete and since volatile compounds may have escaped. The presence of a soluble bromide compound may, if desired, be detected by catching the filtrates in alcoholic potassium hydroxide and determining the inorganic bromide formed after evaporation to dryness and ashing. Whether or not the bromide so found is methyl bromide or a nonvolatile organic halide may be ascertained by making a duplicate set of extractions and evaporating the extracts to about half of their original volume before addition of alcoholic alkali. Methyl bromide is expelled during this process and nonvolatile bromide may be determined on the remainder after hydrolysis and ashing. In this way the authors have found that within experimental error no organic bromide is formed by reaction of methyl bromide with the products that have so far been tested.

To remove any incompletely extracted volatile bromide compounds, the sample remaining after extraction is returned to the original beaker and treated with 15 ml. of methylene chloride. This is evaporated to dryness while being stirred to prevent bumping, but it should not be overheated. When the sample appears dry, the beaker is laid on its side in a warm place, such as on top of an oven, until all the odor of methylene chloride is gone.

Inorganic Bromide. Inorganic bromide may now be determined by the same procedure as described above for total

bromide, practically all of the organic bromide having been removed. However, in order to have further confirmation of the inorganic nature of the remaining bromide, it has been shown in all the authors' tests to be water-soluble. The beaker and its contents are cooled, 30 ml. of water are added with thorough mixing, and then the mixture is filtered on the original crucible. In the case of some foods, such as flour, filtrations are very slow and preliminary separations by centrifuging are desirable. After each separation the solid is mixed with 30 ml. of water and allowed to stand for 15 minutes, then filtered or centrifuged again, until four extractions have been made. The combined filtrates are treated with 3 ml. of saturated sodium chloride solution and evaporated nearly to dryness in a silica dish, then 30 ml. of 2.5 per cent alcoholic potassium hydroxide are added and evaporated and the bromide is determined after ashing as described in the previous paper (*7*).

Unaccounted Bromide. The residue after water-extraction may be placed in a 100-ml. nickel crucible and analyzed for bromide in the manner described under Total Bromide. Generally only a very small fraction of the total bromide will be found in this residue and the amount will be less the more thorough the water washing. Therefore this bromide is usually considered to be inorganic. Only in some experiments in which methyl bromide was adsorbed upon charcoal has the unaccounted bromide been thought to be organic. No food adsorbs methyl bromide as tenaciously as does charcoal.

Application to Fumigated Products

Since one cannot readily prepare standard mixtures for testing proposed separation methods, the procedure outlined above has been tested indirectly:

1. By considering the data obtained when the procedure is applied to a fumigated product at various intervals after fumigation. Since methyl bromide may escape upon airing or may decompose into inorganic bromide, whereas the latter cannot escape, one should always find that the total bromide decreases toward a limit while the inorganic bromide increases toward the same limit. If the inorganic bromide should appear to decrease during aeration, the proposed method would be unsuccessful, since the only way this could occur would be through a change of methyl bromide into inorganic bromide during the separation.
2. By allowing a reactive product such as flour to air for a week or more after fumigation and before analysis. In this time the methyl bromide will be completely volatilized or hydrolyzed. If the analytical method then gives the same values for inorganic and total bromides, one can be sure that it does not introduce negative errors in the determination of inorganic bromide. (An error of 0.10 ml. of 0.01 *N* thiosulfate, or about 0.0003 per cent bromine on a 5-gram sample, is considered allowable.)
3. By fumigating a nonreactive material such as pure dry sugar charcoal and analyzing for inorganic bromide. None of the latter should be found if no decomposition occurs during the separation.

Table I. Laboratory Fumigation of Wheat Flour (White)

(1.98 pounds of CH_3Br per 1000 cu. feet for 12 hours at 80–90° F.)

Aired after Fumigation	Total Bromide	Inorganic Bromide	Organic Bromide
Hours	%	%	%
0.5	0.0224	0.0135	0.0089
4	0.0175	0.0135	0.0040
24	0.0159	0.0151	0.0008
48	0.0159	....	....
96	0.0154	0.0149	0.0005
168	0.0149	0.0148	0.0001
Control, not fumigated	0.0010	0.0009	0.0001

Table II. Laboratory Fumigation of American Process Cheese

(2.00 pounds of CH_3Br per 1000 cu. feet for 12 hours at 76–77° F.)

Aired after Fumigation	Bromide in Outer 0.25-Inch Layer		
	Total	Inorganic	Organic
Hours	%	%	%
0.5	0.0114	0.0047	0.0067
4	0.0093	0.0052	0.0041
24	0.0075	0.0062	0.0013
48	0.0077	0.0072	0.0005
96	0.0071	0.0076	....
168	0.0079	0.0080	....
Control, not fumigated	0.0005	0.0004	0.0001

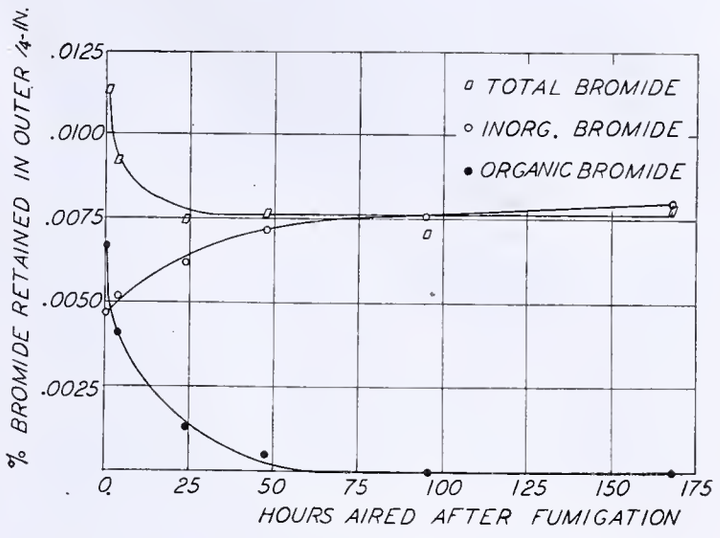

Figure 2. Retention of Bromide by American Cheese

Any procedure to be satisfactory must conform in all the above points, as well as give consistent results in the analysis of duplicate samples. However, there still might be a possibility that the method allows some reaction of methyl bromide with the food constituents. To minimize this chance, one can only compare various "satisfactory methods" on duplicate portions of a reactive food taken at the same time shortly after fumigation. The method which under these conditions gives the lowest value for inorganic bromide is the one which allows the least conversion of methyl bromide into inorganic bromide. This has been the final criterion in the choice of the above procedure.

To show the types of airing curves obtained, fumigations of some typical foods as well as of sugar charcoal have been carried out, and the products analyzed after definite intervals. In Table I and Figure 1 are presented data obtained in the laboratory fumigation of white wheat flour. The retention of bromide is greater in fumigations performed on a laboratory scale than in those made commercially, as will be shown in a separate publication; hence these values are higher than would normally be obtained, but the general shape of the airing curves is the same. It is evident that most of the methyl bromide disappears within a day and that it is completely gone within a week. The decrease in methyl bromide content is due mainly to volatilization, only a small part of it decomposing into inorganic bromide after removal from the fumigation chamber.

Table II and Figure 2 represent a laboratory fumigation of a sample of American process cheese. Half a pound of the unwrapped cheese in the form of a single cube was fumigated and the portions for analysis were taken from the outer layer, to a depth of 0.64 cm. (0.25 inch). It is of interest to note that methyl bromide does not penetrate process cheese, no more bromide being found in the interior portions than in unfumigated controls. During aeration the cheese dries out, hence the bromide results reported in Table II have been corrected back to the as-received basis. The final value of 0.0080 per cent bromine in the outer layer corresponds to 0.0043 per cent based on the entire sample and would be even lower if a larger piece of cheese had been fumigated. Relatively more of the methyl bromide taken up is eventually decomposed to inorganic bromide in the case of cheese than with flour, but the total retention is lower.

To test the method on a sample containing adsorbed methyl bromide relatively free from inorganic bromide, sugar charcoal was fumigated and analyzed at intervals while airing. Because the charcoal adsorbs so much more fumigant than do foods, a greatly reduced dosage of methyl bromide

TABLE III. LABORATORY FUMIGATION OF SUGAR CHARCOAL

(0.0267 pound of CH_3Br per 1000 cu. feet for 12 hours at 73–75° F.)

Aired after Fumigation	Total Bromide	Inorganic Bromide	Unaccounted Bromide	Organic Bromide
Hours	%	%	%	%
0.5	0.0197	0.0002	0.0036	0.0159–0.0195
4	0.0188	....	....	....
24	0.0188	0.0002	....	....
96	0.0183	0.0001	0.0025	0.0157–0.0182
192	0.0180	....	....	....
336	0.0174	....	....	....
720	0.0144	....	....	....
1008	0.0120	....	....	....
2400	0.0113	0.0001	0.0037	0.0075–0.0112
Control, not fumigated	0.0002	0.0000	0.0000	0.0002

was employed. The results, shown in Table III, demonstrate that within experimental error no methyl bromide is falsely reported as inorganic bromide. Some of the bromide is found in the residues after separation and is therefore labeled as unaccounted; these amounts are not large when the difficulty of removing adsorbed methyl bromide from charcoal is realized. Thus 720 hours of airing removed only 27 per cent of the original methyl bromide, whereas 81 per cent was quickly removed by the analytical procedure outlined in this paper. At the end of the 720-hour period, a duplicate sample was evacuated for 26 hours at 2 mm. of mercury, air being admitted ten times during the evacuation to wash out liberated methyl bromide. This treatment lowered the total bromide percentage only from 0.0144 to 0.0122.

Comparison of the data obtained on charcoal with those on flour and cheese emphasizes the fundamental differences in the ways in which bromide is retained by a food and by an efficient adsorbent. Methyl bromide taken up by an adsorbent remains as such for a long time, while free methyl bromide present in a food can escape readily by volatilization and only the inorganic bromide produced by decomposition remains. The analytical method here proposed is capable of being applied to a wide variety of foods and has been found of value for indicating the time of airing which should be allowed after fumigation.

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Determination of Citric Acid

In Pure Solutions and in Milk by the Pentabromoacetone Method

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OF THE methods that have been used for the quantitative determination of citric acid, the pentabromoacetone method has produced the most satisfactory results. It is based upon the fact that when citric acid is oxidized with potassium permanganate in the presence of bromine, under controlled conditions, the acid is converted quantitatively into pentabromoacetone, $CBr_3.CO.CBr_2H$, the amount of which can be determined gravimetrically.

Although the detection of citric acid through the formation of a bromine derivative of a product of its oxidation with potassium permanganate was noted by Stahre (*9*) in 1895, Kunz (*5*) was the first to utilize the reaction to determine the citric acid content of milk, wines, and other fruit products.

Hartmann and Hillig (*1–4*), Reichard (*8*), Lampitt and Rooke (*7*), and others also studied the reaction to determine the conditions that would result in a quantitative conversion into and isolation of the pentabromoacetone. In general, the procedures adopted have been those used by Kunz, with particular attention being given to the temperatures used during the oxidation and bromination procedure, the formation of the precipitate, and the isolation, washing, and drying of the product. The results obtained were generally low in value, especially with solutions containing sugars, and corrections were usually made to account for the losses incurred. The variations in percentage recovery in most cases were also greater than should be expected.

After a study of different methods and various modifications of the pentabromoacetone method, Lampitt and Rooke recommended the following procedure with solutions of citric acid and with milk serums. It is given in detail because it forms the basis for the work herein described.

To a solution containing citric acid or to 50 ml. of milk serum are added 10 ml. of sulfuric acid (1 to 1 by volume) and 5 ml. of potassium bromide solution (37.5 per cent). Potassium permanganate solution (5 per cent) is added dropwise from a buret with constant shaking until a brown precipitate persists, 10 ml. usually being required for 0.10 gram of citric acid and 25 ml. for a milk serum. The mixture is allowed to stand at room temperature for 1 hour, further additions of permanganate being made if the brown precipitate disappears. Sufficient ferrous sulfate solution (20 per cent in 1 per cent sulfuric acid solution) is then added slowly till a pale yellow solution containing a white precipitate is obtained and the mixture is cooled in an ice chest overnight (16 hours).

The precipitate is removed by filtration through a sintered-glass crucible (size 10 G 4), the reaction flask being washed out with the filtrate to remove the last traces of precipitate, and the washings passed through the crucible. The precipitate is then washed with portions of 10, 10, and 5 ml. of cold water. The crucible is dried to constant weight in a vacuum desiccator (about 16 hours). The precipitate is dissolved out of the crucible with industrial spirits followed by 20-, 10-, and 10-ml. portions of ether. The crucible is again dried in a vacuum desiccator and weighed, the loss in weight being taken as pentabromoacetone.

$$\text{Citric acid (anhydrous)} = 0.424\left(W + \frac{0.005V}{100}\right)$$

where W represents the difference in weight of the crucible before and after treatment with industrial spirit and ether, and V the original volume of filtrate from reaction mixture, less the total volume of washings.

In Pure Solutions

In the application of the method as modified by Lampitt and Rooke to citric acid solutions, the authors obtained results which were not unlike those reported by these and other workers in the percentage recovery of citric acid (Table I).

In several experiments wherein the excess of reagents was not discharged prior to storage, it was noted that where the excess of potassium permanganate was slight the manganese

dioxide had disappeared during the 16 hours' storage. This was taken to indicate that the reaction was not completed in the 1 to 2 hours allowed by the Lampitt and Rooke and other procedures. The following modification was, therefore, introduced.

After addition of the bromine, potassium permanganate was added in slight excess as recommended, the mixture was allowed to stand at room temperature for approximately 1.5 to 2 hours, and more reagent was added if the excess disappeared. The mixture was then placed in the icebox for 16 hours or more and the excess of permanganate was discharged at the conclusion of this period.

Table I. Percentage Recovery of Citric Acid by Pentabromoacetone Procedure

Citric Acid Present	Recovery		
	Minimum	Maximum	Average
Gram	%	%	%
Lampitt and Rooke Procedure			
0.10 (5 detns. in triplicate)	95.20	98.84	97.42
0.15 (1 detn. in duplicate)	94.8	97.0	95.90
0.20 (2 detns. in triplicate)	96.2	98.4	97.30
0.30 (1 detn. in duplicate)	90.66	94.0	92.33
Modified Lampitt and Rooke Procedure			
0.10 (5 detns. in triplicate)	97.20	102.3	99.65
0.20 (5 detns. in triplicate)	101.60	101.95	101.77

With this modified procedure the results shown in Table I were obtained upon citric acid solutions. These seem to confirm the conclusion that a longer time is required for the completion of the oxidation and bromination than that specified by the various authors. This seems also to be borne out by a comparison of the bromine content of the products obtained by Lampitt and Rooke and those obtained by the authors. Pentabromoacetone products isolated by Lampitt and Rooke had a bromine content of 86.8 per cent when prepared from pure citric acid solution and 85.5 per cent when prepared from milk serum, and had a melting point of 71° to 73° C. Two similar products isolated by the present authors in the course of regular determinations upon pure citric acid solutions had bromine contents of 87.88 and 87.79 per cent and melting points of 74° and 74.5° C.

Pure pentabromoacetone contains 88.28 per cent of bromine and has a melting point of 76° C.

With the use of Lampitt and Rooke's procedure, except for the noted modification in the time of reaction, the results obtained upon solutions of pure citric acid containing 0.20 gram or more per determination were consistently from 1 to 2 per cent higher than the theoretical values. This could result from insufficient washing of the precipitate, or the application of a correction of greater value than the losses incurred.

To ascertain if the high values were the result of one or both of these factors, the following experiments were carried out.

Losses in Weight of Precipitate. *Washing of Precipitate.* The Lampitt and Rooke procedure specifies the use of 25 ml. of cold water (portions of 10, 10, and 5 ml.) to wash the precipitate. The authors' experiences indicate that this is sufficient in most cases. However, in many instances the weights of precipitates were greater than the theoretical value and their differences in duplicate determinations greater than should be expected.

Rewashing these precipitates with 25 ml. of cold water produced as a rule values in good agreement with one another, and further washings with similar quantities resulted in a constant loss for each washing.

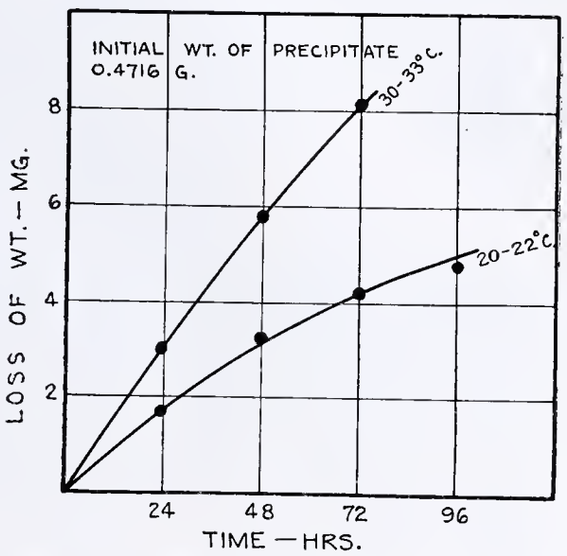

Figure 2. Effect of Drying on Loss in Weight of Pentabromoacetone Precipitates

These results indicate that in many cases all of the extraneous materials are not removed by 25 ml. of wash water, but that they are completely removed by 50 ml.

The difference in the ease with which precipitates wash free of extraneous material is no doubt a result of the differences in their physical structures, the finer and more closely packed crystals being more difficult to free of extraneous products. To ensure complete washing of the precipitates the authors have, therefore, used 50 ml. (20, 20, and 10 ml.) of water at a temperature of 1° to 3° C. That the use of water of this temperature is necessary to keep the losses at a low value is indicated by Figure 1.

Drying of Precipitate. Hartmann and Hillig dried the precipitates by aspiration of the crucibles with air for 0.5 hour. They found, however, that rapid aspiration resulted in low values.

During the drying of a pentabromoacetone precipitate in vacuum there is a progressive loss of weight because of the volatility of the compound itself. The amount of loss is dependent upon the time and temperature of drying; hence, these factors should be constant in order that the losses in drying may not vary too greatly. The results given in Figure 2 indicate the losses in weight of pentabromoacetone precipitates during storage in vacuum desiccators at 20° to 22° C. and 30° to 33° C. for various lengths of time.

While overnight drying, as specified and at a temperature of 20° C., is not necessary in most cases to attain a practically

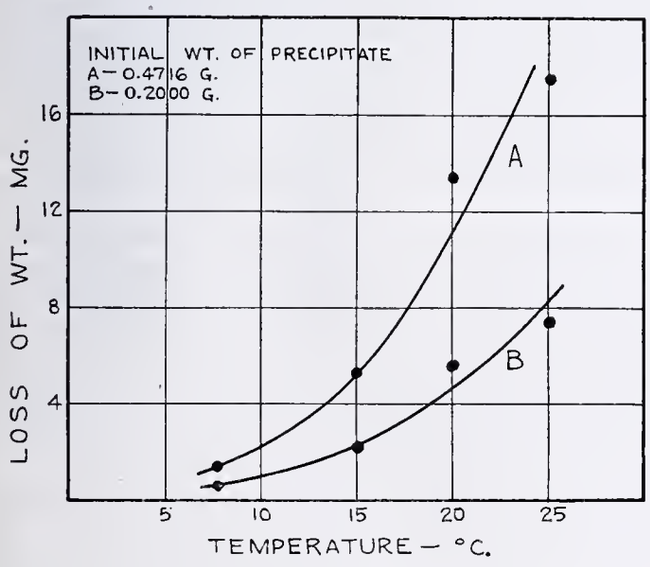

Figure 1. Effect of Washing on Loss in Weight of Pentabromoacetone Precipitates

Washed with 50 ml. (20, 20, and 10 ml.) of water at different temperatures

constant weight of the precipitate, this time factor (approximately 16 hours) has been chosen for use because of its convenience.

Solubility of Product in Reaction Mixture. Reichard has given the solubility of pentabromoacetone in 100 ml. of a 10 per cent sulfuric acid solution plus 100 mg. of bromine as nil at 5° C. and 5 mg. at 15° to 18° C. Lampitt and Rooke have determined the loss of weight of the substance through solubility in the reaction mixture and through its washing and drying "under the conditions of the experiment" and give this loss as 5 mg. per 100 ml. of reaction mixture. They later state that this is the solubility of pentabromoacetone in the reaction mixture at 0° C. Hartmann and Hillig (*1*) found that the losses were equivalent to 4 mg. per 100 ml. of the reaction mixture, under experimental conditions somewhat different from those employed by Lampitt and Rooke and later also suggested the use of an empirical factor in the correction of the results (*3*).

Table II. Loss of Pentabromoacetone

Through solubility in reaction mixture and wash water, and through volatilization during drying)

Pentabromoacetone	Loss (100-Ml. Reaction Mixture)
Gram	*Gram*
0.30	0.0030, 0.0033, 0.0037 0.0030, 0.0038, 0.0037
0.20	0.0037, 0.0024 0.0032, 0.0044

To determine the losses in weight of the product through the experimental procedures adopted by the authors, samples of pentabromoacetone were added to mixtures of water, potassium permanganate, and bromine solution. These mixtures were comparable in the quantities of these reagents to those used in a determination. The mixture was held at a temperature of approximately 2° C. for from 4 to 10 days with shaking at frequent intervals. The solutions were then treated with ferrous sulfate and filtered in the usual manner and the crystals were washed with 50 ml. (20, 20, and 10 ml.) of water at a temperature of 1° to 3° C., and dried in a vacuum desiccator overnight (18 hours). Table II indicates the results obtained.

Under the stated conditions, the loss of weight of pentabromoacetone because of solubility in 100 ml. of reaction mixture and in 50 ml. of wash water at a temperature of 1° to 3° C., and because of volatility during drying, is approximately 3.5 mg.

Table III. Percentage Recovery of Citric Acid

(Pentabromoacetone method as modified by authors)

Citric Acid Present	Citric Acid Recovered	Loss	Recovery Uncorrected	Recovery Corrected
Gram	*Gram*	*Mg.*	%	%
0.10	0.0988	1.2	98.80	100.30
0.10	0.0984	1.6	98.40	99.90
0.10	0.0986	1.4	98.60	100.10
0.10	0.0986	1.4	98.60	100.10
0.10	0.0986	1.4	98.60	100.10
0.10	0.0984	1.6	98.40	99.95
0.10	0.0991	0.9	99.10	100.60
0.10	0.0977	2.3	97.70	99.20
0.10	0.0981	1.9	98.10	99.60
		Av. 1.5+		
0.20	0.1985	1.5	99.25	100.00
0.20	0.1978	2.2	98.90	99.65
0.20	0.1984	1.6	99.20	99.95
0.20	0.1982	1.8	99.05	99.85
0.20	0.1987	1.3	99.35	100.10
0.20	0.1983	1.7	99.15	99.90
0.20	0.1980	2.0	99.00	99.75
0.20	0.1993	0.7	99.65	100.40
0.20	0.1991	0.9	99.55	100.30
		Av. 1.5+		

In view of the foregoing results the authors have adopted the following procedure:

Procedure Adopted. After addition of the reagents to the citric acid solution the mixture is allowed to stand at room temperatures for 1.5 to 2 hours, and more permanganate is added if the excess disappears. The mixture is then placed in an ice chest (1° to 3° C.) overnight and the excess of permanganate is discharged with ferrous sulfate immediately before filtration.

Table IV. Percentage Recovery of Citric Acid Added to Decitrated Milk

Citric Acid Present	Citric Acid Recovered Minimum	Maximum	Average
Gram	%	%	%
(Pentabromoacetone method modified by Lampitt and Rooke)			
0.10 (4 detns. in duplicate)	90.0	99.0	95.10
0.20 (2 detns. in duplicate) (3 detns. in triplicate)	96.30	99.20	97.13
0.30 (5 detns. in duplicate)	92.15	99.30	95.26
0.40 (3 detns. in triplicate)	92.15	94.22	93.06
(Pentabromoacetone method modified by authors)[a]			
0.10 (2 detns. in duplicate)	100.0	100.75	100.37
0.20 (2 detns. in duplicate)	98.5	99.9	99.20
0.30 (2 detns. in duplicate)	99.75	101.0	100.37

[a] Results calculated by formula of Lampitt and Rooke.

The pentabromoacetone precipitate is washed with 50 ml. (20-, 20-, and 10-ml. portions) of water at a temperature not over 3° C. At the conclusion of the filtration and washing the temperature of the filtrate should not be over 7° C.

The precipitates are dried in a vacuum desiccator as described by Lampitt and Rooke, but at a temperature not over 20° C.

In calculation of the results a correction of 3.5 mg. of pentabromoacetone per 100 ml. of reaction mixture is used when pure citric acid solutions are analyzed.

Application of Modified Procedure. The procedure of Lampitt and Rooke, modified as stated, was applied to nine 50-cc. aliquots of each of two citric acid solutions, one containing 0.10 and the other 0.20 gram of citric acid per aliquot. The corrected values were calculated by the following formula and the data are given in Table III.

$$\text{Citric acid (anhydrous)} = 0.424\left(W + \frac{0.0035\ V}{100}\right)$$

where W equals weight of precipitate, and V equals the original volume of the filtrate from the reaction mixture, not inclusive of the volume of the washings.

Determination of Citric Acid in Milk

Lampitt and Rooke found that the pentabromoacetone method, as modified by them, when applied to citric acid solutions containing lactose gave results that in general were lower in value than those obtained with pure solutions. In three determinations upon dried skim-milk suspensions to which citric acid had been added to the extent of 0.18, 0.46, or 0.92 per cent these authors recovered 0.13, 0.44, and 0.91 per cent, respectively. They claim, however, that the method yields values "less than 2 mg. low for weights of citric acid up to 0.11 gram in the presence of milk serums".

In an experiment of this type, it is necessary to make a correction for the initial citric acid content of the product, the determination of which may in itself be in error. To obviate the necessity of making such a correction, the authors have used skim milks sterilized by intermittent heating and in which a citrate-fermenting lactose-nonfermenting bacterium had been grown until no citric acid could be detected in the milk. The organism used was isolated from Swiss cheese by Dr. Burkey of these laboratories and many of its characteristics are similar to those of *Lactobacillus leichmanii*, Bergey *et al.*

During the growth of this organism the milk was tested at intervals for the presence of citric acid. When the citrates had been fermented the action of the organisms was stopped by heating the milk. Continued action of the organism after disappearance of the citrates seemed to result in decomposition of some milk constituents, the resulting compounds of which were readily oxidized by the permanganate, and interfered with the complete oxidation of the citric acid. The pH of the decitrated milk was usually approximately 6.2.

In a series of experiments upon decitrated milk, to which citric acid had been added, analyses by the Lampitt and Rooke procedure gave the results of Table IV. These are in agreement with the observations of Lampitt and Rooke, that in the presence of lactose and in milk the percentage recovery tends to be less than with the pure citric acid solutions.

Using the authors' modification—namely, allowing the reaction mixture to remain at room temperature for 2 hours and then at icebox temperature overnight (16 to 20 hours) prior to the discharge of excess potassium permanganate with ferrous sulfate—another series of experiments was carried out (Table IV).

These results, like those upon pure citric acid solutions, indicate that a longer time of reaction of the potassium permanganate and bromine yields values of greater magnitude than do the procedures employed by other workers.

Losses in Weight of Precipitate. The losses of pentabromoacetone when reaction mixtures, prepared with water, are used has been determined as 3.5 mg. per 100 ml. of the mixture. The experiments of Table IV, wherein a correction of 5 mg. per 100 ml. of reaction mixture was used, seem to indicate that the losses are greater when a milk substrate is used. The correct value to be applied for the losses sustained under the conditions employed was determined as follows:

To decitrated milk serum were added the reagents used in a determination. To 100 ml. of this mixture a weighed amount of pentabromoacetone was added and the mixture was allowed to stand at ice-chest temperatures, with frequent agitation, for from 24 to 96 hours. The crystals were filtered off and washed with 50 ml. of water (20-, 20-, and 10-ml. portions) at a temperature of 1° to 3° C., and dried overnight in a vacuum desiccator at 20° C.

Table V. Solubility of Pentabromoacetone in Reaction Mixture Prepared with Decitrated Milk Serum

Pentabromoacetone Used	Loss of Weight
Gram	*G./100 ml.*
0.10	0.0053, 0.0032
0.20	0.0040, 0.0034, 0.0077 0.0060, 0.0074, 0.0037 0.0092
0.30	0.0034, 0.0040, 0.0037 0.0040, 0.0040, 0.0032
	Av. 0.0048

The results given in Table V seem to indicate that the solubility of the pentabromoacetone in a reaction mixture prepared with decitrated milk serum is slightly greater than in that prepared with water. There is also a greater divergence in the values obtained in these experiments than in those obtained with reaction mixtures prepared with water, which may be the result of variations in the rates of establishing solubility equilibria.

Table VI. Recovery of Citric Acid Added to Decitrated Milk

(By modified procedure)

Citric Acid Present	Citric Acid Recovered	Recovery
Gram	*Gram*	%
0.06	0.0589	98.16
0.06	0.0589	98.16
0.06	0.0594	99.00
0.06	0.0594	99.00
0.06	0.0595	99.16
0.06	0.0595	99.16
0.06	0.0596	99.33
0.06	0.0598	99.66
0.06	0.0602	100.33
0.06	0.0603	100.50
0.06	0.0606	101.00
0.06	0.0609	101.50

Application of Modified Procedure. One hundred and fifty grams of decitrated skim milk, to which had been added 0.30 gram of citric acid, were treated in the following manner, described by Lampitt and Bogod (*6*), to obtain the serum.

One hundred and fifty grams of milk are heated to 50–60° C. in a 250-ml. graduated flask, 25 ml. of potassium oxalate (2 per cent) are added, and the contents are shaken. Twenty milliliters of sulfuric acid (1 to 1) are added, and the contents are shaken and allowed to cool; 10 ml. of a phosphotungstic acid solution (10 per cent) are then added and the contents are made up to 250 ml., thoroughly shaken, and filtered.

Fifty milliliters of serum were used in each determination, which represented 30 grams of skim milk and contained 0.06 gram of citric acid. This is approximately the concentration of citric acid in milk.

The results of 12 determinations in duplicate are given in Table VI.

The formula used to obtain the corrected values was similar to that given above, except that the correction used was 0.005 instead of 0.0035 gram per 100 ml. of reaction mixture.

Summary

The determination of pentabromoacetone formed by the action of potassium permanganate and bromine upon citric acid under controlled conditions has been used as a basis for the quantitative determination of citric acid by various authors. The recovery of citric acid by the methods employed has usually been less than the theoretical value, especially where sugars have been present. That the losses incurred may in part be the result of incomplete conversion of citric acid is indicated by the fact that allowance of a longer time for the reaction than has hitherto been used results in recoveries of greater percentages of the citric acid present.

In addition to losses incurred through incomplete conversion of citric acid to pentabromoacetone, losses may occur also because of volatility of the precipitate under the usual conditions of drying and of solubility of the precipitate in the reaction mixture and the wash water used.

Because of these facts no absolute method can be prescribed for the complete recovery of citric acid as pentabromoacetone under all conditions. The method must be standardized with respect to the conditions and products employed and corrections made for the losses incurred in each case.

The losses sustained through the use of different amounts of wash water and wash water of different temperatures and those resulting from drying the precipitate at different temperatures have been determined to ascertain the effect of variations in these procedures. With the use of a standardized procedure for handling the precipitate the correction for losses was found to vary with the nature of the substrate, because of variations in the solubility of the pentabromoacetone precipitate in different reaction mixtures. Thus, with pure solutions the losses were found to be 3.5 mg. while with milk serum they were approximately 5 mg. of pentabromoacetone per 100 ml. of reaction mixture.

The modified method applied to aliquots of solutions containing from 0.10 to 0.30 gram of citric acid gave results that were consistently within ±0.50 per cent of the theoretical values. When the quantity of citric acid was less than 0.10 gram the percentage recoveries were slightly less than the value given and the results were not so consistent as those obtained with the use of larger amounts.

In the case of milk the quantity of serum that can be conveniently used in each determination is such that the approximate amount of citric acid present is less than 0.10 gram. The results obtained upon decitrated milks to which citric acid in these amounts had been added were usually within ±1.00 per cent of the theoretical values, though the differences between duplicate determinations were usually greater than those in which larger amounts were present.

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Estimating the Density of Tobacco Tissue

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A KNOWLEDGE of the density of cured tobacco tissue is of importance to the agronomist (*4, 5, 6*) and to the tobacco manufacturer (*1*). While a number of attempts to determine the density of tobacco have been made, these have apparently not resulted in the development of a rapid, accurate method which will give easily reproducible results. In view of this, the method of Tschapek (*7*) was studied as applied to tobacco, including the limits of allowable variation, which would not affect the use of the density values obtained by it for comparative purposes.

The sample is placed in a tared pycnometer, weighed, and covered with liquid. The pycnometer is next placed in a suitable container, which is evacuated to below the vapor pressure of the liquid used. After evacuation, the vacuum is broken, and the pycnometer is filled with the liquid, brought to a definite temperature, and weighed. The density is calculated by the formula

$$d = \frac{P_2}{P_1 + P_2 - P_3} d_0 \qquad (1)$$

where d = calculated density of the sample, d_0 = density of liquid used, P_2 = weight of sample, P_1 = weight of pycnometer plus liquid, and P_3 = weight of pycnometer plus liquid plus sample.

Preparation of Samples

All samples used were the cured unaged leaf of flue-cured tobacco which had been air-dried to approximately 2 per cent moisture content. The midribs were removed from the lamina material by hand and the lamina was removed from the veins by rubbing the leaf fragments on sieves, using a rubber stopper as a pestle.

The nature of the material, the quality and location of the leaf on the stalk, and the particle size are indicated in Table I.

TABLE I. MATERIAL USED IN TESTS

Sample No.	Part of Leaf	Quality of Tobacco	Location on Stalk	Particle Size
				Mesh
1	Lamina	Poor	Bottom	100
2	Lamina	Good	Middle	100
3	Lamina	Fair	Top	100
4	Midribs	Good	Middle	1-cm. length
5	Midribs	Good	Middle	20
6	Midribs	Good	Middle	60
7	Midribs	Good	Middle	100
8	Lamina	Good	Middle	2-cm. diameter
9	Lamina	Good	Middle	20
10	Lamina	Good	Middle	100

Pycnometers

Specific gravity bottles of 25-, 50-, and 100-cc. capacity of both the Gay-Lussac and Hubbard type gave equally satisfactory results if rapid changes in pressure were avoided and the tobacco did not occupy over one-fourth of the volume of the pycnometer. The 25-cc. pycnometers proved most satisfactory because of the smaller quantity of liquid required and the greater ease of weighing.

The evacuation pressure employed did not seem to be significant if it was less than the vapor pressure of the liquid.

The size of the portion of tobacco placed in the pycnometer was not significant, provided it was representative of the sample. Thus the average of several determinations on sample 2 carried out with different weights of tobacco were as follows: with 1 gram, 1.452; with 3 grams, 1.451; and with 5 grams, 1.455.

Unless otherwise stated, all data given were obtained on approximately 5-gram samples of tobacco in 25-cc. Hubbard pycnometers, using C. P. xylene to cover the tobacco. The vacuum was applied for 15 minutes, and the pycnometer was filled with xylene, brought to 30° in a thermostat, and weighed.

Experimental

Table II indicates that density data accurate to about four units in the third decimal place can be obtained by Tschapek's method if the samples are well prepared and ground to pass a 100-mesh sieve.

TABLE II. REPRODUCIBILITY OF DENSITY DATA OBTAINED WITH XYLENE FOR FINELY GROUND TOBACCO

	Sample 1	Sample 2
	1.552	1.456
	1.557	1.456
	1.557	1.456
	1.556	1.453
	1.557	1.457
	1.555	1.454
	1.558	1.453
		1.455
Average	1.556	1.455
Deviation from average	0.003	0.002

TABLE III. EFFECT OF LIQUID ON DENSITY

(Sample 2 used)

C. P. Benzene	C. P. Toluene	C. P. Xylene	Redistilled Water
1.451	1.455	1.456	1.533
1.447	1.453	1.457	1.525
1.454	1.451	1.453	1.536
	1.457	1.455	1.543
Av. 1.451	1.454	1.455	1.534

Culbertson and Dunbar (*2*) and Culbertson and Weber (*3*) have pointed out that different values may be obtained for the apparent density of a material if different liquids are employed as buoying fluids. Therefore density values for sample 2 were obtained with several different liquids, in order to find a suitable one for convenient use in a routine procedure. The liquid employed should not be readily volatile at room temperature, but should have a vapor pressure which will allow it to boil gently under reduced pressure. It should exercise but little solvent action on the tobacco, and its viscosity should be such that it will completely penetrate the tobacco tissue.

Benzene, toluene, and xylene approach these requirements, and were tried, as well as a mineral oil, diphenyl ether, and water. The mineral oil was not suitable because of its high boiling point. Diphenyl ether failed to give satisfactory results because of its high freezing point. With persistent effort the apparent mechanical difficulties with water can be overcome, but the density value is appreciably greater than that obtained when employing the organic liquids. A

TABLE IV. EFFECT OF IMPURE XYLENE ON DENSITY VALUES

(Sample 3 used)

C. P. Xylene	Xylene Extract of Tobacco
1.426	1.428
1.422	1.426
1.424	1.427
1.426	1.426
Av. 1.425	1.427

Table V. Effect of Particle Size on Apparent Density

Sample No.	4	5	6	7	8	9	10
Approximate wt. of sample, grams	2	5	5	5	2	5	5
Part of leaf	Midrib	Midrib	Midrib	Midrib	Leaf without midrib	Leaf without midrib	Leaf without midrib
Size of particle	1 cm. long	20-mesh	60-mesh	100-mesh	2-cm. diameter	20-mesh	100-mesh
Density	1.175	1.545	1.600	1.599	1.388	1.427	1.456
	1.229	1.546	1.606	1.607	1.391	1.429	1.457
	1.260	...	...	1.600	1.396	1.430	1.458
	1.304	...	...	...	1.400	1.431	1.459
	Av. 1.242	1.546	1.603	1.602	1.394	1.429	1.458

similar increase in the apparent density obtained with water above that obtained with benzene in determining the density in the case of silica, an insoluble material, is attributed by Culbertson and Weber (*3*) to the difference in electric moment of the two liquids.

Tobacco is somewhat soluble in water and in the organic liquids employed. It is, however, more soluble in the water, and this may be the cause of the observed 5 per cent increase in the apparent density. The density data obtained with the different liquids are given in Table III.

One hundred grams of tobacco were extracted with C. P. xylene for several days, the density of the extract was determined, and the extract was used to determine the density of portions of sample 3. The data given in Table IV indicate that the solvent action of the xylene on the tobacco does not, within the experimental error, affect the value obtained for the apparent density of the tobacco. They also tend to show that a technical grade of xylene could be used if its density were known or were accurately determined.

Table V indicates that the particle size affects the apparent densities obtained: the smaller the particle size up to 60-mesh, within the limits studied, the greater the density. The variation in these results with the particle size can probably be explained by the failure of the xylene to penetrate completely all cavities within the structure of the large pieces of tobacco tissue and the consequent resulting failure to remove all gaseous materials from these cavities during evacuation.

The data in Table VI were obtained by evacuating the samples for 20 minutes, and determining the density. Then the pycnometer and contents were placed under vacuum again for 40 minutes and the density was redetermined. This procedure was repeated on the initial portions at 2- to 3-hour intervals for a total of 20 hours or until constant results were obtained. These data indicate that the application of vacuum for 20 minutes will give constant values for density on samples ground to pass 100-mesh sieves, and that constant values probably cannot be obtained on samples composed of large-sized pieces of tobacco.

The density data given in Table VII show that a variation of 10° C. in the temperature of the tobacco from 20° to 30° does not result in density differences greater than the experimental error.

Table VI. Increase in Density with Increase in Time of Evacuation

Hours Evacuated	Sample 4, 2 Grams, Midrib — Particle Size — 1 cm. long	Sample 7, 5 Grams, Midrib — Particle Size — 100-mesh	Sample 8, 2 Grams, Lamina and Veins — Particle Size — 2-cm. diameter	Sample 10, 5 Grams, Lamina and Veins — Particle Size — 100-mesh
0.33	1.300	1.602	1.407	1.455
1	1.308	1.603	1.410	1.456
3	1.329	...	1.421	1.458
5	1.334	...	...	...
7	1.341	...	1.423	...
10	1.346	...	...	...
11	1.348	...	1.423	...
13	1.354	...	...	...
15	1.356	...	...	...
17	1.360	...	...	...
20	1.365	...	...	...

Method

The following procedure, which has been used on numerous samples of tobacco in this laboratory, has been found to give reproducible results in all cases.

One to 5 grams of tobacco, ground to pass a 60- to 100-mesh sieve, are placed in a 25-cc. tared Hubbard type pycnometer and covered with C. P. xylene. The pycnometer and contents are placed in a vacuum desiccator and the pressure of the system is reduced to 10 mm. of mercury or less for 15 minutes. The vacuum is broken, and the pycnometer is filled with xylene, brought to 30° C. in a thermostat, and weighed at a room temperature between 20° and 30° C., and the density is calculated by Formula 1.

Table VII. Effect of Temperature on Density

20° C.	25° C.	30° C.
1.457	1.454	1.456
1.458	1.452	1.456
1.451	1.455	1.456
1.454	1.452	1.453
Av. 1.455	1.453	1.455

Discussion of Data

The data show that results accurate to the third decimal place are obtainable by the method and that comparable results can be obtained in the temperature range of 20° to 30° C. If the sample is finely ground the period of evacuation need not be over 15 minutes. The size of the sample used is not significant if the sample is homogeneous. Liquids of similar structure will give comparable results, whereas those of dissimilar structure will not give results that can be used to compare different tobaccos.

The method of Tschapek as applied to tobacco is of value primarily in obtaining comparative results on samples that have been prepared in a uniform manner as to particle size. If the particle is small, the tobacco loses its identity as to the form or structure of its leaf, lamina, or rib material, the forms in which it is generally used in manufactured products. The method as used does not give a measure of "filling power", this being the volume which tobacco in a certain physical state will occupy per unit weight, the tobacco retaining its identity as to tissue structure and retaining such admixed gaseous and liquid materials as were present in its original condition.

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Determination of Particle Size Distribution

For Particle Sizes between 40 and 1 or 2 Microns

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WITH the increasing demand from the arts and industries for finer and finer grinding it becomes essential to have a simple method of determining the fineness of pulverized material where the particle size is beyond the range of the standard testing screens—i. e., finer than 38 microns.

A number of methods have been proposed to accomplish this, most of them involving the use of expensive apparatus, and many of them taking a day or more for one determination.

There are three general methods of making quantitative particle size determinations in the subscreen sizes: microscopic, elutriation, and sedimentation.

The microscope is capable of giving accurate results in skilled hands and with considerable expenditure of time. It has the inherent disadvantage of using an extremely minute sample which may not be at all representative of the product as a whole. On the other hand, it gives an actual visual picture of the individual particles, their distribution, and their shape, which latter may be very important.

The elutriation methods which separate out the particle sizes by means of currents, usually of air, moving with various velocities, are not very familiar to the author, but appear to give very accurate results. They involve considerable expensive apparatus, a skilled operator, and an exacting technique.

The sedimentation methods, on a whole, appear to be the best adapted to use where rapid determinations are essential and an unskilled operator must be used.

In developing the method presented here, several conditions were considered essential:

The method must be adaptable to a wide range of materials without any calibration of equipment.

It must rapidly give a sufficient number of determinations at different points to plot a curve.

It must be capable of giving at least closely corresponding results, whether used by an experienced operator in New York or a novice in Timbuctoo, and it must give a reasonably close check with other methods.

The apparatus should be compact and preferably not too costly.

The sedimentation methods using Stokes' law seemed to offer the best chance of satisfying these conditions. In investigating the best way to use this law, the author had no knowledge of methods used by others, except a very general idea of the photoelectric processes, and so had no hesitation in discarding one method if a better one appeared possible. A considerable number of methods were considered, and most of them were tried.

After the method presented here had been perfected far enough to be put to practical use, it developed that a good many of the discarded methods were being used by others, and a description of some of them had been published. This method is a variation of the pipet method, discussed by Oden (*1*), which was actually used by the author for a time without knowing that a description of it was in print.

The difference is that previously the pipetted sample was evaporated to dryness and the residue weighed, while the present method determines the amount of solids by the use of the specific gravity bottle. The older method is mathematically more accurate, but it takes a good deal more time and effort, the practical difficulties are greater, and it is very difficult, if not impossible, to use with many suspension mediums, such as kerosene.

Like all other methods using sedimentation, the present one depends upon Stokes' law, which in effect states that small spheres falling freely through a viscous medium maintain a velocity, which if other conditions are equal is proportional to the square of their diameter.

If we take a liquid with which the substance being investigated will not react, determine its specific gravity, and in a known quantity suspend a known quantity of the solid, we can by determining the specific gravity of the suspension derive the specific gravity of the solid. Knowing the specific gravity of the solid and of the liquid, we can by transposing the same equation find the amount of solid material in a given quantity of the suspension at any time.

From Stokes' law we can obtain the time at which the last particle of a given diameter will settle below a given plane.

If we determine the amount of solid material in a given volume of suspension before any settling has taken place, and then take a sample at some plane at the instant that the last particle of a given size settles past it, and determine the amount of solids in the same volume of suspension as previously used, the ratio of the second value to the first, multiplied by 100, is the per cent of material finer than the size under investigation at that point and time.

Most sedimentation methods use this ratio of the amount of solids in a given volume before settling takes place and at a given depth after a calculated time. The difference in the means used to make these determinations constitutes the differences between the various methods.

The more elaborate apparatus utilizes a beam of light and a photoelectric cell, determining the density of the suspension by its light absorption (Wagner turbidimeter and Central Scientific photelometer).

The densities may also be obtained with a hydrometer (*2*). Since the density of the suspension varies throughout the entire depth of the hydrometer, complicated figuring and some assumptions are necessary.

Another method is to suspend a weight from a scale beam and measure the change in displacement, but here, unless the weight is withdrawn each time and cleaned, the values may be thrown so far out by material settling on it as to be absolutely worthless, and if the weight is withdrawn and cleaned the method becomes a little more cumbersome than the pipet method described here.

Others take a slightly different approach, and instead of measuring the densities, either weigh or measure (*3*) the sediment as it settles. This involves much more intricate calculations, and in the case of measurements the increments are so small as to make accurate readings extremely difficult.

The method presented here is not intended to replace the expensive turbidimeter and photelometer methods, which may make a dozen determinations between 7.5 and 60 microns in half an hour, but to make possible rapid determination of particle size distribution at as many as six different points from a single sample, which is sufficient to plot a smooth curve and for most cases is plenty. It has been found to give results which check with different operators and with other far more elaborate, costly, and time-consuming apparatus.

The equipment required is available in almost any laboratory, or can be purchased from any chemical supply house, at a cost, excluding the balance, of less than five dollars. The balance will cost about thirty dollars, since it need weigh only to 1 mg., although a more sensitive one is preferable.

MATERIAL REQUIRED. One balance, sensitive to 1 mg., or less. One specific gravity bottle, 10-cc. adjusted. One pipet of 10 cc.

or more (not calibrated). One cylindrical graduate of 250 cc. or more, preferably at least 500 cc. with ground-glass stopper. A thermometer, a watch, and a viscometer (Ostwald or similar).

The calculations are very simple, and the procedure is not at all laborious, requiring much less care than most quantitative determinations.

The time required varies with the specific gravity of the material and the fineness at which the determinations are being made. With ordinary materials (specific gravity 2 or more) determinations to as low as 5 microns may be made in about an hour, with readings, say, at 20, 10, and 5 microns. To obtain the reading at 2.5 microns will probably take another 2 hours. For any smaller sizes the time increases rapidly and disturbing influences become more important. However, there is no need for using fixed depths, and the graduate may stand overnight for the finer sizes and the depth may be figured for any given time.

Dispersing Agents

The solid must be thoroughly dispersed in the settling medium, so that the particles settle individually and not as agglomerates. This will ordinarily not be possible without the addition of a small amount of dispersing agent to the fluid.

Sodium metaphosphate, or tetrasodium pyrophosphate, will disperse a great many mineral materials, such as clay, limestone, etc., in water.

A lignin compound, lignin dispersing agent 4-A put out by the Marathon Chemical Co., Rothschild, Wis., will disperse carbon products, such as coal, graphite, or the ashes of these products in water.

Dispersing agents 7027C and 7027D put out by Palo Myers, Inc., New York, N. Y., will disperse coal and many carbon products.

Oleic acid may be used to disperse cement in kerosene.

Other dispersing agents for special materials may be obtained from the chemical manufacturers, and considerable experimentation is sometimes necessary to obtain satisfactory dispersion.

Only a fraction of a per cent of any of these agents need be added for satisfactory dispersion. Too much may be detrimental.

With any material or any dispersing agent considerable agitation is necessary to ensure dispersion. For ground materials, which are the present author's main problem, a couple of minutes' violent shaking in the graduate is usually sufficient, but if an attempt is being made to disperse a filter cake or a dried material to its ultimate particle size, nothing short of prolonged agitation with a high-speed mixer such as a malted milk mixer should be considered.

When starting with a new material it is often advisable to try several dispersing agents. If all give approximately the same results it seems reasonable to assume that dispersion is complete, or if different results are obtained that the finer values indicate the more nearly complete dispersion.

Only in a very unusual material, with an extremely steep particle distribution curve, will the suspension settle with a visible line of demarcation, between a clearer portion above and a denser portion below, if dispersion is complete. Such a line, which often occurs, definitely indicates an incomplete suspension. An incomplete suspension is also indicated by a flocculent appearance of the suspension, or by small agglomerates adhering to the sides of the graduate.

Another way to check the suspension, and the accuracy of the work as a whole, is to plot a curve of values on semilog paper, plotting the particle size on the log scale and the per cent of the material by weight greater (or less) than that particle size on the linear scale. These points should result in a regular curve which takes the form of a straight line through the central portion, curving off toward a tangent with the 0 and 100 per cent lines at the bottom and top (Figure 1).

If the straight-line portion shows a change of more than 40 points for a doubling of the size of the particle, it should be looked on with suspicion. (It will usually be between 20 and 35.)

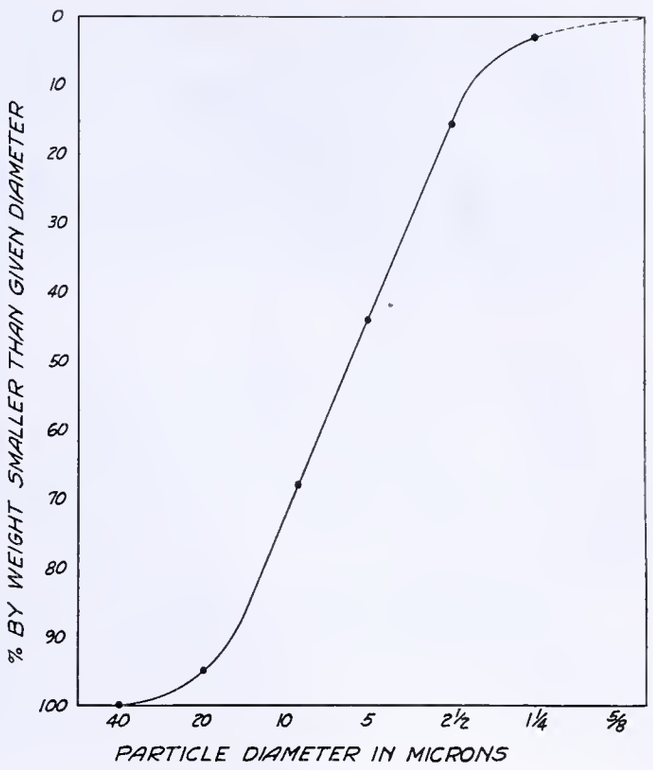

Figure 1. Particle Size Distribution Curve for Iron Oxide

Symbols Used

t = time, seconds
h = depth of fall, cm.
η = viscosity of medium, poises (water = 0.01 at 20° C.)
ρ_1 = density of solid material, grams per cc.
ρ_2 = density of medium and dispersing agent (suspending medium), grams per cc.
g = acceleration due to gravity, 980 cm. per second per second
d = average diameter of particle, microns (actually the reciprocal of the mean of the reciprocals of the diameters)
T = tare weight of specific gravity bottle, grams
B = gross weight of bottle, filled with suspending medium, grams
m = grams of solid in specific gravity bottle filled with dispersion before settling takes place
M = gross weight of bottle filled with dispersion before material has started to settle, grams
C_h = gross weight of bottle filled with dispersion pipetted from depth h at time t, grams
W = weight of sample used, grams
Q = volume of dispersion after solid has been added and dispersed, ml.
V = volume of specific gravity bottle, ml.

Calculations

Stokes' law may be stated mathematically as:

$$t = \frac{18h\eta\ 10^8}{(\rho_1 - \rho_2)gd^2}$$

or: The time of fall of a particle in a viscous medium varies directly as the depth of fall, directly as the viscosity of the fluid, inversely as the difference between the specific gravities of the solid and fluid, and inversely as the square of the particle size.

Steps in Determination. 1. Determine the tare weight of the specific gravity bottle, T. This need be determined only once during the life of the bottle, although an occasional check is advisable to guard against loss of weight from chipping or gain of weight from insoluble deposits.

2. Dissolve from a 0.25 to 0.5 per cent of dispersing agent in the fluid which is to be used. Bring to room temperature and weigh for B.

3. Determine the viscosity of the dispersing medium—i. e., fluid plus dispersing agent. If distilled water is used at 20° C. this value is 0.01 poise. The difference in using ordinary tap water is usually very slight but should be occasionally checked. A difference in temperature, however, may make a considerable difference in viscosity. The temperature should not be allowed to change more than 2° C. during the test. Viscosities may be determined rapidly and accurately enough for this purpose with a very reasonably priced viscometer.

4. From (1) and (2) determine the specific gravity of the solid, $\frac{B-T}{V} = \rho_2$.

5. $m = WV/Q$. When a 25-gram sample is dispersed to form 500 cc. of dispersion and a 10-ml. specific gravity bottle is used, $m = 0.5$.

6. Accurately weigh quantity of material W and thoroughly agitate with settling medium until complete dispersion is obtained. It is best to make a little more than Q dispersing medium and keep some in the specific gravity bottle, so that the graduate may be filled up to quantity Q after the solid is added. Thoroughly disperse the solid in the dispersing medium (see remarks under Dispersing Agents).

7. Before material has time to start settling, draw off sample in pipet, fill specific gravity bottle, and weigh for M. This, like all other samples withdrawn for weighing, is discarded after weighing.

8. From the values determined above determine ρ_1 (density equals mass/volume).

$$\rho_1 = \frac{mP_2}{(B-T)-(M-T-m)}$$

9. We now have all the quantities necessary for substituting in the formula for Stokes' law. It is only necessary to substitute in this formula for one depth and particle size. Other particle sizes can be found by simple proportions. It is convenient to use for the substitution in the formula 10 microns at 10 cm. Then:

$$t_{10} = \frac{(18)(10)(0.01)(10^8)}{(\rho_1-\rho_2)(980)(100)} = \frac{1835}{\rho_1-\rho_2}$$

If some other fluid than water is used at 20° C., the suitable viscosity will have to be inserted in the above equation.

Other convenient depths are 20 cm. for 20-micron particles, 7.5 cm. for 5-micron particles, and 5 cm. for 2.5-micron particles. If these values are used the figuring is very simple: t_{20} for 20 microns at 20 cm. is one-half of t_{10}. t_5 for 5 microns at 7.5 cm. is three times t_{10}. $t_{2.5}$ for 2.5 microns at 5 cm. is eight times t_{10}.

It is well to take samples for larger particles at deeper depths than for small particles, partially for increased accuracy and time-saving due to the long time it takes small-sized particles to settle, and partially to avoid removing any of the fluid above the point where a sample is to be taken, which would introduce a slight error.

10. Obtain samples with the pipet at the depths and times figured, fill specific gravity bottle, using precautions suggested later, and weigh. Obtain the quantities $C_h - B$ for each of these positions.

11. Get the quantity $M - B$. This is 100 per cent.

12. Divide each $C_h - B$ by $M - B$. The result multiplied by 100 is the percentage by weight smaller than the particle size under consideration at that particular point.

Stokes' law is valid only over a certain range of particle size and no attempt should be made to use it outside this range. Particles larger than about 44 microns (325-mesh screen) settle with turbulent or partially turbulent flow, instead of the viscous flow as postulated by Stokes' law, and their fall is slower.

Particles smaller than 1 or 2 microns begin to feel the effect of the random molecular movement of the suspending fluid (Brownian movement) and may move in any direction. In this size, too, the slightest convection current will disturb the settling.

If particles larger than the 325-mesh screen or at very most the 200-mesh screen (74 microns) are present, they should be screened out and the settling test run on the material through the screen. The actual particle distribution on the original material can then be figured, knowing the percentage of large particles which remained on the screen.

Sample Calculation. Sample, red iron oxide (25 grams used). Medium, tap water at 72° F. Dispersing agent, sodium metaphosphate (Calgon).

Tare weight of bottle, T, 8.021

Gross weight of bottle, water, and dispersing agent, B, 18.022

Gross weight of bottle with dispersion, M, 18.401

Amount of solid in 10-ml. dispersion, $\frac{(10)(25)}{500}$, m, 0.5

B	18.022	M	18.401
T	8.021		8.021
$B-T$	10.001	$M-T$	10.380
		m	0.5
		$M-T-m$	9.880

$$\rho_1 = \frac{(0.5)\ (10.001)}{10.001 - 9.880} = 4.13$$

Hence for 10 μ at 10 cm. substituting in Stokes' equation

$$M - B = 0.379 = 100 \text{ per cent}$$

Material screens 99.98 per cent through 400

$$t = \frac{(18)(10)(0.01)(10^8)}{(4.13-1)(980)(100)} = 586 \text{ seconds} = 9.75 \text{ minutes}$$

For 40 μ at 20 cm.[a] t_{40} = 9.75/9 = 1.2 minutes
For 20 μ at 20 cm. t_{20} = 9.75/2 = 4.9 minutes
For 10 μ at 15 cm. t_{10} = (1.5) (9.75) = 14.6 minutes
For 5 μ at 10 cm. t_5 = (4) (9.75) = 39 minutes
For 2.5 μ at 7.5 cm. $t_{2.5}$ = (3) (39) = 117 minutes
For 1.25 μ at 5 cm. $t_{1.25}$ = (8) (39) = 312 minutes

[a] Sample returned to graduate and graduate shaken again before taking reading for 20 microns.

The sample for determining the specific gravity of the material is usually also returned to the graduate and reshaken before proceeding. This reduces the chances of getting below the area of free fall on the 20-cm. readings.

μ	h	t (min.)	C	$C-B$		
40	20	1.2	18.401	0.379	0.379/0.379 = 100%	< 40 μ
20	20	4.9	18.381	0.359	0.359/0.379 = 95%	< 20 μ
10	15	14.6	18.281	0.259	0.259/0.379 = 68%	< 10 μ
5	10	39.0	18.190	0.168	0.168/0.379 = 44%	< 5 μ
2.5	7.5	117	18.081	0.059	0.059/0.379 = 15.5%	< 2.5 μ
1.25	5	312	18.034	0.012	0.012/0.379 = 3%	< 1.25 μ

In actual routine work the calculations can be so condensed that two complete tests can be readily recorded on a sheet of 4 × 7 inch notebook paper.

Precautions

Most of these precautions would be automatically adopted by any one used to this type of work, and should not be regarded as burdensome. After two or three determinations they become automatic.

The graduate or other vessel must have a diameter large enough to enable the pipet to draw all its charge from approximately the same plane, and it must be deep enough to give satisfactory depths without getting below the range of free fall.

When the specific gravity bottle containing the suspended material is filled, an excess should be drawn from the graduate in the pipet and used to rinse out the bottle. This washes out any sediment from previous weighings.

The top of the pipet should always be kept closed when being inserted, until the bottom reaches the correct level.

The top of the stopper should be wiped immediately after inserting, but if the warmth of the hand forces out more fluid, this should not be wiped away. Otherwise the bottle must be perfectly dry before weighing, and some effort should be made to keep the elapsed times from filling the bottle to reading the weight approximately equal, in order to equalize evaporation.

Although a known specific gravity of material may be used for figuring time of settlement, it should never be used for figuring M.

The sample should never be drawn from so close to the bottom of the container that the free fall of the particles has begun to be checked. The longer the material has settled, the greater this distance should be. Seven or 8 cm. is the minimum for the first reading after about 7 or 8 minutes, and should be increased for the succeeding readings. If any weight is greater than the origi-

nal weight before settling started and no mistake has been made in either weighing, it is proof positive that samples are being taken from too close to the bottom. The graduate should not sit in a position where hot or cold air can strike part of it to set up convection currents, nor should the temperature change more than 2° C. during the course of the test.

Distilled water may increase the accuracy slightly, and if distilled water at 20° C. is used with a weighed quantity of dispersing agent, the first weighing of the specific gravity bottle with the clear solution may be omitted, since under those conditions this weight will always be the same.

If care is used, this method is just as accurate and as rapid (probably a great deal more rapid) as with equipment costing hundreds of dollars, where not more than four or five different sizes are wanted from one sample. A 500-ml. graduate is not satisfactory for more than this number of determinations. Using a larger sample and a deeper settling container additional determinations could be obtained.

Great cleanliness of the containers is not necessary, as in methods measuring the transmission of light through the suspension.

Calculations of percentages should not be carried into fractions of a per cent, since the accuracy of this method (or any other method) probably does not warrant it.

Where some other material than water must be used for the settling medium, its viscosity may be determined with a cheap viscometer (Ostwald or similar).

The specific gravity is readily obtained with the specific gravity bottle.

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Recording Color of Opaque Objects

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To compare the colors of opaque objects, such as fish fillets, photographic color transparencies are prepared and their spectral distribution curves obtained, using a photoelectric spectrophotometer. Errors due to variations in illumination during exposure and in processing of the film are eliminated by taking pictures of objects to be compared on the same negative.

COLOR of transparent liquids can conveniently be recorded by obtaining a spectral distribution curve, utilizing equipment which is now available in many chemical laboratories for colorimetric analyses. Opaque objects present more of a problem, and no simple yet entirely satisfactory method is available that does not require special equipment for reflectance measurements.

In connection with studies on changes in color of salmon caused by cooking and by cold storage, a method was developed in which color photographs were taken on Kodachrome film, the transparencies were placed in a Coleman spectrophotometer, and spectral distribution curves were obtained. While such curves are not absolute records of the color of the original object, they are very useful in recording relative changes in color—for example, pictures of two identical objects taken on the same negative and processed together will give substantially identical curves. If the two objects—e. g., fish fillets—are subjected to different treatments, such as storage at different temperatures, the relative change in color of the two objects will be recorded and the effect of the treatment upon the color change can be determined.

If the change in color takes place over a relatively short period of time, the photographs of the object before and after the color change has taken place can be made on the same roll of film, and if reproducible illumination and exposure conditions are used the curves can be directly compared. If color changes slowly over a period of weeks or months, the original and subsequent photographs must be made on separate film, and slight differences in processing will render a direct comparison unreliable. Even in such a case, however, color changes between a control and one or more variable conditions can be photographed on the same negative, and the relative color change recorded.

Photographs were taken using equipment whereby illumination and exposure conditions could be readily duplicated. A 35-mm. camera with f 3.5 anastigmatic lens was mounted on a rigid upright attached to a large wooden base, which was placed inside a light-tight box without a top and of such a height that the camera was slightly above the open top of the box.

Two lights in metal reflectors were rigidly attached above the camera and pointing inward slightly. Five hundred-watt, clear, 3200° Kelvin lamps were utilized. These lamps are corrected for Eastman type B film which is available only as cut film in the larger sizes. The type of film employed with the 35-mm. camera is designed for use with photoflood lamps, but can be used with the 3200° Kelvin bulbs with only a slight difference in results. This disadvantage is more than compensated by the fact that the 3200° Kelvin lamps have a far more constant illumination over their life (30 hours) than the photoflood lamps. The voltage to these lamps was adjusted to 120 volts by means of an autotransformer.

Two squares of opal flash glass were supported on the top of the box directly beneath the lamps but leaving an opening at the center for the camera. This resulted in a fairly uniform illumination of the object to be photographed. The camera was mounted so that it could be raised or lowered to obtain a field of varying dimensions. By adjusting the field of the camera properly, it was possible to obtain a photograph of the object of a size very slightly larger than the exit slit of the spectrophotometer. If these conditions are fulfilled the color of the entire object will be measured; this is an important consideration in obtaining reproducible results, if the color of the object is not uniform throughout.

If the photograph of the object is considerably larger than the exit slit of the spectrophotometer, only a portion of the image will be in the path of the light beam, and the color of that portion only will be recorded. If there is considerable variation in color from one part of the object to another, different results will be obtained, depending upon the portion of the photograph placed in the beam of light. On the other hand, it is imperative that the photograph of the object be at

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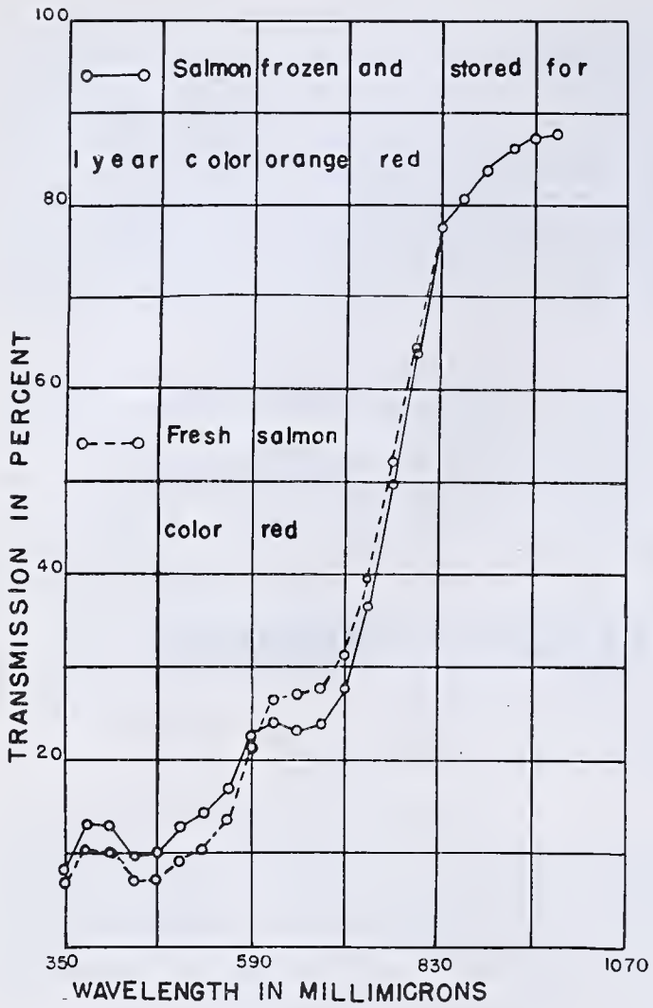

FIGURE 1

least as large as the light beam; otherwise the color of a portion of the background will also be recorded. Therefore, it is best to make the photograph of the object slightly larger than the beam of the spectrophotometer.

In obtaining reproducible exposure for consecutive pictures, the setting of the lens opening and speed of the shutter are very important. With the particular camera employed (Kodak 35 with f 3.5 lens) the setting of the shutter speed could be duplicated accurately, whereas it was impossible to reproduce the setting of the lens opening with any degree of certainty. Accordingly, exposures were always made with the lens opened wide and a suitable shutter speed was selected to give the appropriate exposure. A magnifying glass was used in setting the shutter speed, to obtain exactly the speed desired, but even with these precautions it was difficult to obtain reproducible exposures. Each time the shutter speed lever was moved, a number of exposures had to be made before equilibrium was re-established and the desired speed once more attained. For example, if a picture were taken at $^1/_{25}$ second, and the speed lever was moved to another setting and then at once moved back to exactly $^1/_{25}$ second, a second picture would not have exactly the same exposure as the first. Subsequent exposures taken without moving the shutter speed lever gradually approach that of the initial exposure and finally become constant. This error can be reduced by adjusting the shutter speed before winding the shutter or eliminated by making the necessary adjustments, snapping the shutter a large number of times before loading the film, and then not altering the adjustments during the series of pictures on that roll. Examples of this error are shown in Table I, in which per cent transmission of the film at a wave length of 590 millimicrons is used as a measure of the exposure of the film. This same effect was obtained with two different cameras of the same type and make.

The considerable error made if the camera is at different temperatures for the different exposures (Table II) can be eliminated by placing the camera in an incubator at a standard temperature before taking the pictures.

After processing, the film can be cut and the photograph of each object mounted separately on a suitable cardboard form containing a hole over which the transparency is carefully centered so as to coincide with the exit slit of the spectrophotometer.

An extensive series of pictures was taken at different exposures. The red end of the spectrum was most affected by differences in exposure. It was the purpose of the present work to study gradations from red colors to yellow, and under these circumstances at least the greatest difference in transmission curves was obtained when the pictures were overexposed with about twice as much light as gave the best picture by visual inspection. In any case it is better to overexpose than to underexpose, and in order to allow a sufficient margin of safety, doubling the correct exposure is an adequate precaution.

With the particular arrangement of camera, stand, and lights used in these experiments, an exposure of $^1/_{25}$ second at f 3.5 gave the best results.

Using all the precautions described, a fairly high degree of precision is attainable—for example, a series of pictures of the same object, taken on the same film, gave the following per cent transmission: 23.9, 23.6, 23.8, 23.6, 23.9, 23.6. The same picture taken with one-half the exposure time gave a per cent transmission of 34.7.

One example of the application of this method is shown in Figure 1, which gives spectral distribution curves for transparencies of a fresh silver salmon fillet and one which had been frozen and stored for one year. The protracted storage period had caused a discoloration from the normal red color to an orange-red hue. In the blue and yellow portion of the spectrum (350 to 600 millimicrons) the stored fish had a higher per cent transmission than the fresh fish, while at the red end of the spectrum the reverse was true.

In the application of this method two points are of especial importance if erroneous conclusions are to be avoided. First, unless the control color is very similar to that of the color

TABLE I. EFFECT OF SHUTTER-SPEED ADJUSTMENT ON REPRODUCIBILITY OF EXPOSURE

Shutter Setting *Second*	Exposure at 590 Millimicrons *% Transmission*
1/25	28.3
Shutter moved to 1/50 then back to 1/25	22.4
No change	27.6
No change	28.3
No change	28.3
Shutter moved to 1/10 then back to 1/25	23.0
No change	28.0
No change	28.0
Shutter moved to about 1/27 then back to 1/25	24.0
No change	27.0
No change	27.4
Shutter set at 1/50	35.4
Shutter set at 1/25	22.8
No change	28.1
Shutter set at 1/10	9.6

TABLE II. EFFECT OF TEMPERATURE OF CAMERA AND FILM ON EXPOSURE

Temperature	Exposure at 590 Millimicrons	
	Picture 1	Picture 2
° *C.*	*% Transmission*	
5	32.3	40.0
20	31.1	37.3
30	29.6	36.9

under consideration, entirely misleading results will be obtained. For example, if an artificial, painted color standard were used with some object such as fish, results obtained could only lead to confusion and misleading interpretation.

The second point to be stressed is the necessity of restricting all comparative data to results obtained on a single roll of film. Even if it were possible to eliminate differences in processing (which ordinarily would be excessive for photometric work), there would still remain the uncertainty of differences in the original rolls of unexposed film. Improvements in color film are constantly being made which undoubtedly would markedly affect the results obtained by this method.

Accordingly, the method as described is of value only when a control very similar to the object under investigation is photographed, preferably simultaneously on the same negative but at any rate within a short period of time on the same roll of film.

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The Analysis of Peppermint Oil

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IN THE routine examination of a large number of peppermint oil samples, some interesting observations were made on pharmacopeial as well as unofficial methods of assay. This paper serves as a summary of these observations and as a supplement to other reports (*19*) concerning the production, collection, and storage of the oil samples. For general reference purposes, the reader is referred to (*9, 10, 16, 17, 22, 24*).

Historical

In a brief history (*6*) of the inclusion of oil of peppermint in the United States Pharmacopoeia, it is pointed out that a chemical assay for this product was not included in the monograph until the eighth revision, 1900.

In the sixth revision, 1880, the committee on essential oils included in the official definition the statement that the oil is a "colorless, or yellowish, or greenish-yellow liquid becoming darker and thicker by age and exposure to air", which indicated that the effects of poor storage conditions had already been noted by investigators. The seventh revision, 1890, advised that the oil be kept in a cool place in well-stoppered bottles. This revision also included a number of qualitative tests which were intended to eliminate the practice of adulterating the oil with foreign substances or with oils from other species of mint.

In the eighth revision, 1900, following the work of Power and Kleber (*18*), the committee on essential oils defined the product as "the volatile oil distilled from the fresh or partly dried leaves and flowering tops of peppermint, rectified by steam-distillation and yielding, when assayed by the process given below, not less than 8 per cent of ester, calculated as menthyl acetate, and not less than 50 per cent of total menthol, free and as ester. It should be kept in well-stoppered, amber-colored bottles, in a cool place, protected from light." The process for menthyl acetate involved the saponification of a weighed sample of oil with alcoholic potassium hydroxide solution and subsequent titration of the residual alkali to determine the amount of esters present. The saponified oil was then treated with acetic anhydride and sodium acetate to convert the menthol to menthyl acetate. After washing and drying, the acetylated sample was treated with excess alcoholic potassium hydroxide for 1 hour, the mixture was back-titrated with normal acid, and from the amount of base used, the per cent of total menthol was determined. In this revision a solubility test using 70 per cent alcohol, the degree of rotation, −25° to −33° in a 100-mm. tube at 25° C., and a qualitative test for dimethyl sulfide intended to detect nonrectified oils were included. Limits for the specific gravity of the oil were included, 0.894 to 0.914 at 25° C.

In the ninth revision the ester requirement was lowered to 5 per cent, no change was made in the menthol content, and the optical rotation was changed to −23° to −33°. Separate portions were taken for the ester and menthol assays, but the committee failed to take into consideration the menthol in the esterified state in the calculation of the total menthol. In the tenth revision, 1920, the formula was changed to include the menthol combined as menthyl acetate, and limits for the refractive index were included.

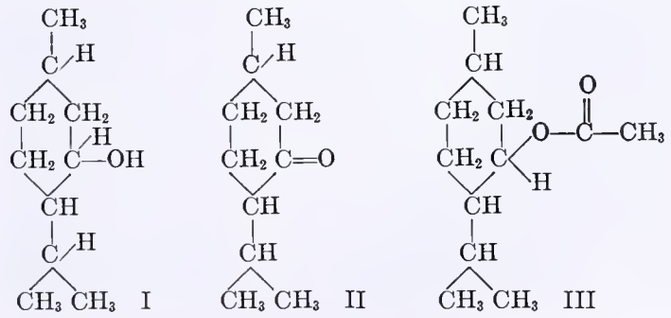
Composition of Oil of Peppermint

In the earliest elaborate research of Power and Kleber (*18*) on the composition of American peppermint oil the following compounds were identified in the oil:

Acetic aldehyde	CH_3CHO
Acetic acid	CH_3COOH
Alpha-pinene	$C_{10}H_{16}$
Limonene	$C_{10}H_{16}$
Menthone	$C_{10}H_{18}O$
Menthyl acetate	$C_{10}H_{19}.C_2H_3O_2$
Cadinene	$C_{15}H_{24}$
A menthyl ester	$C_{10}H_{19}.C_8H_{11}O_2$
Isovalerianic aldehyde	C_4H_9CHO
Isovalerianic acid	C_4H_9COOH
Phellandrene	$C_{10}H_{16}$
Cineol	$C_{10}H_{18}O$
Menthol	$C_{10}H_{19}.OH$
Menthyl isovalerianate	$C_{10}H_{19}.C_5H_9O_2$
A lactone	$C_{10}H_{16}O_2$

In addition to these compounds a heavy, resinous material has been observed, particularly in older oils or in those which have been stored improperly. Amyl acetate and dimethyl sulfide, the latter particularly in nonrectified oils, have been detected in traces.

Concerning the biogenesis of these compounds in peppermint, the reader is referred to a discussion by Hall (*11*), who also included a brief history of the theories proposed to explain the formation of these substances by the plant. Inasmuch as citronellal, an open-chain unsaturated aldehyde obtained chiefly from oil of citronella, is now being used for the production of synthetic menthol, further proof is given to the theory proposed by Kremers (*13*) that citronellal serves as the precursor of menthol in the plant.



Menthol (I), menthone (II), and menthyl acetate (III) constitute the major portion of peppermint oil distilled from mature plants. That the other components are necessary for oils of pleasing aroma and taste is apparent when organoleptic tests are applied to natural as well as to synthetic oil of peppermint. By careful removal of the lower-boiling terpene fractions, either by fractional distillation under reduced pressure

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or by countercurrent extraction with immiscible solvents, terpeneless oils are obtained which command a higher resale price and in which the tendency to form terebinthinate odors is lessened.

Experimental Procedures

Inasmuch as the methods included in the U. S. Pharmacopoeia for determining physical and chemical constants of essential oils are used in most laboratories, tests for identity and purity and the assays (*24*) are incorporated in this paper, as a supplement to other material on various phases of oil production.

Any variation must be checked against the standard method, as outlined in the Pharmacopoeia, and in any controversy involving the Pure Food and Drug law, the analysis of the oil is to be made in strict accordance with the method outlined in the U. S. Pharmacopoeia. In analyzing a large number of samples, however, strict adherence to the standard method may be time-consuming. Slight variations will often facilitate the analyses without sacrificing the accuracy of the procedure. In the routine examination of oils in the oil-producing areas, the completeness of the investigation is limited primarily by the amount of equipment available in the laboratory, and secondarily by the degree of purity suspected by the analyst and/or indicated by the integrity of the seller or grower. The total menthol content, ester content, optical rotation, and solubility in 70 per cent alcohol are usually determined. The refractive index and specific gravity are included, if the apparatus is available and time permits. The U. S Pharmacopoeia does not include an assay for menthone, but several methods for its determination are in use among analysts. Variations in the per cent of this component occur when identical samples are submitted to different analysts who use their own methods. For this reason only limited importance can be attached to the assay for this compound.

Oil of Peppermint (*24*). Oil of peppermint is the volatile oil distilled with steam from the fresh, overground parts of the flowering plant of *Mentha piperita Linne* (*Fam. Labiatae*), rectified by steam distillation. Oil of peppermint yields not less than 5 per cent of esters, calculated as menthyl acetate ($C_{10}H_{19}.C_2H_3O_2$), and not less than 50 per cent of total menthol ($C_{10}H_{19}.OH$), free and as esters.

Description and Physical Properties. A colorless liquid, having a strong, penetrating odor of peppermint, and a pungent taste, followed by a sensation of cold when air is drawn through the mouth.

Tests for Identity and Purity. The oil is soluble in 4 volumes of 70 per cent alcohol, by volume, showing not more than a slight opalescence and no separation of oil globules (dementholized or impure peppermint oil).

Specific gravity, from 0.896 to 0.908 at 25° C.

Optical rotation, from −18° to −32° in a 100-mm. tube at 25° C.

Refractive index, 1.4600 to 1.4710 at 20° C. A solution of the recently rectified oil in 70 per cent alcohol (1 in 4) is neutral to moistened litmus paper. Distill 1 cc. from 25 cc. of oil of peppermint, and carefully superimpose the distillate on 5 cc. of mercuric chloride T. S. in a test tube: a white film does not form at the zone of contact within one minute (dimethyl sulfide found in nonrectified peppermint oils). Mix in a dry test tube 3 drops of oil of peppermint with 5 cc. of a solution of 1 volume of nitric acid in 300-cc. volumes of glacial acetic acid and place the tube in a beaker of boiling water. In from 1 to 5 minutes the liquid develops a blue color, which, on continued heating, deepens and shows a copper-colored fluorescence, and then fades, leaving a golden, yellow solution (distinction from oil from *Mentha arvensis*).

The solubility test of the Pharmacopoeia was originally intended to serve for the detection of adulterants added to the oil to increase bulk. Some common adulterants are lard oil, kerosene, and turpentine, all of which could cause an opalescence when the adulterated oil is mixed with 70 per cent alcohol in the proper proportions. Although one would expect old oils showing a high content of resinous material to be insoluble in 70 per cent alcohol, there appears to be no correlation between solubility and resin content. Several samples with high resin contents were completely soluble, while other samples low in resin were very insoluble. Obviously the solubility test cannot be considered too reliable in grading oil of peppermint.

The degree of opalescence, whether "very slight", "slight", or "opalescent", rests on the opinion of the analyst and on the diameter of the column of the mixture of oil and alcohol.

To facilitate the tabulation of solubility data, the following key has been used. This is based upon a solution of one volume of oil in four volumes of 70 per cent ethyl alcohol.

A. Completely soluble, solution clear
B. Completely soluble, solution very slightly opalescent
C. Completely soluble, solution slightly opalescent
D. Completely soluble, solution opalescent
E. Completely soluble, solution very opalescent
F. Not completely soluble

The Essential Oil Association of the U. S. A. has recommended that oil of peppermint be completely soluble in four volumes and more of 70 per cent alcohol, either before or after distillation. The committee also suggests an additional requirement that 1 cc. of oil mixed with 4 cc. of 70 per cent alcohol in a glass-stoppered, 10-cc. graduate should show no separation of oily globules after standing overnight.

Some essential oil dealers also determine the solubility of this oil in three volumes of 70 per cent alcohol, and in 80 per cent ethyl alcohol.

The specific gravity of older oils usually reaches or exceeds the upper pharmacopeial limit. New or freshly distilled oils stay well within the limits. Bosart (*4*) has furnished directions for determining the specific gravity of oils at room temperatures with subsequent correction to 25° C. For the temperature range 15° to 35° C. the variation in specific gravity per degree Centigrade is 0.00076, additive to convert the specific gravity to 25° C. if the temperature at which the measurement is made is between these limits. The specific gravity of an oil can be determined accurately either with a pycnometer or with a Westphal balance. For routine analyses, hydrometers covering the limits of oil of peppermint and calibrated for any temperature can be obtained.

The optical rotation of oil of peppermint is due to the optical activity of the components making up the oil. If the menthol content of oil of peppermint is increased by the addition of synthetic menthol, from which the dextrorotatory isomers have not been removed, the resulting oil may not have so high a levorotation as would normally be expected in an oil with high menthol content. Natural menthol and menthyl acetate are levorotatory, the former having a specific rotation of −50°; the latter, −79.42°.

A change has been proposed for the refractive index of this oil. Very few oils exceed the upper limit, 1.4710, whereas the lower limit is too high to permit the inclusion of many samples of unquestioned purity. In the examination of 100 samples of oil, it was observed that 21 were below the lower limit for refractive index, but that 18 of the 21 were high in menthol content and of unquestioned purity. In the redistillation of oils to remove highly refractive terpenes the refractive index of the final product is lowered sharply, often below the lower limit, particularly if the original oil does not have a fairly high initial value. The present Pharmacopoeia requires redistillation by steam of oil of peppermint; it has been proposed to include vacuum distillation of the oil in the revised monograph. While this would effect a better fractionation of the oil, it would cause a marked decrease in the refractive index, making more imperative a change in the lower limit permitted for the oil. Bosart (*5*) has determined the constants for calculating the refractive index from a given temperature to that at any other temperature for a considerable number of essential oils and odorants. For oil of pepper-

mint, the correction, 0.0004 per degree Centigrade between 20° and 40° C., is to be added to the observed reading to convert it to the refractive index at 20°, to be subtracted if temperature is below 20° C. The determination of the refractive index is of special value in detecting adulteration of oil samples with denatured alcohol.

Assay for Esters. Place 10 cc. of oil of peppermint in a tared 125-cc. Erlenmeyer flask, and weigh it accurately. Add 25 cc. of 0.5 *N* alcoholic potassium hydroxide, connect the flask with a reflux condenser, and boil the mixture on a water bath for 1 hour. Allow the mixture to cool, disconnect the flask from the condenser, and titrate the excess of alkali with 0.5 *N* sulfuric acid, using 10 drops of phenolphthalein T. S. as the indicator. The number of cubic centimeters of 0.5 *N* alcoholic potassium hydroxide consumed in the saponification, multiplied by 0.09912, indicates the number of grams of esters, calculated as menthyl acetate, in the oil taken for the assay.

Table I. Variation in Saponification Value of Acetylated Peppermint Oil with Age of Acetic Anhydride

Age of Acetic Anhydride	Sample Weight	Volume of 0.5 *N* Alcoholic KOH	Saponification Value
	Grams	*Ml.*	
One year	4.4617	22.15	139.4
	4.4436	22.05	139.3
Two months	4.4485	22.05	139.1
	4.4478	22.05	139.1
Fresh	4.4422	22.05	139.3
	4.4496	22.10	139.4

The ester determination is similar to that for total menthol (below). According to the pharmacopeial standard, oil of peppermint should contain not less than 5 per cent of esters. Reliable sources have stated, however, that the ester content of American oils can be as low as 3 per cent. The ester content is calculated as menthyl acetate, although menthyl esters of other organic acids are known to exist in the oil. Those esters containing acyl radicals of higher molecular weight are highly resistant to saponification with alkali. Menthyl isovalerianate requires at least 2 hours of boiling with alkali for complete saponification.

Esters in essential oils are believed to originate in the green parts of the plant by the action of free acids on the alcohol. Earlier investigators observed that the dehydration of the alcohols and the formation of esters were related to the intensity of chlorophyll action. It has also been observed that the esterification is more rapid if the absorption of moisture by the roots is diminished, a fact which would help to explain the high ester content of many oils distilled during recent hot seasons.

In order to determine the amount of combined menthol in any sample of peppermint oil, it is necessary to multiply the per cent of ester by the fraction 156/198—that is, the molecular weight of menthol divided by the molecular weight of menthyl acetate.

Assay for Total Menthol. Place 10 cc. of oil of peppermint in an acetylization flask of 100-cc. capacity and add 10 cc. of acetic anhydride and 1 gram of powdered anhydrous sodium acetate.

Inasmuch as the volume of oil specified by the Pharmacopoeia does not provide sufficient acetylated material for making duplicate determinations of total menthol content, it is suggested that the amounts specified be increased by 50 to 100 per cent in order to provide for duplicate or triplicate checks. It has been pointed out (*15*) that the sodium acetate should be freshly fused and kept in tightly stoppered bottles, since the presence of water in the salt caused variations in the analyses of a sample of oil by different analysts.

In order to determine whether the age of the acetic anhydride used for the acetylation of oil of peppermint would cause a variation in results, a sample of oil was acetylated with three different lots of acetic anhydride: one which had stood in a partly filled, glass-stoppered bottle for 1 year after opening; the second which had been stored in a similar manner for 2 months; and the third from a bottle which had never been opened since leaving the chemical plant. The results of this investigation are presented in Table I.

Boil the mixture gently for 1 hour, cool, disconnect the flask from the condenser, transfer the mixture to a small separator, rinsing the acetylization flask with three successive 5-cc. portions of warm distilled water, and add the rinsings to the separator. When the liquids have completely separated, reject the aqueous layer and wash the remaining oil with successive portions of sodium carbonate T. S., diluted with an equal volume of distilled water, until the last washing is alkaline to 2 drops of phenolphthalein T. S. Dry the resulting oil with anhydrous sodium sulfate (prepared by drying sodium sulfate to constant weight at 110° and powdering), and filter it.

The length of acetylation time and the temperature used in the process vary in different laboratories. Inasmuch as the boiling point of acetic anhydride is 140° C., heating facilities should be used to give constant temperatures at or above the boiling point of this reagent. Some laboratories use sand baths thermostatically controlled; others employ a free flame for heating. In the analytical procedures for this report an oil bath was used. Within the temperature range 150° to 160° C., active uniform refluxing of the mixture was observed and the color of the acetylated oil did not undergo pronounced darkening. Although some investigators have recommended an acetylation time of 2 hours, 1 hour appears to be sufficient for oil of peppermint. Care must be observed in maintaining a more definite saponification time than the acetylation time (*2*).

Upon cooling, the acetylation mixture usually solidifies, although occasional samples will remain liquid after acetylation and cooling. In transferring the acetylated product to the separatory funnel, 30- to 40-cc. portions of hot water, 70° to 80° are used. The U. S. Pharmacopoeia specifies that the term "moderately warm" indicates temperatures slightly above 25° C. It has been found that water at this temperature will not decompose acetic anhydride at a sufficiently rapid rate for routine analyses or for the examination of an oil in a limited amount of time. Acetic anhydride, soluble in both acetylated peppermint oil and water, will be distributed between these solvents according to the partition-coefficient law and only upon long-continued contact with water at normal temperatures or upon treatment with water at higher temperatures will acetic anhydride be decomposed to form acetic acid. It is recommended that the acetylated product be washed twice with hot water at 70° to 80° C., vigorous shaking being employed throughout. Following this treatment, the sample should be vigorously shaken with sodium carbonate solution, 6.25 per cent, until the washings are alkaline to phenolphthalein test solution (1 per cent phenolphthalein in 95 per cent ethyl alcohol). A further advantage in washing the acetylated oil with hot water lies in the fact that a sharper and more rapid separation of the oil and water takes place at higher temperatures, particularly in the case of old oils which show a tendency to form emulsions. Some laboratories recommended washing with water twice after the carbonate renders the mixture alkaline. Care in the removal of acetic anhydride and acetic acid from the oil cannot be overemphasized, since the presence of traces would increase the amount of alkali used in the estimation of total menthol.

In a previous investigation of this problem, Elliot (*8*) treated each of four portions of an acetylated mixture in a different manner to remove excess anhydride. The first was washed with successive portions of 6 per cent sodium carbonate solution until alkaline; the second was refluxed with two volumes of water followed by washing with 6 per cent sodium carbonate solution; the third was refluxed four times with two volumes of water and alkalinized; while the fourth was washed with carbonate until

alkaline, refluxed with two portions of water, and rewashed with carbonate.

The Essential Oil Association of the U. S. A. has recommended refluxing the acetylated oil with water for 15 minutes on a water bath, followed by washing successively with three 60-cc. portions of 10 per cent sodium chloride solution and sodium carbonate test solution, 6.25 per cent, until the mixture is alkaline. A final wash with distilled water is followed by drying the acetylated oil with sodium sulfate. The salting-out effect of the sodium chloride tends to minimize loss of water-soluble components in the washing process.

Recently in a study of isomeric menthols (*12*) the acetylation mixture was refluxed with water for 30 minutes on a steam bath, followed by two washes with water until the washings were neutral. Much time can be saved in the analysis of a large number of samples if washing with hot water can be substituted for refluxing, provided that excessive volatilization of the oil is avoided in the washing process.

For removing small amounts of water from the acetylated oil, anhydrous calcium chloride appears to work as well as anhydrous sodium sulfate. If an obstinate emulsion forms, the use of calcium chloride results in the formation of a thick sludge in the mixture, from which the oil must be removed and again dried. If care is taken to effect a sharp separation of oil and water, either dehydrating agent can be used. Drying is usually complete from 3 to 4 hours; the process can be expedited by centrifuging the oil in contact with the drying agent for 4 or 5 minutes. Filtration of the oil ensures a clear, clean sample for saponification.

Transfer 5 cc. of dry acetylized oil to a tared 100-cc. Erlenmeyer flask, note its exact weight, add 50 cc. of 0.5 *N* alcoholic potassium hydroxide, connect the flask with a reflux condenser, and boil the mixture on a water bath for 1 hour.

The 0.5 *N* alcoholic potassium hydroxide solution is prepared by dissolving the base (35 grams of 85 per cent potassium hydroxide per liter) in aldehyde-free ethyl alcohol. The U. S. Pharmacopoeia directs that this solvent be prepared by treating 1 liter of alcohol with lead acetate (2.5 grams in 5 cc. of water) and potassium hydroxide (5 grams in 25 cc. of warm alcohol). After standing overnight, the alcohol is decanted and distilled. The lead hydroxide serves to oxidize the aldehydes in the alcohol. It has been observed that certain samples of ethyl alcohol now on the market need not be purified and that certain denatured alcohols available can be used as solvents for the base. The presence of aldehydes is manifested by a darkening of the solution. The U. S. Pharmacopoeia directs that the solution be kept in bottles with well-fitting stoppers of rubber or of paraffin-coated cork, be protected from light, and be restandardized whenever it is used. For convenience the solution may be checked by titration when each group of samples is analyzed. This titration with acid establishes a ratio between the acid and the base and enables the analyst to express the strength of the base in terms of the acid normality. The Pharmacopoeia also emphasizes the precaution that the solution be used at the same temperature at which it is standardized. Recently, diethylene glycol (*20*) has been recommended as a solvent for potassium hydroxide, inasmuch as the saponification of the acetylated oil with this reagent can be carried out in one fourth to one fifth of the time required when using ethyl alcohol as a solvent.

Previous work by Baldinger (*1*) has shown that water-bath temperatures are necessary for the saponification of acetylized peppermint oil. A hydrolysis time of exactly one hour is important. Apparently side reactions take place if the oil is treated with potassium hydroxide for longer periods. In the work of Hall, Holcomb, and Griffin (*12*) it was shown that the acetic ester of *d*-neomenthol required at least 3.5 hours of refluxing for complete hydrolysis, but that the esters of *l*-menthol and *d*-isomenthol were hydrolyzed in one hour.

Allow the mixture to cool, disconnect the flask from the condenser, and titrate the excess of alkali with 0.5 *N* sulfuric acid, using 10 drops of phenolphthalein T. S. as the indicator. Calculate the per cent of menthol by the following formula:

Per cent of total menthol in the oil tested =

$$\frac{A \times 7.808}{B - (A \times 0.021)} \times [1 - (E \times 0.0021)]$$

A is the result obtained by subtracting the number of cubic centimeters of 0.5 *N* sulfuric acid required in the above titration from the number of cubic centimeters of 0.5 *N* alcoholic potassium hydroxide originally taken. *B* is the weight of acetylized oil taken, and *E* is the percentage of esters calculated as menthyl acetate.

The 0.5 *N* sulfuric acid may be standardized by any of the common methods. If the analyst does not wish to adjust the solution to the exact normality, the normality factor multiplied by the volume of acid used in the titration will give the value for *A*. In actual practice, it is more convenient to perform a titration of the alcoholic base in terms of the acid value. In the titration of freshly distilled or rectified oils the end point with phenolphthalein is easily detected. In the case of old or badly oxidized oils, the marked darkening of the sample upon refluxing with alkali renders difficult the estimation of the end point. Baumgarten (*3*) has suggested a modification of the titration procedure in which a few drops of methylene blue solution (1 per cent in 95 per cent alcohol) are added to the titration mixture. The color change is from reddish violet to green or olive green.

Some laboratories report good results using a mixture of 2 drops of 0.02 per cent phenol red with 6 drops of 1 per cent phenolphthalein. If the saponified sample is very dark, it can be diluted with water before titration or diluted with water to a definite volume, 250 or 500 cc., with aliquot parts being taken for titration. The error involved in this procedure is commensurate to that involved in the titration of the undiluted mixture. That the titration of samples involves errors in human judgment is evidenced by the following communication from an essential oil company.

We carry the back-titration to the absence of red color. At times two different operators may think this takes place at slightly different points, which would cause some difference in their results. We have found it hard to obtain consistent checks when the same samples are tested in different laboratories, and for that reason we usually allow up to 1 per cent for experimental error on menthol determinations.

The formula for calculating the per cent of total menthol in oil of peppermint, first introduced into the tenth revision of the U. S. Pharmacopoeia, takes into account the menthol already present in the oil in the form of menthyl acetate, and the weight of acetyl radical added to the oil in the acetylation process (*14*).

A more recent method (*7*) for the estimation of menthol in oil of peppermint involves the action of measured amounts of acetic anhydride on weighed samples of oil in the presence of pyridine or *n*-butyl ether as carriers. This process, by eliminating the acetylation, washing, and drying processes, is less time-consuming. The *n*-butyl ether used in this determination must be carefully purified to remove all hydroxylated compounds. This method determines free menthol only, so that it is necessary to calculate from the ester content (determined by saponification) the amount of menthol in the combined state, which, when added to the free menthol content, will give total menthol, free and combined. Grove (*10a*) and Wilson (*25*), independently, have made investigations similar to those of Brignall on the determination of hydroxyl groups in oil of peppermint and in other volatile oils. Pyridine and xylene as solvents, and acetic anhydride, acetyl chloride, and phthalic anhydride as acylating agents, were used in their work.

Determination of Menthone in Oil of Peppermint

Menthone is a ketone, $C_{10}H_{18}O$, which occurs with menthol in oil of peppermint. When natural *l*-menthol is oxidized, *l*-menthone is produced. This substance is easily converted to *d*-menthone by the action of acids.

Earlier investigators advanced the theory that menthone was formed in the plant by the oxidation of menthol and that oils distilled late would show a high menthone content. Recently, however, Strauss (*23*) has stated:

> Peppermint for oil production is best cut after full bloom has been reached, not at the beginning of flowering. In this way it is possible to obtain a maximum yield of oil with a menthone content not exceeding 15 per cent. According to Rutovskiĭ and Travain (*21*) the menthol content of the plant increases during growth, whereas the menthone content decreases. It appears that, under the influence of the sunlight during the growth of the plant up to the time of full bloom, menthol is slowly formed from menthone, to which the bitter aftertaste of peppermint oil is due.

The author's observations on the menthone content of numerous samples of oil collected at different stages of growth agree with the findings of Strauss. Apparently the biogenetic process in the plant synthesizes menthone as well as menthol and a phytochemical reduction changes the former substance into the corresponding alcohol, menthol, as the plant matures. Although the significance of the observation can be questioned, it is interesting to note that the sum of the menthol and menthone percentage in oil of peppermint will be within the range of 75 to 85 per cent.

For determination of menthone content, the hydroxylamine hydrochloride method has proved to be most popular and best adapted to give consistent results. This method involves the reaction between the ketone and hydroxylamine hydrochloride to form menthone-oxime hydrochloride which decomposes to form menthone-oxime and hydrogen chloride.

Titration of the liberated hydrogen chloride with a standard alcoholic base serves as a basis of the calculation of menthone content, since each mole of hydrochloric acid liberated is equivalent to one mole of menthone.

The technique of applying this method is subject to much variation in different laboratories. The use of different indicators for the end point in the titration of the liberated acid and the manner of adding the alcoholic base constitute the chief differences. The following method, admittedly open to criticism for several reasons, has worked satisfactorily for routine analyses.

> Oil of peppermint (2.5 cc., 2 to 3 grams), accurately weighed, is treated with 20 cc. of hydroxylamine hydrochloride solution [5 grams of hydroxylamine hydrochloride in 9 cc. of hot water, 80 cc. of 90 per cent alcohol, 2 cc. of bromophenol solution (0.1 gram of bromophenol blue with 3 cc. of 0.05 *N* sodium hydroxide, water to make 25 cc.), and enough 90 per cent alcohol to make 100 cc.]. From time to time the mixture is titrated with 0.5 *N* alcoholic potassium hydroxide solution to maintain the blue color of the indicator. At the end of 4 or 5 hours a slight excess of the standard base is added and the mixture back-titrated with 0.5 *N* sulfuric acid. The number of cubic centimeters of 0.5 *N* alcoholic potassium hydroxide used in the reaction, multiplied by 7.7 and divided by the weight of oil taken, indicates the per cent of menthone. For establishing the relationship between the base and acid, it is recommended that 20 cc. of hydroxylamine hydroxide be added to the base before the acid is added in the blank titration.

A similar method for determining menthone advises the addition of 20 cc. of 0.5 *N* base immediately after adding the hydroxylamine hydrochloride solution, with titration of the residual base after 2 or 3 hours. It was observed in this modification upon titration of the blank that the titer of the mixture changed as the hydroxylamine hydrochloride remained in contact with the excess base. For the assay of a single sample this method would offer no disadvantages, provided both the sample and the blank were back-titrated at the same time. Inasmuch as the titer may change as much as 3 cc. of 0.5 *N* acid over a period of 1 hour, it is obvious that the titration of ten or more samples to give consistent results would be difficult. The study of the determination of this component is being continued in a number of laboratories.

Determination of Resinous Material in Oil of Peppermint

In a personal communication from Schimmel and Company, Inc., New York, N. Y., the method used for determining resinous material is described:

> A carefully weighed quantity of oil is placed in a flask and subjected to steam-distillation until all volatile material has passed over. The residue is extracted with ether and the ethereal extractions are allowed to evaporate in a tared beaker. The weight of the resinous material remaining divided by the weight of the oil originally taken indicates the fraction of resinous material present.

In order to eliminate the use of large quantities of ether and to speed up the determination when a large number of samples are to be examined, the following modification has been found to give good results:

> Five cubic centimeters of oil, carefully weighed in a tared flask (125-cc. Erlenmeyer) are steam-distilled until all volatile material has passed over. From 100 to 150 cc. of water should distill through the apparatus to remove all volatile material. After removing the flask from the steam generator, it is placed in an oven having good air circulation and maintained at a temperature between 90° and 100° C. After thorough drying of the contents, the flask is allowed to cool and is weighed. The difference in weight represents the amount of resinous material in 5 cc. of oil.

Oil samples containing fair amounts of resinous material are characterized by a yellow to brownish-yellow color and a terebinthinate odor and are almost devoid of the sharp, minty odor of fresh oil. Poor storage conditions are responsible for the high resin content in many oils, although occasionally an oil freshly distilled through a dirty coil will be dark and of poor quality. Thickening of the oil usually accompanies aging and darkening in color; both appear to be due to oxidation, polymerization, and resinification of the terpene constituents of the oil.

If a few drops of the original oil and a few drops of the steam-redistilled oil are treated with 1 cc. of concentrated sulfuric acid (95 per cent), a distinct darkening of the oil-acid mixtures will result. If the resin content does not exceed 2 per cent, the darkening of the original oil and of the redistilled oil is of the same intensity. Above a 2 per cent resin content, the color of the original oil solutions deepens from dark red to black. Unfortunately, the depth of color does not appear to be proportional to the amount of resin; the test is only roughly qualitative for the presence and amount of resin. The work on this problem of color determination is being continued.

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The Komarowsky Color Reaction for Aliphatic Alcohols

Limitations and Sensitivity

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Limitations of the Komarowsky color reaction for fusel oil and certain precautions to be observed in its quantitative application to fusel oil determination, using *p*-dimethylaminobenzaldehyde, are pointed out. Means of increasing the test's sensitivity, so that it can be used on ethanol, are described. The colored solutions obtained as a result of the Komarowsky reaction have been studied with the Coleman spectrophotometer, and photographed on Kodachrome.

OF THE several methods described in the literature for the determination of fusel oil, a colorimetric method based on the Komarowsky (*5*) reaction seemed to have obvious advantages over all other methods (*7*), the two most outstanding being rapidity and satisfactory sensitivity of the reaction for most purposes. However, constant progress in the design of rectifying columns has produced an ethanol in which the higher aliphatic alcohol content collectively is of the order of 1 to 5 parts per 100,000. A re-examination of the colorimetric method of analysis was therefore necessary with a view to increasing its sensitivity, the study being based on Penniman's (*7*) adaptation of the reaction.

Komarowsky Reaction

This reaction involves the interaction of the higher alcohol, concentrated sulfuric acid, and an appropriate cyclic aldehyde. It is stated (*3*, *6*) that the color is given by hydrocarbons of the C_nH_{2n} series except ethylene. In general, the reaction is shown by higher alcohols of the paraffin series (*1*, *4*), esters of these alcohols, hydroaromatic alcohols, phenols, and ethylene compounds when present as a straight chain (*2*). Polyalcohols, ortho- and para-substituted phenols, alcoholic and phenolic acids, and aromatic derivatives having an ethylene group and carboxyl group on the side chain do not give the test. It has been assumed that the color formation is due to a dehydration of the alcohol by the sulfuric acid, the unsaturated hydrocarbon then combining with the aldehyde.

The authors have not found the reaction to be so simple as this assumption, because pentene-2, even in high concentration, does not produce a color with *p*-dimethylaminobenzaldehyde on prolonged boiling. If concentrated sulfuric acid is added to this mixture, the color develops only when the acid reaches a definite concentration. The acid is necessary for the reaction. Sirupy phosphoric acid may be substituted for sulfuric acid, but the intensity of the developed color is less.

The intensity of the color depends not only on the concentration of the higher alcohols but also on that of the reagent

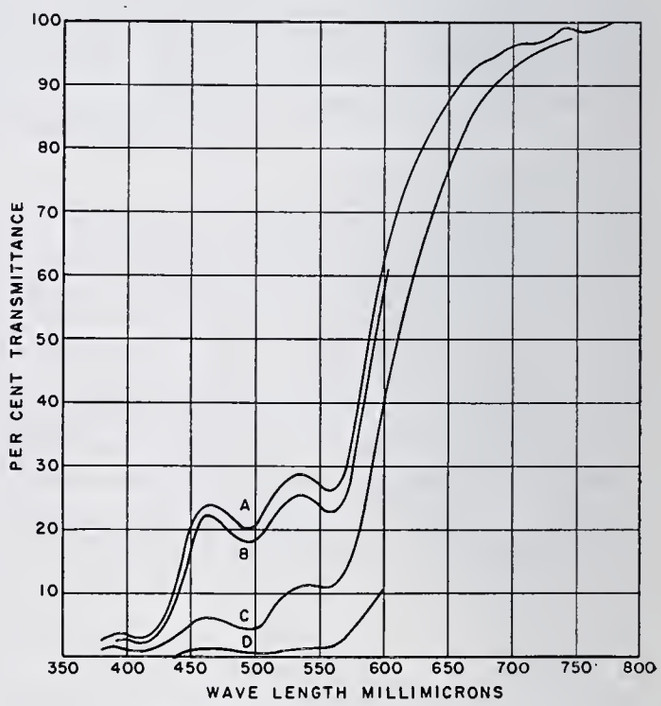

FIGURE 1. SPECTRAL TRANSMITTANCE

A. 5 grams per 100 liters of isopropyl alcohol
B. Same as *A*, but 2 hours later
C. 5 grams per 100 liters of isoamyl alcohol
D. Same as *C*, but 24 hours later
Reagent, *p*-dimethylaminobenzaldehyde. Standard, sulfuric acid plus reagent (in ethanol)

aldehyde and sulfuric acid. The color is more intense on heating, shifts toward greater intensity with time, occurs with all alcohols, and can be followed with a spectrophotometer (a Coleman double monochromator spectrophotometer with 7.5 mμ slit and square cells, Figure 1). The colored complexes cannot be extracted from the sulfuric acid with solvents, but they can be adsorbed on fuller's earth to give blue zones. The colored layer may be removed and washed with water until most of the acid is removed, and the colored compounds eluted with alcohol or acetone. The solvent may then be reduced in volume and the color intensity determined with a colorimeter. This procedure offers a way of concentrating the colored compounds from minute amounts of the alcohols present in ethanol, thus overcoming the inability of the colorimeter to give accurate results with weakly colored solutions.

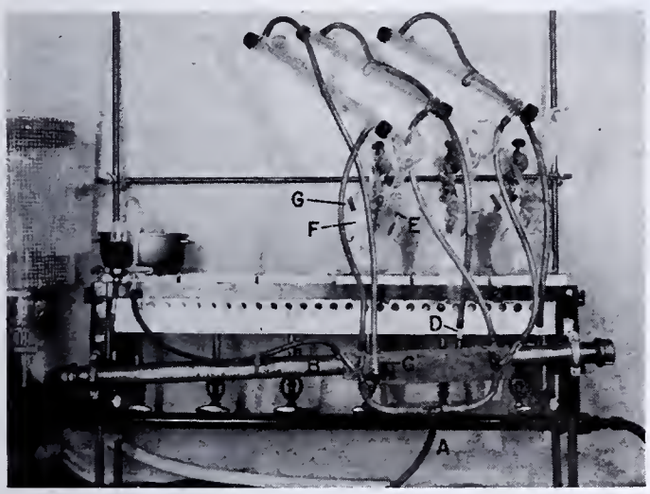

FIGURE 2. FUSEL OIL APPARATUS IN REFLUXING POSITION

The Komarowsky color complexes, after elution from fuller's earth with alcohol, show color changes when the reaction is shifted from an acid pH to a basic pH or *vice versa*.

Apparatus and Technique

Penniman's (*7*) procedure for the preparation of the sample has been changed in a few respects for the determination of aliphatic alcohols in fermented mashes.

A 75-cc. sample of the filtered mash is used and the final dilution with (1 + 1) sulfuric acid is omitted because of the very low percentage of the alcohols.

Seventy-five cubic centimeters of sample are placed in a 500-cc. round-bottomed flask. Silver sulfate (0.5 gram) and 1 cc. of (1 + 1) sulfuric acid are added, and the total volume is made up to 110 cc. The contents are gently refluxed for 15 minutes. The solution is next made alkaline with 5 cc. of (1 + 1) sodium hydroxide solution and it is then refluxed for 30 minutes, a little granulated zinc being used to minimize bumping.

After the saponification the sample is distilled, 75 cc. of distillate being collected, 2 cc. of the distillate are accurately pipetted into a 125-cc. Florence flask, and 20 cc. of concentrated sulfuric acid are carefully added, the flask being swirled in a bath of ice water during the addition. Then 2 cc. of a solution of *p*-dimethylaminobenzaldehyde in 95 per cent ethyl alcohol (10 mg. per cc.) are added, the flask being again swirled in the ice water.

Similar flasks containing 2 cc. of standard fusel oil solutions are prepared with acid and reagent. (These standards are conveniently prepared from a natural fusel oil resulting from a similar fermentation and may be secured from a distillery. The standards are made by dissolving the natural fusel oil in 95 per cent alcohol to give concentrations of 2, 3, 5, 10, etc., grams per 100 liters. The proper concentrations for comparison with the unknowns are found by trial.)

The flasks are placed simultaneously in a bath of vigorously boiling water and are transferred after 20 minutes to an ice-water bath. When cold, the solutions are ready for comparison in the colorimeter (100-mm. cups) or spectrophotometer.

The de-aldehyding refluxing with silver sulfate (the authors do not find any of the methods suggested for removal of aldehydes from alcohol entirely satisfactory) is carried out in the apparatus shown in Figures 2 and 3, which is an adaptation of a suggestion of Stout and Schuette (*8*).

Figure 2 shows the all-glass apparatus in a refluxing position. A gas-heated Kjeldahl heating shelf is used. The condenser water is introduced through tubing *A*, and pipe *B* acts as drain. The condenser tubing carrying the waste water has a piece of glass tubing *C*, which is simply inserted into the copper tubing attached to the main drain as shown at *D*. The condenser and the distilling head are in one piece and can be rotated at the glass joint, *E*. The granulated zinc is placed in the small-bore glass tubing, *F*, which passes through the wall of the flask, and then is washed into the flask with sodium hydroxide from a 5-cc. pipet. The end of the small-bore glass tube is covered with a rubber policeman, *G*, during the refluxing. At no time is it necessary to disconnect the condenser from the flask or to cool the flask as extensively as in the Penniman procedure.

Figure 3 shows the apparatus in distillation position. The rotation takes place at the glass joint. The distilling head, *A*, is so designed that no sodium hydroxide can spatter or be thrown up into the condenser.

Penniman has fully discussed the variables controlling the color reaction, stressing the necessity of accurate time intervals in all steps and accurate volume measurements. All stirring or shaking of the flask (because of possible bubble formation) is avoided after the heating stage, which is carried out in a suitable water bath permitting the simultaneous heating of six flasks under exactly the same conditions. After heating, the flasks are immersed simultaneously in an enameled pan for cooling.

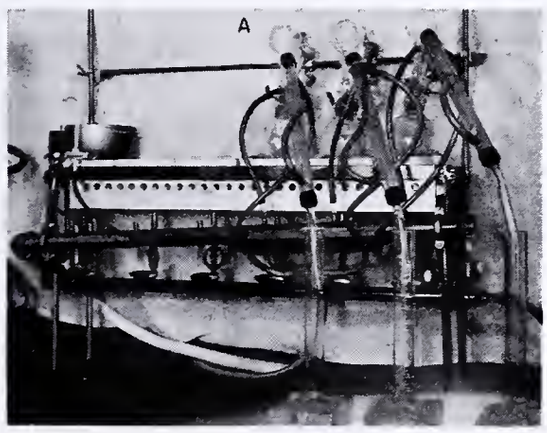

FIGURE 3. FUSEL OIL APPARATUS IN DISTILLATION POSITION

The aromatic aldehyde *p*-dimethylaminobenzaldehyde has been chosen for this work, for reasons explained in a later paper.

FUSEL OIL IN ETHANOL. The following details have been worked out as a result of about five hundred tests.

Samples (10 cc.) of 190-proof ethanol are measured by volumetric pipet into 2.5 × 20 cm. (1 × 8 inch) Pyrex test tubes which are immersed in ice water.

Twenty cubic centimeters of chilled concentrated sulfuric acid are added by volumetric pipet to the sample contained in each tube. This sulfuric acid contains the crystalline color reagent (*p*-dimethylaminobenzaldehyde) dissolved in it in such an amount that 20 cc. of acid contain a total of 0.5 mg. per cc., or 10 mg. (This acid solution deteriorates rather rapidly and should be freshly prepared.)

The contents are well mixed with a glass stirring rod, and the tube is placed in boiling water for exactly 20 minutes.

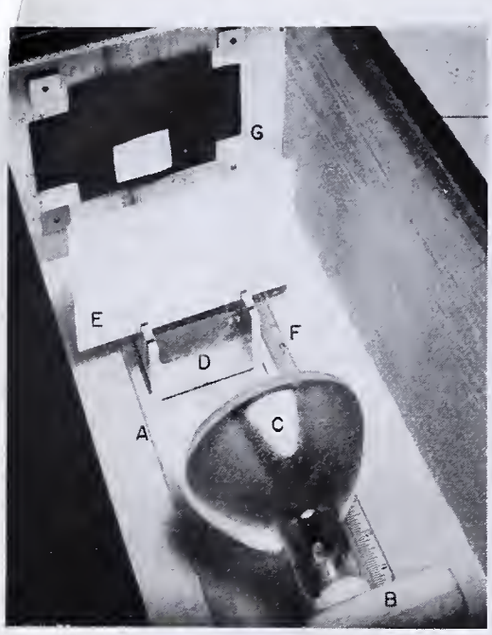

FIGURE 4. REAR VIEW OF CAMERA BOX

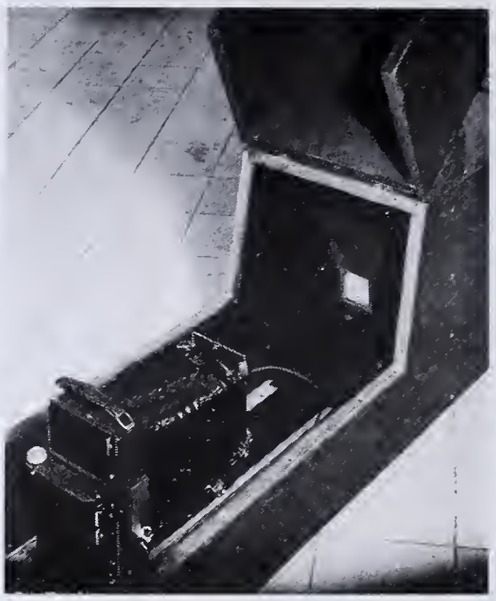

FIGURE 5. FRONT VIEW OF CAMERA BOX

The tubes are immediately immersed in ice water, each tube being removed from the ice water only when its turn comes for testing. It is allowed to come up to room temperature (25° C.). Colorimeter or spectrophotometer readings are then immediately made. The colors are compared, in the colorimeter, with the 3 gram per 100 liter standard, and with the spectrophotometer the standard is made up and treated in exactly the same manner as given above, except that no color reagent is added. The standard has the same proof in alcohol as the unknowns.

About 10 grams of fusel oil per 100 liters is the lower limit of the Penniman procedure, applied to ethanol, at which the visual colorimeter comparisons are reliable. The above directions have increased the sensitivity to 1 gram of the alcohols per 100 liters. The employment of the spectrophotometer increases the sensitivity still more and, at least with alcohols above the propanols, concentrations of 1 gram per 1000 liters may be determined.

The spectrophotometric examination of the color produced with the Komarowsky reagents cannot be used as a means of qualitatively or quantitatively determining the constituents of a complex mixture of alcohols. Peaks are present in the same regions (395, 465, and 535 mμ) for all the alcohols. The method would be very valuable for simple mixtures of two higher alcohols.

Kodachrome Pictures

The authors have found it useful and convenient to photograph the Komarowsky color solutions on Kodachrome, not only as an additional record of the work but also for visual comparison of different fermentation results from week to week. Figures 4 and 5 illustrate the arrangement of the camera box for the Kodachrome photography.

The over-all dimensions of the box are 29.4 × 29.4 × 131.9 cm. (11.75 × 11.75 × 52.75 inches). Clear Idaho white pine, 1.88 cm. (0.75 inch) in thickness and 30 cm. (12 inches) wide, is used. The box is varnished and has a stand constructed of angle iron.

Figure 4 shows two bracket-shaped aluminum strips, *A*, screwed to the bottom of the box, to permit a sliding arrangement for the holders of the lamp and the diffuser. The wood mount, *B*, on a sliding platform, is for the socket of the G. E. 150-watt, 115-volt Mazda tungsten lamp, *C*, with the silvered reflector inside the lamp. An aluminum holder, *D*, with slots, holds the 12.5 × 17.5 cm. (5 × 7 inch) opal glass diffuser, *E*. A ruler, *F*, fastened to the box, permits the position of all equipment to be accurately determined and reproduced. A second opal-glass diffuser, *G*, partly blanked out with photographic black paper, covers the hole in which the optical cell, containing the colored solution, rests. The inside dimensions of this part of the box are 25 × 25.6 × 58.12 cm. [10 (width) × 10.25 × 23.25 inches]. The top is fitted with a sliding panel.

Figure 5 is the other half of the camera box. When the lid is closed, the inside dimensions are 25 × 25.6 × 68.12 cm. [10 (width) × 10.25 × 27.25 inches]. The interior is painted black and felt is tacked to the edges of the box to make it lightproof. This side is also provided with bracket-shaped aluminum strips to act as a runway for the platform supporting the camera, which is held rigid thereon by an aluminum upright and a screw. There is also a ruler on this side.

The camera used is a Kodak Recomar No. 33 with miniature attachment f 4.5 lens, and is particularly well adapted for this kind of work. No. K828A film is used.

The optical cell for holding the colored solutions is made of clear glass plates, ground, polished, and cemented with a special acidproof cement. The outside dimensions (in mm.) are: 55 long, 15 wide, and 53 high. In order that the film may not be exposed to reflections from the sides and bottom of the optical cell, the cell is placed in a black metal holder which masks the edges. After development, each frame of the unmounted film is cut crosswise into four or more sections, which are then mounted in envelopes containing cellophane windows for comparison and filing. Except for very faint colors, the Kodachrome film is sensitive to slight changes in shade. No filters are used, and standards of approximately the same concentration are photographed on each roll of film. It has been found that the film can be used to check colorimeter readings. It furnishes a permanent visual record of shade variations, differences which are not apparent from colorimeter readings.

Summary

The Komarowsky color reaction, as exemplified in Penniman's procedure, has been somewhat limited as a means of determining higher aliphatic alcohols in ethanol because of its lack of sensitivity toward very small concentrations of the alcohols, particularly the propyl alcohols. Slight changes in the Penniman method, coupled with the use of the spectrophotometer, have increased the reaction sensitivity about one hundred times. Types of apparatus which eliminate some of the variables and speed up the determination are illustrated.

The spectral curves of the Komarowsky complexes of the higher alcohols do not offer a means of quantitatively analyzing a mixture of the alcohols. These curves shift toward greater intensity with time. The color can be adsorbed on fuller's earth and then eluted with a suitable solvent. This method offers a means of concentrating minute amounts of the colored compounds for study. The use of Kodachrome for recording results is suggested.

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Conductometric Determination of Ammonia

Application to Nitrogen Distribution Studies

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IN THE course of a sulfur dioxide fumigation experiment in 1935 it was suspected that some ammonia was being evolved, presumably from decaying organic matter under the crop. The inference was based on the fact that a sulfur dioxide recorder (*10*) showed a slight decrease in conductivity when analyzing the air from a "check" plot. Evidently the dilute acid in the absorber was being neutralized. The inference was confirmed by the Nessler test, both qualitatively and as to order of magnitude of concentration, though the amount of ammonia in the absorbing solution represented the extreme limit of sensitivity of nesslerization.

This experience suggested that the conductivity technique might be applied to the determination of ammonia, particularly in the range of microquantities, which are difficult to titrate accurately, though larger amounts might also be determined. For this purpose it would generally be preferable to employ a weak acid which would increase in conductivity, rather than a strong acid which would decrease in conductivity on conversion to the salt. Boric acid with a dissociation constant of 6.4×10^{-10} should be ideal for this purpose because its conductivity is very small and practically independent of its concentration, whereas ammonium borate has the conductivity of a well-ionized salt. Though boric acid has been proposed many times (*3*, *5*, *15*, *17*) as an ammonia absorbent, so far as the writers are aware no one has previously suggested a conductometric measurement of the system. If a strong acid were employed it would be necessary to know beforehand the approximate amount of ammonia to be absorbed, so that the proper quantity of acid could be taken and satisfactory results obtained.

In this paper Hendricks and Thomas are responsible for the data on air analysis and the distillation method; Stout and Tolman are responsible for the aspiration method.

Air Analysis

Mixtures of ammonia and air have been analyzed by means of the sulfur dioxide autometer (*10*), which was used without modification. The mixtures were prepared by sending gas from a tank of liquid ammonia through a calibrated capillary flowmeter into a large measured air stream. The ammonia concentration ranged from 0.3 to 50 p. p. m. by volume in the air. The analytical machine automatically drew a measured sample of the gas through an absorber and recorded the resistance of the solution throughout the absorption period. The absorbing solution was 100 ml. of 0.04 *M* reagent grade boric acid in redistilled water. Absorption of the gas was practically complete in one absorber at an aspiration rate of 10 liters per minute. With two absorbers in series the second absorber collected only 0.5 per cent of the amount of gas absorbed by the first absorber at both 50 and 14 p. p. m. At lower concentrations the loss from the first absorber was hardly detectable. The conductivity recorder had a range from 40,000 to 125 ohms. The electrodes were a pair of platinum plates, 18 mm. square, spaced about 2 mm. apart, with a cell constant of about 0.069. The recorder was calibrated by adding ammonia to the boric acid solution in the range from 10^{-5} *N* to 3×10^{-3} *N*.

In Table I are given the results of a group of experiments in which the analytical concentrations are compared with values calculated for the synthetic mixtures. The close concordance of the results indicates the reliability of the method. There is no suggestion that the range of concentrations in Table I approaches either the upper or lower limits, which can be conveniently handled by this technique, though the lower limit would depend to a large extent on the quality of the distilled water used in the absorbing solution. In the analysis of 50 p. p. m. of ammonia, 140 liters of air were aspirated, raising the pH of the boric acid solution from 5.8 to 8.1. The efficiency of the absorbing solution remained unimpaired. With larger amounts of ammonia it would be necessary to use a stronger boric acid solution, as indicated below.

TABLE I. AUTOMATIC DETERMINATION OF KNOWN MIXTURES OF AMMONIA AND AIR BY MEANS OF A RECORDING ANALYZER

Concentration of NH_3 in Air			
Calculated	Found	Calculated	Found
P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*
51	52	3.5	3.4
52	49	3.5	3.5
50	50	Av. 3.5	Av. 3.45
Av. 51.0	Av. 50.3		
		2.34	2.54
13.5	13.5	2.34	2.56
13.5	13.2	Av. 2.34	Av. 2.55
13.5	13.8		
Av. 13.5	Av. 13.5	1.31	1.26
		1.31	1.28
14.3	13.4	Av. 1.31	Av. 1.27
14.3	13.7		
Av. 14.3	Av. 13.6	0.42	0.49
5.9	5.9		
5.9	5.7		
Av. 5.9	Av. 5.8	0.30	0.30

Vacuum Distillation and Conductometric Determination of Ammonia

In the analysis of biological material it has been found (*4*, *6*, *14*) that the ordinary distillation of ammonia, even with a mild base like magnesia, causes partial hydrolysis of glutamine and asparagine. Vacuum distillation has therefore been proposed and it has been established that the rate of distillation is rapid, particularly when the distillation is aided by more or less aspiration of air through the system. For example, the vacuum distillation by Stanford (*8*) was completed at 35° C. in 10 minutes, during half of which time air was aspirated through the system; though Watchorn and Holmes (*16*) preferred to heat the distillation flask to 60° C. without any aeration. Pucher, Vickery, and Leavenworth (*4*) suggested a 15-minute distillation at 40° C. with the aspiration of 2 to 3 bubbles of air per minute.

In the foregoing distillations the ammonia was received in sulfuric or hydrochloric acid. It was necessary to study the behavior of boric acid under these conditions, since Kober and Graves (*2*) reported that boric acid was not so efficient as sulfuric acid for absorbing ammonia.

The following apparatus, resembling somewhat that of Pucher *et al.* (*4*), was used: A 150-ml. distillation flask was attached by means of a ground-glass joint to the still head, which carried an intake tube (with stopcock), that reached to the bottom of the flask. The receiver was a 250-ml. Erlenmeyer flask with a sealed-in intake tube extending to the bottom at one side and ending with a 15-mm. porous glass disk. A 10-mm. outlet tube was sealed on the opposite side of the flask near the top. A 12-cm. condenser connected the still head with the receiver through two standard taper joints. In this assembly ground-glass joints are convenient, but not essential. Good results have been obtained with rubber connections. The carbon dioxide absorber already described (*11*) has also been satisfactory.

TABLE II. STABILITY OF AMMONIUM BORATE SOLUTIONS

Subjected to vacuum distillation and aspiration for 20 minutes at 13- to 15-mm. pressure)

	Mixture before Distillation						
		Molality				NH_3 Distilled	
Temp.	pH	H_3BO_3	NH_3	Ratio	Excess H_3BO_3	N	%
° C.					Mg.	Mg.	
15		0.1	0.0047	21	0.095	1.32	0.00	0
25	7.63	0.1				1.32	0.015	1.2
40		0.1				1.31	0.335	25.6
15		0.1	0.0094	10.5	0.091	2.62	0.02	0.8
25	7.94	0.1				2.60	0.085	3.3
40		0.1				2.51	0.47	18.7
15		0.4	0.0188	21	0.381	5.24	0.00	0
25	6.90	0.4				5.24	0.00	0
40		0.4				5.24	0.056	1.1
15		0.4	0.047	8.5	0.353	13.18	0.025	0.2
25		0.4				13.16	0.145	1.1
40	7.55	0.4				13.01	0.695	5.3
25[a]		0.4	0.047	8.5	0.353	13.18	0.06	0.5
40[a]		0.4				13.12	1.66	12.7

[a] Solution containing butyl alcohol distilled from flask having porous glass bubbler.

TABLE III. VACUUM DISTILLATION OF AMMONIA AT 40° TO 50° C.

(Conductometric measurement in boric acid solution)

NH_3 from NH_4Cl		Amide NH_3 from Asparagine Hydrolysis			
N Taken	N Recovered	Time	Temp.	N Taken	N Recovered
Mg.	Mg.	Hours	° C.	Mg.	Mg.
		3	96	0.466	0.400
0.01	0.010	16	96	0.466	0.465
0.03	0.029	1	115	0.466	0.435
0.05	0.051	1.5	115	0.466	0.465
0.10	0.099	16	115	0.466	0.470
0.50	0.505	3	115	0.932	0.955
1.00	0.983	3	115	1.40	1.40
1.00	0.485[a]	3	115	1.86	1.75
1.00	1.015[b]	..	..	...	...
1.00	1.000	..	..	...	...
2.00	1.99	..	..	...	...
3.73	3.70	..	..	...	...
5.22	5.17	..	..	...	...

[a] Solution contained phosphate, made alkaline with MgO.
[b] Solution contained phosphate, made alkaline with 0.1 *N* NaOH.

The stability of ammonium borate solutions was studied by distilling mixtures of boric acid and ammonia in vacuum at 15°, 25°, and 40° C. for 20 minutes according to a definite procedure. The pressure was maintained at 13 to 15 mm. and air was allowed to bubble through the flask at 50 cc. (90 bubbles) per minute (650-mm. pressure). The temperature was kept constant by immersing the flask in a large beaker of water. Condensation of moisture in the still head above the water bath was prevented by occasionally playing a stream of hot water on that part of the apparatus. Two absorbers, each containing 10 ml. of 0.4 *M* or 0.2 *M* boric acid, were usually employed but only traces of ammonia ever reached the second absorber.

The data are summarized in Table II. Ammonium borate solutions are unstable unless a large excess of boric acid is present and room temperature is not exceeded. The table indicates that for a given boric acid–ammonia ratio the stronger solutions are the more stable ones. The presence of butyl alcohol affected the stability only at elevated temperatures. It may be expected that boric acid will absorb ammonia without appreciable loss at the rate of about 0.1 mg. of nitrogen per ml. for the 0.1 *M* solution, or about 0.7 mg. of nitrogen per ml. for the 0.4 *M* solution. The absorbing solution tends to be cooled by evaporation during the distillation, and this circumstance favors absorption.

In accord with these observations that ammonium borate solutions are not entirely stable, it has been necessary to employ a sintered-glass disk of medium porosity to produce small bubbles in all absorbers, except those used for air analysis. Using the glass disk, quantitative absorption has been obtained with boric acid under conditions which permitted 10 to 30 per cent of the ammonia to escape through the Van Slyke-Cullen absorber (*13*), though absorption was satisfactory with the latter apparatus if sulfuric acid was the absorbent. In most of the analyses reported in this paper (except the air analyses), the absorbing solution contained about 1 per cent of butyl alcohol, which caused some foaming and increased the time of contact between the gas and liquid. Early work with 0.04 to 0.1 *M* boric acid solutions showed that the alcohol was definitely helpful in raising the efficiency of the absorption. Later work with stronger solutions of boric acid indicated that the alcohol was unnecessary.

Typical data, showing the recovery of ammonia after vacuum distillation and conductivity measurement, are presented in Table III. The distillations were conducted in the apparatus described above. The volume of the liquid in the flask was 15 to 25 ml. and magnesia was used in all cases, except one. The observation of Pucher *et al.* (*4*), that low results were obtained when phosphate and magnesia were present together, was confirmed. Distillations were complete in 15 to 20 minutes at 40° or 50° C. if water was prevented from condensing in the still head. Eighty-eight to 97 per cent of the ammonia was distilled in 5 minutes even when no air was allowed to bubble through the system. In other experiments the rate of distillation from a volume of 100 ml. was found to be about half the rate from 20 ml. *l*-Asparagine was not completely hydrolyzed by normal sulfuric acid in 3 hours at 96° C. in the boiling water bath, but the amide group was split off in 1.5 hours in the autoclave at 115° C. Longer heating had no further effect.

The absorbing solution, containing a definite amount of boric acid—(e. g., 10 ml. of 0.2 *M* or 0.4 *M*)—was diluted in a volumetric flask to 25 to 250 ml. according to the amount of ammonia present, and its conductivity determined using glass-petticoated dip electrodes of 0.069 cell constant, similar to the one described above. A conductivity recorder or a Leeds & Northrup alternating current bridge was employed to measure the conductivity. Temperature correction could be applied using the compensator of the instrument, though it was preferable to bring the solution to a definite temperature before placing it in the cell. An empirical calibration curve was prepared for each boric acid concentration, by adding known amounts of standardized ammonia solution to the boric acid, diluting to volume, and reading the resistance. The reciprocal of the resistance (or conductance) was then plotted against the concentration of ammonia, because the relation between conductance and concentration is nearly linear. The cell constant was checked occasionally, using 0.001 *N* potassium chloride, and analyses were run to determine the reagent blanks.

In dealing with microquantities of ammonia it is essential to employ ammonia-free water with a specific resistance of at least 3×10^5 ohms. Water of this quality can readily be obtained by a single redistillation, in glass, of ordinary distilled water, discarding the first fractions until the desired specific resistance is reached. The water can be handled freely and even stored for a long time in Pyrex without appreciable change, because it is evidently in equilibrium with the carbon

TABLE IV. CHANGES IN CONDUCTIVITY OF SULFURIC AND BORIC ACIDS DUE TO ADDITION OF AMMONIA

(Dilution 100 ml.)

Concentration		NH_3 N per	Conductivity						Recorder Shift	
				H_2SO_4 + NH_4OH			H_3BO_3 + NH_4OH		With	With
H_2SO_4	NH_4OH	100 Ml.	H_2SO_4		Diff.	H_3BO_3		Diff.	H_2SO_4	H_3BO_3
$N \times 10^5$		Mg.	Mhos. × 10^4						Div.	Div.
..	366.5	5.0	...	...	...	0.55	45.5	45.0	..	81.8
90	71.5	1.0	49.5	24.0	25.5	0.55	10.65	10.1	24.7	34.5
10	7.15	0.10	5.65	3.10	2.55	0.55	1.61	1.06	11.0	4.8
1	0.71	0.010	1.25	1.00	0.25	0.55	0.66	0.11	0.8	0.5

dioxide of the atmosphere. Recrystallization of reagent grade boric acid resulted in only a slight improvement.

There is uncertainty of about 10 per cent in the conductance value of 10 micrograms of ammonia. The distillate was diluted to 25 ml. in this case. It would be practicable to enlarge the electrodes and to modify the absorber and cell so that a final dilution of 5 or 10 ml. would suffice, in which case still smaller amounts of ammonia could doubtless be accurately determined. In this connection the distillation technique of Conway and Byrne (*1*) would be particularly effective. Some additional sensitivity could also be obtained by using sulfuric acid as the absorbent, as indicated below. With larger amounts of ammonia the error of the conductance reading approached 1 per cent without taking special precautions to secure greater accuracy. In this connection the speed and ease with which conductivity measurements can be made should be emphasized.

Boric acid was selected as the ammonia absorbent because it supplied the convenience of a direct conductance-concentration relationship, and avoided the necessity for a number of accurately standardized solutions of a strong acid—the proper one to be selected according to the amount of ammonia to be determined. On the other hand, the strong acids are more efficient absorbents of ammonia than boric acid and they also show a greater change of conductance. It is probable, therefore, that they will be more suitable than boric acid for some purposes—for example, in the analysis of microquantities of ammonia.

The data in Table IV indicate that the relative change in the conductance of sulfuric acid due to the absorption of ammonia is 2.5 times that of boric acid. It is evident that the molecular conductivity of ammonium sulfate is nearly constant over the range considered, whereas the values for ammonium borate fall off appreciably at higher concentrations. The relative sensitivity of the recorder with the two acids is also indicated in Table IV. When measurements were made with microquantities in the same conductance range, the recorder sensitivity was greater with sulfuric than with boric acid. The reverse was true in dealing with 1 mg. of ammonia nitrogen per 100 ml., because the measurements were made in the high conductance range with the former acid and in the low range with the latter. The range of the recorder (minimum resistance, 125 ohms) did not permit measurement of more than 1.5 mg. of ammonia nitrogen per 100 ml. of sulfuric acid solution without additional dilution or decrease in the size of electrodes. Eight milligrams could be measured in boric acid under comparable conditions.

Finally, the method has been applied to nitrogen distribution studies on alfalfa leaves and on sugar beet seeds (*9*), using the procedure of Vickery and co-workers (*4*, *14*). Reproducible results were obtained and there was no indication that any other volatile conducting material besides ammonia interfered with the method. It is of course possible that in certain plant material appreciable amounts of volatile organic bases other than ammonia may be encountered, and these may interfere with the method.

Aspiration Method

The chief advantage of the aspiration method for the separation of ammonia from plant extracts and solutions is its adaptability to the routine analysis of large numbers of samples. The principal objection to the method has been the length of time necessary for complete recovery of the ammonia (*12*). Incomplete absorption has been reported with boric acid (*2*).

A satisfactory aspiration apparatus, shown in Figure 1, consisted of 12 units connected in series. The evolution tubes were supported in a compact water bath, the temperature of which was controlled by the rate of feeding water into an "instantaneous" heating coil. The rate of aspiration (200 to 300 cc. per minute)

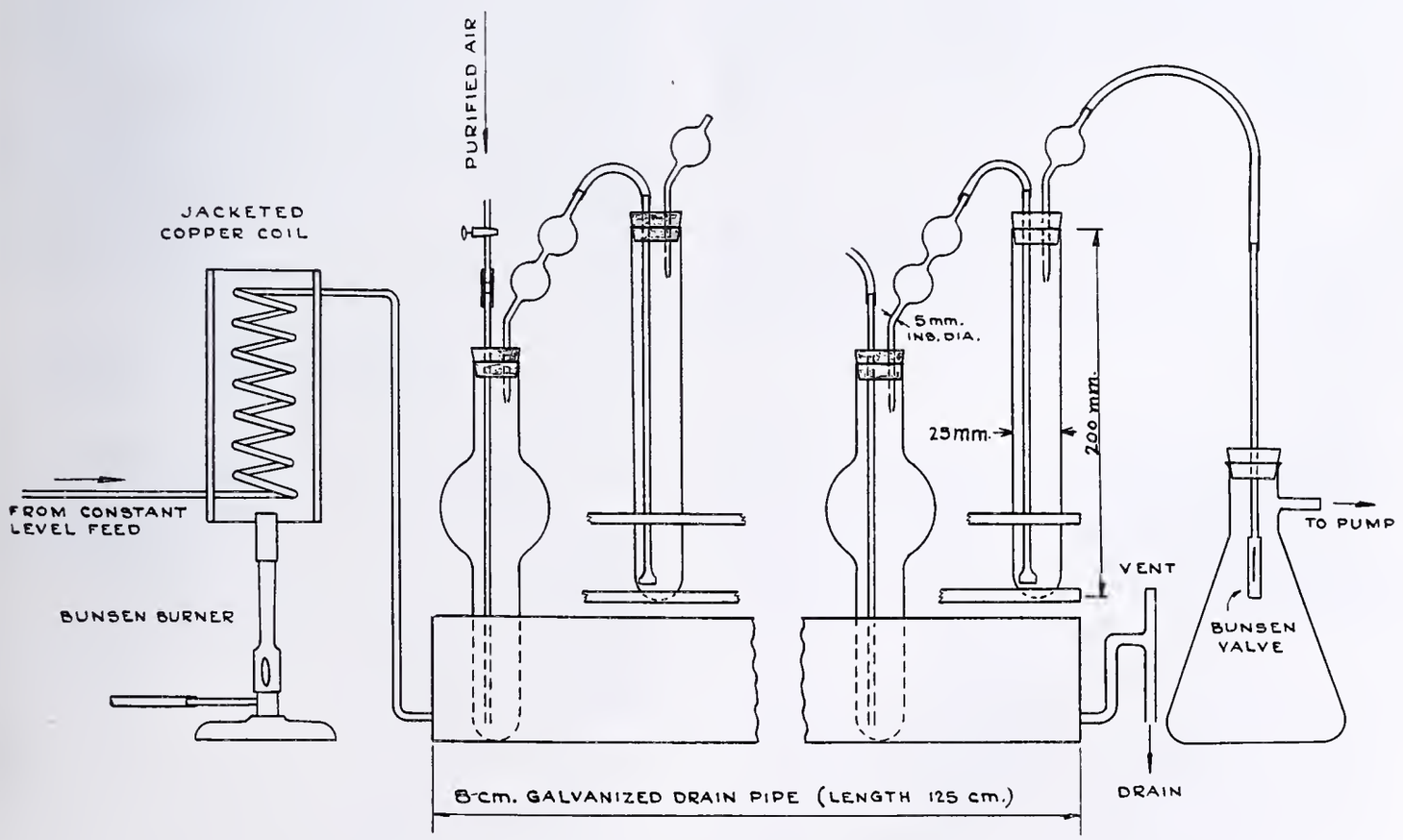

FIGURE 1. ABSORPTION APPARATUS WITH ACCESSORY EQUIPMENT

Absorbers should be supported in cold bath for rapid separations at increased temperature and reduced pressure (Table II).

Table V. Relationship of Temperature and Alkaline Reagent to Time of Aspiration

(Time necessary to effect complete recovery of ammonia from ammonium sulfate solution containing 2.00 mg. of nitrogen in 10 ml. of sample.)

Aspiration Period	Temperature of Aspirated Solution	Average Aspiration Rate	Nitrogen Recovery with Different Bases: MgO, 50 mg.	NaOH, 1 ml. 3 N	K_2CO_3, 1 ml. 50%	NaOH-$Na_2B_4O_7$, buffer[a]
Hours	° *C.*	*Cc./min.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
1	65	196	1.93	1.97	1.99	1.97
1	35	184	1.32	1.33	1.31	1.32
2	65	215	1.99	1.99	2.00	2.00
	35	213	1.80	1.86	1.81	1.80
3	63	222	1.99	2.00	2.00	2.01
	35	223	1.95	1.97	1.96	1.95
4	65	243	2.01	2.00	2.00	2.01
	35	202	1.98	2.00	1.97	1.99
5	65	313	...	...	...	2.01
	35	226	1.98	2.00	2.00	2.00
6	35	235	1.99	2.00	2.00	2.01
Av.	65		9.50	11.15	10.40	10.15
pH	35		9.80	11.35	10.10	10.10

[a] 3 ml., 50 grams of $Na_2B_4O_7$ made to 1 liter with 0.5 N NaOH.

Table VI. Nitrogen Distribution in a Seed-Ball Extract

(With and without the addition of ammonium sulfate)

Solution Volume 20 Ml.	Nitrogen Recovered: Ammonia 1st aspiration, 4 hours	Ammonia 2nd aspiration, 3.5 hours	Amide aspiration, 4.0 hours	Residual by Kjeldahl	Total: Sum	Total: By analysis
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
$(NH_4)_2SO_4$	2.01	0.005	0.008[a]	...	2.023	....
Extract	1.78	0.043	1.052	8.166	11.041	10.990
Extract corrected	1.74[b]	...	1.12[c]	...	....	....
Extract + $(NH_4)_2SO_4$	3.77	0.057	1.012	8.223	13.062	....
Extract + $(NH_4)_2SO_4$ corrected	3.72[b]	...	1.11[c]	...	....	....

[a] Reagent blank.
[b] Subtract ammonia obtained in second aspiration assuming 1 was derived from amide.
[c] Add decomposed amide value less reagent blank.

through the apparatus was controlled by a stopcock on the intake tube or by regulation of the filter pump. The sample volume was 25 ml. or less and the absorbing solution was 15 ml. of 0.4 M boric acid containing 2 drops of butyl alcohol. The delivery tube in the absorber ended with a 6-mm. sintered-glass disk of "coarse" porosity. The method of connecting and disconnecting the apparatus was similar to the procedure of Sessions and Shive (7). The ammonium borate solutions were measured conductometrically, as described above. Two dip cells, one having a constant of 0.05 and the other 0.15, served conveniently to cover the range from 0.01 to 10 mg. of ammonia when the absorbing solution was made up to 100 ml.

Experiments summarized in Table V indicate that 2 mg. of ammonia were completely volatilized in 2 hours or less at 60° C. At 35° C., 4 to 5 hours were required. (At 25° C. the process may require 24 hours.) The different bases gave nearly identical results, and illustrate the reliability of the procedure. The final values were within 0.5 per cent of the amount of ammonia taken. In other experiments, slightly low results were obtained when phosphate and magnesia were present together.

An extended investigation (9) was carried out by the aspiration method of certain nitrogen fractions in aqueous extracts of sugar beet seed balls, before and after the extracts had been in contact with germinating seeds. In this study the ammonium salts were first determined; then the amide nitrogen was hydrolyzed in the autoclave at 15 pounds' pressure for 3 hours, using 10 per cent sulfuric acid, and the hydrolyzed ammonia was removed by another aspiration after making the solution slightly alkaline with sodium hydroxide.

The nitrogen fractions of a fresh seed-ball extract are considered in Table VI. To one aliquot of the original solution was added an ammonium sulfate solution containing 2.02 mg. of ammonia nitrogen. Another aliquot was analyzed for total nitrogen by the Kjeldahl-Gunning method, as were also the solutions after the amide determinations. The results show that the ammonia volatilized by aspiration, plus the residual nitrogen, corresponds closely with the total nitrogen value, thus suggesting that the aspiration procedure did not volatilize conducting substances other than ammonia. It is improbable that other conducting materials would show the same conductivity as the estimated amount of ammonia in the system. This was verified by nesslerizing the solutions after the conductivity measurements. It was found that the conductivity and Nessler values agreed within about 1 per cent. The results also show complete recovery of the added ammonium salt. Slight hydrolysis of other nitrogen compounds, most of which are presumably amides, is suggested by the second aspiration rather than incomplete removal of ammonia in the first. Assuming that the ammonia obtained in the second aspiration was derived from amides, the values for ammonium and amide nitrogen have been corrected in accordance with the continuous amide decomposition observed by Schlenker (6).

Summary

The absorption of ammonia by boric acid was satisfactorily accomplished after vacuum distillation or volatilization by aspiration, if sintered-glass bubblers and sufficient excess of boric acid were employed. It may be necessary to use butyl alcohol in the absorbing solution in some cases. The change in the conductance of the solution provides a simple and accurate method of measuring the amount of ammonia absorbed. Sulfuric acid is a better ammonia absorbent than boric acid and gives a larger change of conductance. Boric acid is preferred in general use because of its convenience. Sulfuric acid may be more suitable in some cases, particularly in microanalyses.

The method has been applied to nitrogen distribution studies of plant materials and to automatic analysis of traces of ammonia in air.

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Determination of Moisture in Sugar Products[1]

Maltose and Dextrose

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CORN sirup contains maltose and dextrose, both of which form hydrates. A literature survey reveals little information in regard to the determination of dry substance of dextrose and dextrose hydrate, leading one to believe that they offer no serious analytical difficulties. On the contrary, there is considerable controversy over the dry substance of maltose and its hydrate. Apparently the difficulty in the determination of dry substance is the reason why no generally accepted values for specific rotation and reducing power exist for this sugar. Since these constants are indispensable in sugar analysis, and both sugars are constituents of corn sirup, it was deemed advisable to apply the new drying technique to these products.

Important early work done on maltose and its anhydride was reported in 1894 by Lobry de Bruyn and Van Laent (*7*). These authors called attention to the observations of Soxhlet, who stated that maltose failed to lose its water of crystallization at 100° C. unless heated in a vacuum. They checked this observation by drying maltose several hours at 100° to 105° C. in an air oven and followed this with a specific rotation determination on the dried material which was found to be 130° (temperature not specified, concentration 1 gram in 10 cc., *D* line used). For vacuum drying, they made use of a device consisting of two flasks connected by a curved tube, one containing the maltose and the other phosphorus pentoxide. The flask containing the maltose was heated at 105° C. for 8 hours. The specific rotation of the maltose was found to be 137.7°. These authors called attention to the stability of maltose by stating that the anhydride could be produced by placing a solution of maltose in a platinum dish and heating in a calcium chloride bath held at 130–135° C. They stated that the sirup must be stirred during evaporation and the heating continued 1 to 2 hours after the last traces of water have been removed. Again the evidence that the anhydride had been obtained was the specific rotation found.

Brown, Morris, and Millar (*2*) in their classic work on products formed by diastatic conversion of starch, experienced great difficulty in driving off the last traces of moisture from these carbohydrates. After many attempts, they finally adopted a form of apparatus similar to the one recommended by Lobry de Bruyn and Van Laent (*7*) and were able to render crystallized hydrated maltose completely anhydrous in a few hours at 105–106° C. without any signs of fusing or discoloration. Hydrated dextrose was still more easily made anhydrous and crystallized levulose completely lost all traces of adherent moisture below the fusing point.

The same workers determined the specific rotation of maltose, drying hydrated maltose in the vacuum apparatus to constant weight, the temperature being gradually raised from 80° to 108° C. The specific rotation value was based upon actually weighed quantities of dried anhydrous maltose and an average value at 15.5° C. for 2 to 20 per cent solutions was 137.93°. This value was compared to that of Ost (*9*), which was 137.04° for a temperature of 20° C., by applying Meissl's (*8*) correction for temperature to Ost's number. The following comparison was obtained: $(\alpha)_D$ of maltose at 15.5° (2 to 20 per cent concentrations), Ost 137.96, Brown, Morris, and Millar 137.93.

The determinations of Ost were all made on weighed quantities of hydrated maltose which had been brought to a constant weight in a desiccator over sulfuric acid. The anhydrous maltose was then calculated on the basis that the composition of the substance being used was $C_{12}H_{22}O_{11}.H_2O$, and that it contained exactly 5.00 per cent water of crystallization. Brown, Morris, and Millar (*2*) found that hydrated maltose, although readily coming to a constant weight when dried in this manner, invariably contained an amount of water greater than that corresponding to one molecule, even after several weeks of drying. This was shown by the loss in weight when the hydrate was dried carefully in a vacuum over phosphorus anhydride in the Lobry de Bruyn apparatus in such a manner as to prevent any decomposition. The average amount of water retained by the hydrate was 0.46 per cent in excess of that corresponding to one molecule. Using this value, a final comparison was made by Brown, Morris, and Millar between their value and that of Ost: $(\alpha)_D$ of maltose at 15.5° C. (2 to 20 per cent concentrations), Ost corrected 138.12, Brown, Morris, and Millar 137.93.

Lintner and Düll (*6*) examined starch hydrolytic products and obtained the amount of solids in solution from the specific gravity, the weight constant being determined by drying 2 to 3 grams of a 10 per cent solution at 100° C. or sometimes at only 60° C. until the weight of the residue was constant. Brown, Morris, and Millar (*2*) showed that it was impossible to remove the last traces of water from starch hydrolytic products in this manner and that nothing short of heating in vacuum over phosphorus anhydride at a temperature above 100° C. was effective.

In a more recent publication, Walker (*10*), co-author of the Munson-Walker tables, reports on the determination of the copper number of maltose. The data obtained on maltose are important because the values for reducing power obtained in this work are those appearing in the Munson-Walker tables (*1*) and more recently adopted by the International Sugar Commission. The maltose used was prepared by recrystallizing Merck's C. P. maltose three times, and drying below 80° in vacuum. This gave a specific rotation of 130.48°, corresponding to 137.2° for the anhydrous maltose, which was stated to show a very pure preparation.

In view of the criticism of this method of preparation by Brown, Morris, and Millar in an earlier paper, it is unlikely that a pure hydrated product was obtained.

The purpose of this investigation was not to determine the specific rotation of maltose, as such a study would entail the preparation of pure maltose, which is the subject of controversy, but to examine the drying technique employed in the past with particular reference to the newer drying methods of the present authors. No claims other than those of the manufacturer are made for the maltose used, nor is the dried material defined as to its α- or β-maltose content. It is believed that the maltose was substantially pure material as it is commonly known. The methods and technique are presented with the belief that they constitute more efficient and reliable means of drying difficult hydrates than have hitherto been used.

Materials

Commercial dextrose hydrate (Cerelose).

Pfanstiehl C. P. maltose. Several lots of maltose hydrate varying in moisture content from 4.9 to 6.1 per cent have been obtained from this firm. The specific rotation from dry substance values obtained have always checked to the first decimal place.

Apparatus

Weighing bottles, Pyrex glass, 40 × 65 mm., standard-taper stoppers.

General vacuum oven, an older type fitted with rubber gasket closure and used with individual pump, but not suitable for high-vacuum work. The pressure maintained was approximately 60 to 70 mm. Tests were made on this equipment, as it is of the type more often found in laboratories and usually deemed adequate for vacuum oven determinations.

Weber lead gasket, a new oven fitted with lead gasket and employed with a Mega-Vac pump whereby pressures of 1 mm. or less were readily obtainable.

Air oven, Elconap, double wall with temperature regulations ±1° C.

Lobry de Bruyn apparatus, as described (*4*).

Procedure

Approximately 5 grams of the maltose hydrate or dextrose hydrate were weighed into the weighing bottle. Tests were made at 60°, 70°, 80°, 90°, 100°, and 110° C. in the vacuum oven, and at 100° C. in the air oven. Vacuum was released by introducing air through an extended drying train. Weight loss was determined at definite intervals over an extended period of time, depending upon the temperature of the test.

[1] This is the third article in a series on this subject. The first two appeared in IND. ENG. CHEM., ANAL ED., 13, 855 and 858 (1941).

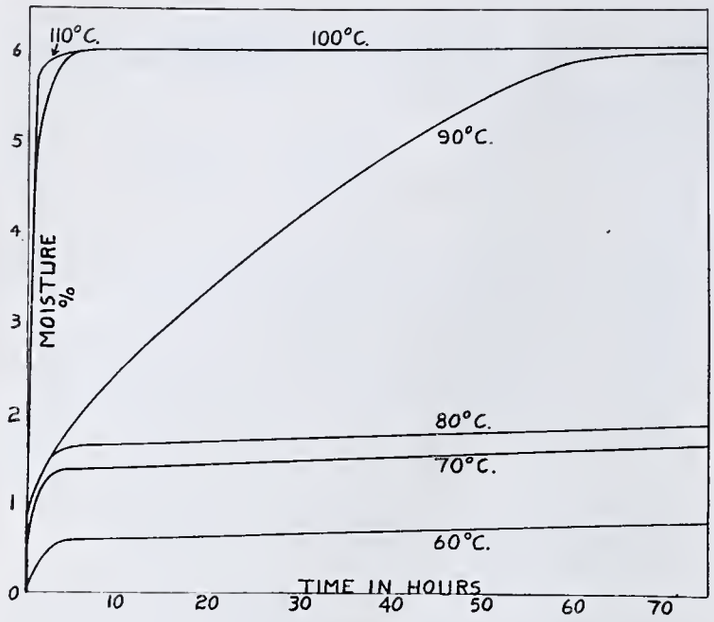

FIGURE 1

The data obtained are best shown by Figure 1. With maltose hydrate, temperatures of 90° C. and above produced an active loss of water. Temperatures of 100° and 110° C. yielded identical values for moisture within the experimental difficulties of weighing such a hygroscopic material. Below 90° C. water was lost with difficulty, and at 60° C. the water loss gave indication of reaching the theoretical hydrate with 5.00 per cent only after extended drying time. The slowness of removing the adsorbed water is attributed to the leaking oven and the hygroscopic nature of the material.

The specific rotation was determined on the material obtained at 90°, 100°, and 110° using a 10 per cent solution (10.000 grams per 100.00 ml.) with the following results:

Temperature ° C.	Time Necessary to Reach Constant Weight *Hours*	Moisture Lost %	$(\alpha)_D$ 20° C.
90	84	6.04	137.5
100	79	6.10	137.42
110	80	6.10	137.38

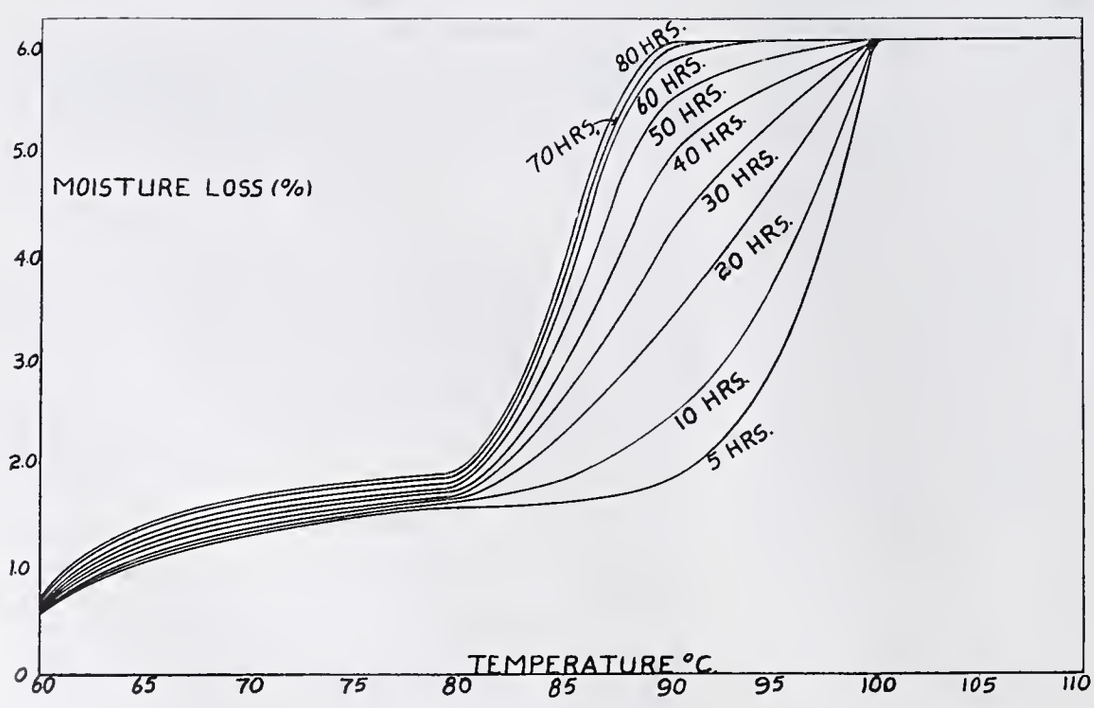

FIGURE 2

The maltose data have been regraphed, taking the moisture obtained at 5, 10, 20, 30, etc., hours at different temperatures, as shown in Figure 2. Under the operating conditions of the general vacuum oven (60 to 70 mm. pressure), there was very little loss of water of crystallization below 80° C. Above 80° C., the rate of loss of hydrate water became increasingly rapid.

The data obtained with dextrose and maltose hydrates employing the Weber oven are shown graphically in Figure 3. As before, 5-gram samples were used and the pressure in the oven was maintained at 0.5 mm. or less, by means of a Mega-Vac pump.

Dextrose proved stable at all temperatures and values obtained could be expressed best by a straight line. The actual data are given in Table I.

TABLE I. DATA ON DEXTROSE

Elapsed Time	Vacuum 60° C.	Vacuum 100° C.	Atmosphere, 100° C.
Hours	%	%	%
10	..	..	7.76
17	7.77	7.77	..
35	7.78	..	..
44	..	7.79	7.76
57	7.78	7.79	..
66	..	..	7.77
103	7.77	..	..

These data are for the crystalline material and do not necessarily follow for solutions where the initial pH of the solution is a function. A series of dextrose solutions ranging from pH 2.0 to 6.0 was dried. Those in the pH range of 4.2 to 5.4 were stable in so far as drying data are a criterion. Solutions above and below this range gave indications of instability based on reduction in reducing power. The authors believe that dextrose solutions should be dried on a dispersing medium (*4*, *5*).

The data on maltose are not comparable in moisture values to Figure 1, as the material was a new lot of maltose hydrate. The specific rotation, however, was the same, as judged by identical drying tests carried out at 100° C.

The rate of moisture removal at 60° C. with the higher vacuum shows a marked increase and probably would have reached the value obtained at 100° C. if the drying time had been extended sufficiently. The data for the atmospheric oven at 100° C. were erratic and did not give a smooth curve. For this reason, the approximate drying curve is shown by the dotted line. The isolated point at 120° C., vacuum oven, gave discolored material, indicative of decomposition.

This difficulty in drying hydrates has been noted before.

Browne (*3*) lists dextrose, maltose, lactose, and raffinose as requiring special precautions. He warns against raising the temperature during the initial stages of drying above the melting point of the hydrate, as otherwise the sugar will liquefy to a thick viscous mass, from which it is difficult to expel the last traces of moisture without decomposition.

For dextrose hydrate, he advises a thin layer of material for several hours at 50° to 60° C. followed by a gradual increase in temperature to 105° C. On the other hand, maltose hydrate loses

water incompletely at 100° C. under atmospheric pressure, so that vacuum dehydration is necessary, and he recommends careful heating at 90° to 95° C. under high vacuum with the completion of dehydration at 100° to 105° C.

The recourse to higher drying temperatures of 105° C. in order to remove the last traces of water seems unnecessary in that it raises the moot question of decomposition. The method of dispersion described for corn sirup (*4*), when applied to a maltose hydrate, gave unexpected results and a new drying technique for such materials.

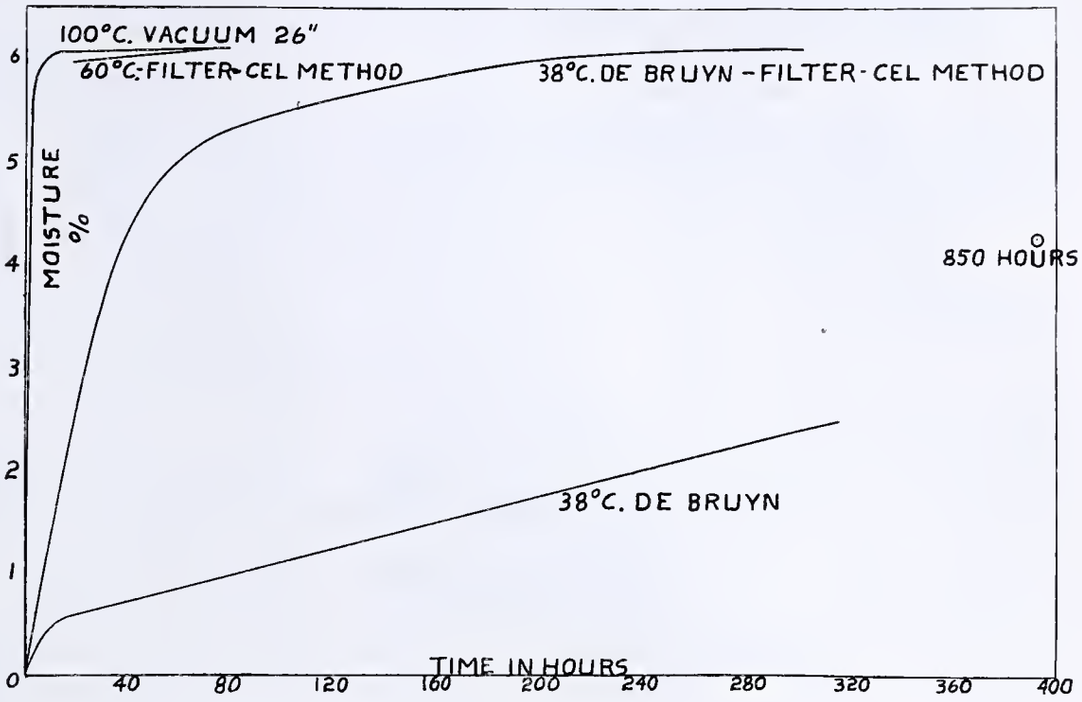

FIGURE 3

The Lobry de Bruyn technique at 38° C. (*4*) was used. In one instance, the crystalline material was used, and in the other the maltose was dissolved and dispersed in Filter-Cel. As a further indication of the effectiveness of dispersion, a sample was dispersed in Filter-Cel and dried in a vacuum oven at 60° C., employing a pressure of 0.5 mm. or less (Figure 4). The maltose was the same material as used for Figure 1.

A comparison of the two Lobry de Bruyn tests is very striking. Even though the pressure on the apparatus containing the crystalline material was reduced to 0.01 mm., the loss of water was exceedingly slow. The test was continued for 850 hours with approximately 25 per cent of the water unremoved. The loss of water from the Filter-Cel sample was more rapid and at the end of 250 hours equaled the value obtained at 100° C. in the vacuum oven, thus establishing identical dry substance values for 38° and 100° C.

The data obtained for the vacuum oven at 60° C., using dispersion, were equally striking. In marked contrast to the behavior of the crystalline material, as shown in Figures 1

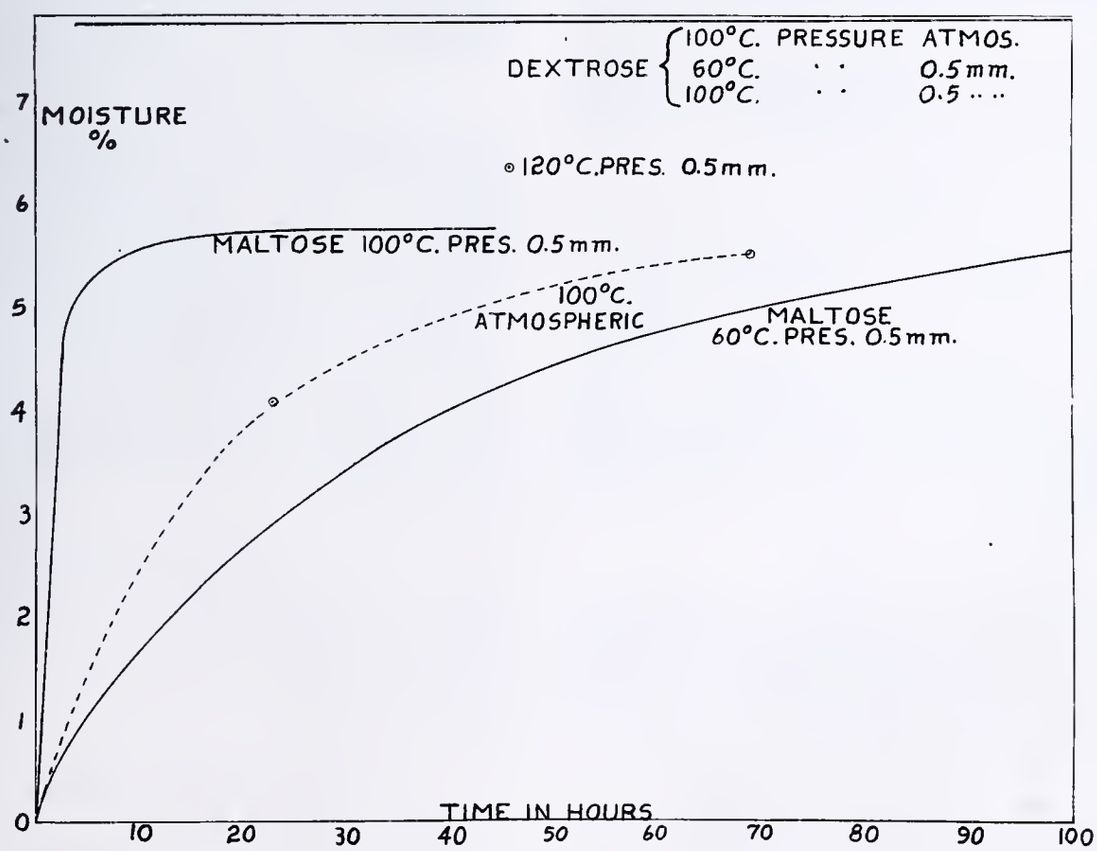

FIGURE 4

and 3, the loss of water was extremely rapid and at the end of 50 hours, the value equaled that at 100° C.

Conclusions

Identical values have been obtained by drying maltose hydrate under low pressures at temperatures of 38° and 100° C.

A new drying technique has been developed for the removal of water of crystallization from sugar.

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Ruthenium Dipyridyl—A New Oxidimetric Indicator

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IT IS well known that various iron complex compounds of the ferrous-phenanthroline type act as high potential redox indicators. These indicators are reversible and reach equilibrium rapidly. Inasmuch as the chemistry of ruthenium parallels that of iron in many respects, an investigation of possible indicator properties of analogous ruthenium complexes has been undertaken. The behavior of ruthenium tridipyridyl dichloride, first prepared by Burstall (*1*), has been examined from this point of view. The results demonstrate that this complex enters into a mobile reversible oxidation in which the reduced divalent form is orange-red and the oxidized trivalent form is green in concentrated solution. At dilutions comparable with those of indicator solutions, the corresponding color change is from yellow to colorless on oxidation. In marked contrast to the behavior of the corresponding iron complexes, both forms of the new indicator exhibit stability towards acid and do not dissociate appreciably even at the boiling temperature.

Materials

Tridipyridyl ruthenium dichloride was synthesized in the manner described by Burstall (*1*). The complex was precipitated as the thiocyanate, which was metathesized with a slight excess of silver nitrate. The resulting solution, which was adjusted to a concentration of approximately 0.02 molar, was used for the determination of the oxidation potential of the complex and as an indicator in the titrations described below.

Solutions of ceric ammonium nitrate in molar nitric acid and ceric ammonium sulfate in molar sulfuric acid were prepared from reagent chemicals. They were standardized against National Bureau of Standards sodium oxalate with phenanthroline-ferrous ion as indicator (*2*).

Oxidation Potential

The oxidation potential of the indicator was determined by titrating 0.002 molar solutions of the nitrate with 0.02 molar ceric sulfate in molar sulfuric acid and with 0.02 molar ceric nitrate in molar nitric acid. Corresponding concentrations of ferrous sulfate and ferrous nitrate were employed in the back-titration. The reference electrode was the quinhydrone in either molar sulfuric acid or molar nitric acid.

The titration curves for the sulfuric acid solutions exhibit a point of inflection at the equivalence point. However, the end point is not sharp, indicating that the potential of the indicator is close to that of the ceric ion in sulfate solution. Thus the indicator cannot be used successfully with this particular oxidant. However, ceric nitrate in nitric acid solution possesses a higher oxidation potential than the corresponding sulfate solution. The end points are sharp with this reagent and the E^0 can be read directly from the curve with ease. The molar oxidation potential found in this manner is 0.58 volt higher than that of the ferric-ferrous system, measured against the quinhydrone reference electrode. The corresponding potential on the hydrogen scale is 1.33 volts, compared with 1.44 volts for ceric sulfate in molar sulfuric acid and 1.61 volts for ceric nitrate in molar nitric acid solution. The indicator solutions reached stable potentials a few seconds after addition of each increment of reagent, indicating a highly mobile, easily reversible redox system.

Application to Titration of Oxalate

The direct titration of sodium oxalate in 2 molar perchloric acid solution at room temperature with ceric nitrate is rapid, precise, and accurate. Two drops of 0.02 molar indicator solution were added to 100 ml. of 2 molar perchloric acid in which from 0.12 to 0.15 gram of sodium oxalate was dissolved. The titrations were then carried out in the cold with 0.1 molar ceric nitrate. At the end point the color changes from yellow to colorless. Successive standardizations of the oxidant solution were in agreement to less than one part per thousand and checked to the same precision standardizations through ferrous sulfate and ceric sulfate, using ferrous phenanthroline as the indicator.

Acknowledgment

The authors wish to acknowledge the aid of George H. Walden, Jr., of Columbia University, who first suggested this problem. One of the authors (J. S.) wishes to thank the American Platinum Co., Newark, N. J., for the ruthenium chloride.

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Titration of Bromide and Iodide Ions with Mercuric Nitrate Solution

Using Diphenyl Carbazide Indicator

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THE determination of iodide by titration with standard 0.1 *N* mercuric nitrate solution using diphenyl carbazide indicator was reported in 1934 by Dubsky and Trtilek (*3*). Roberts (*7*) showed in 1936 that this method could be used successfully in the determination of chloride and made a systematic investigation of the effect of acidity on the end-point. The method of Dubsky and Trtilek using 0.025 *N* mercuric nitrate was found to give erratic results in this laboratory and a modification of Roberts' procedure which is reliable for iodide determinations is offered here. The chloride method is found to be equally reliable in the determination of bromide. The method depends on the formation, from mercuric ion and the alkaline form of the indicator, of a deep blue-violet complex after all the halide ions have been removed in the form of slightly ionized or insoluble mercuric halide. The equilibrium existing between halide ion, mercuric ion, and the indicator has been discussed previously (*7*).

The mercurimetric method for iodide (*3*), using 0.1 *N* mercuric nitrate and diphenyl carbazide indicator, recommends the observation of the blue-violet end point in the presence of the crimson-red precipitate of mercuric iodide. It was found in this laboratory, however, that in titrations requiring 15 to 20 cc. of 0.025 *N* mercuric nitrate the blue-violet color of the indicator complex formed by local excesses of mercuric ion was adsorbed irreversibly by the precipitating mercuric iodide and the end point was completely obscured. Some titrations were carried out by adding the standard mercuric nitrate solution to within 2 to 3 cc. of the end point before adding the indicator; even here, however, the end points were somewhat indefinite because of the brilliant crimson color of the precipitated mercuric iodide.

In the course of a kinetic investigation (*5*) using dioxane-water solutions it was observed that if the titrating mixture for the iodide determination contains 10 to 15 per cent dioxane, the mercuric iodide precipitates in the metastable yellow modification. If in such a titration the diphenyl carbazide indicator is not added until within 2 to 3 cc. of the end point, the color change in this solution at the end point is from canary-yellow to blue-gray and is extremely sharp. The yellow mercuric iodide precipitate does not change appreciably to the more stable crimson form for 5 to 10 minutes, allowing ample time to complete a given titration. There are some objections to the use of dioxane in this determination. On standing it undergoes autoxidation (*6*), forming peroxides which readily oxidize neutral or acidic iodide solutions to iodine. Such impure dioxane has in fact been used by Saifer and Hughes (*8*) in the colorimetric determination of very small concentrations of iodide. Peroxides must therefore be removed from the dioxane. Distillation from sodium (*1*) yields dioxane which does not oxidize an acidified potassium iodide solution for at least 36 hours. For use in mercurimetric determinations of iodide the freshly distilled dioxane may be preserved by the addition of 0.5 gram of hydroquinone per liter; such a solution remains free from detectable quantities of peroxides for at least 2 weeks and the hydroquinone does not interfere with the titration.

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Experimental

MATERIALS AND SOLUTIONS. Primary standards were (1) A. C. S. grade sodium chloride which was reprecipitated, fused in a platinum crucible, pulverized in an agate mortar, and stored over phosphorus pentoxide, and (2) the best grade of mercuric oxide which was reprecipitated and dried for 2 weeks over phosphorus pentoxide. These compounds were checked to 0.2 per cent against each other by analyzing the sodium chloride with mercuric nitrate solution prepared from the mercuric oxide.

Standard mercuric nitrate solution (0.025 *N*) was prepared in a 1-liter volumetric flask from identical weighed samples of the mercuric oxide. Sufficient nitric acid to dissolve all the oxide made the resulting solution 0.008 *M* in acid.

s-Diphenyl carbazide (m. p. 172–173°) from Eastman Kodak Co. was used directly to make a saturated 95 per cent ethanol solution; this solution was the indicator.

1,4-Dioxane was purified as described by Beste and Hammett (*1*) and preserved by the addition of 0.5 gram of hydroquinone per liter.

Silver nitrate, potassium thiocyanate, and saturated ferric alum solutions for the Volhard determinations of halide were prepared from C. P. grade chemicals.

METHOD. In the determination of bromide the method of Roberts (*7*) for chloride, using 0.025 *N* mercuric nitrate, was followed in all respects. Mercuric bromide is sufficiently soluble to cause no interference at the concentrations used here. The indicator blank was determined by the method of least squares applied to a number of titrations requiring different volumes of mercuric nitrate; it amounts to 0.08 cc. of 0.025 *N* mercuric nitrate.

The final volume of the solution being titrated should be 65 ± 10 cc. If the iodide solution to be titrated is acid, it is neutralized with sodium hydroxide solution using phenolphthalein indicator. A calculated amount of 0.2 *N* nitric acid is added, such that the final solution after titration contains the equivalent of 5 cc. of 0.2 *N* acid (*7*). Eight cubic centimeters of peroxide-free dioxane are now added and 0.025 *N* mercuric nitrate is added rapidly to within 2 to 3 cc. of the end point. (The end-point titer is known very approximately as twice the volume of mercuric nitrate necessary to start precipitation of the yellow mercuric iodide. The first half of the mercuric nitrate added produces no precipitate, since the excess iodide forms the soluble complex ion. In some cases it was more reliable to make a preliminary rough determination of the end point.) About 15 drops of the diphenyl carbazide indicator solution are then added and the titration is continued to the end point, which is very sharp. The indicator blank amounts to 0.07 cc. of 0.025 *N* mercuric nitrate as determined by the least squares method.

The standard procedure was followed in Volhard determinations of halide (*4*). In the determination of chloride the modification of Caldwell and Moyer (*2*) was used. A few cubic centimeters of C. P. nitrobenzene were added to each titration mixture; vigorous shaking concentrates the solid silver chloride at the liquid interface and avoids the necessity of filtering. The silver nitrate solution was standardized with solid sodium chloride.

Results

The accuracy of the mercurimetric method in determining halide concentrations is shown by analyses of potassium bromide and potassium iodide solutions by both Volhard and mercuric nitrate methods:

Concentration of KBr solution:	
$Hg(NO_3)_2$ method	0.03255 ± 0.00006 *N*
$AgNO_3$ method (Volhard)	0.03249 ± 0.00007 *N*
Concentration of KI solution:	
$Hg(NO_3)_2$ method	0.02619 ± 0.00003 *N*
$AgNO_3$ method (Volhard)	0.02620 ± 0.00006 *N*

All concentrations and blank corrections were determined by the least squares method applied to a number of samples of the potassium iodide solution requiring different volumes of mercuric nitrate or silver nitrate. A typical set of such data is that for the mercurimetric determination of the concentration of potassium iodide solution, given in Table I.

Table I. Mercurimetric Analysis of Potassium Iodide Solution

Volume of KI Soln.	$Hg(NO_3)_2$ Titer[a,b]
Cc.	*Cc.*
5.25	5.56
5.13	5.47
10.15	10.71
15.15	11.97
20.24	21.29

[a] $Hg(NO_3)_2$ solution is 0.02497 *N*.
[b] Blank correction is 0.07 cc.

The agreement of these results indicates that the mercurimetric method is reliable under the conditions employed here.

The use of peroxide-free dioxane may be a cumbersome and undesirable essential in occasional iodide determinations; however, for frequent routine analyses of iodide solutions in kinetic investigations this method has been found accurate, rapid, and more convenient than the Volhard method.

Acknowledgment

The author acknowledges his indebtedness to L. P. Hammett for helpful guidance in the course of this investigation, and to H. T. Beans for various suggestions.

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Analysis of Divinyl Ether in Blood

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THE prediction of Leake and Chen (*10*) that the unsaturated ethers might have valuable anesthetic properties led to the preparation of divinyl ether by Ruigh and Major (*19*). It has recently been estimated that the number of patients receiving Vinethene anesthesia now exceeds a quarter of a million (*13*). In this paper is described the method used for the determination of divinyl ether in the blood of anesthetized dogs and man. Some of the results obtained have already been published in papers (*3*, *17*, *18*, *19*) which include both experimental and clinical studies of divinyl ether. Root has also used the method described for ethyl ether determinations in dogs (*12*).

A preliminary method employed in determining blood concentrations depended on the initial hydrolysis of the divinyl ether in the blood sample to acetaldehyde by strong mineral acids, followed by precipitation of the protein by the Folin-Wu tungstate reagents, distillation of the aldehyde, and its titration by the method of Neuberg and Gottschalk (*15*). In this way it was found that in a dog the concentration of divinyl ether in the blood during light surgical anesthesia was 29 mg. per 100 cc., while at the point of respiratory failure it was 74 mg. per 100 cc. Because of rather wide variations in the check analyses, its time-consuming technique, and the fact that at these anesthetic concentrations a correction factor of 25 per cent had to be added, the method was abandoned.

The problem of satisfactorily determining the concentration of divinyl ether in the blood of anesthetized animals was finally solved by the development and modification of the iodine pentoxide method based on the reaction discovered by Ditte (*2*) and first applied to the analysis of volatile organic compounds by Henderson and Haggard in the case of ethyl iodide (*6–9*). Haggard (*4*) later applied the method with success to the analysis of ethyl ether in both water and blood. Since the completion of this work the method has been modified and adapted to the analysis of ethyl alcohol by Haggard and Greenberg (*5*) and to cyclopropane by Robbins (*16*).

The original method as applied to ether consisted in aerating the sample of fluid, usually blood, and passing the stream of air carrying the volatile organic vapors over heated iodine pentoxide which oxidized the substance to carbon dioxide and water with the liberation of a stoichiometric amount of iodine. The iodine was absorbed in a solution of potassium iodide and titrated with thiosulfate in the usual manner. A simple calculation then gave the amount of volatile organic substance such as ethyl ether present in the blood or fluid being analyzed. Investigators in other laboratories have had great difficulty in handling the method (*2*, *6*, *7*, *11*, *20*). The main difficulties the author encountered and the means by which they were overcome were:

Tubes filled with alternate layers of iodine pentoxide and glass wool prepared and "conditioned" according to Haggard's directions often failed to give reproducible results. Some tubes gave low and irregular results even after several days of conditioning. This was overcome by the use of a special pumice catalyst support which offered a large surface and obviated the "channeling" effect. Independently, Astapenya, Vapnik, and Zelkin (*1*) discovered the efficacy of a large surface in the use of iodine pentoxide for the analysis of carbon monoxide. With this type of filling, success was immediately obtained with each tube, whereas formerly tubes had to be abandoned even after a week of conditioning and numerous trial analyses.

High and variable blanks were obtained. This was ascribed by Haggard mainly to the quality of the iodine pentoxide. The author's use of a tube of heated copper oxide removed any traces of organic impurities and carbon monoxide present in the air. This purifying train, together with the special contact mass, eliminated completely the necessity for a blank correction.

Variations in the rate of air flow and very rapid rates of flow gave irregular results. The mechanical design of the apparatus

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obviated air pockets and a measured amount of air at a definite slow rate was found necessary to obtain consistent results.

The general method is widely applicable to the analysis of volatile organic compounds in body fluids. The more recent work of Haggard and Greenberg (*5*) on alcohol and of Robbins (*16*) on cyclopropane should be consulted if the method is to be adapted to the analysis of other organic compounds. Both modifications are more rapid than the author's and have about the same order of accuracy but employ very much higher rates of air flow. The limiting factor in his modification is the tendency of the blood sample to froth while air is being bubbled through it. In the alcohol method the evaporation of a 1-cc. blood sample takes about 10 minutes. The 10-cc. samples required with divinyl ether would take proportionately longer. With the gas, cyclopropane, Robbins merely passed a rapid stream of air over the surface of the sample.

Attention should be called to the discovery by Haggard and Greenberg (*5*) that in the case of ethyl alcohol oxidation is incomplete and a titration for hydrogen iodide as well as iodine must be made. Robbins (*16*) found that cyclopropane gave a stoichiometric amount of iodine corresponding to complete oxidation. Although the author has no definite experimental evidence, it seems probable that the ratio of iodine to hydrogen iodide formed and hence the completeness of the oxidation to iodine is due to rate of air flow, surface and nature of iodine pentoxide catalyst mass, and nature of the organic material being oxidized. It is possible that the main reason for an average recovery of only 95 per cent in the author's method with both ethyl and divinyl ethers is the formation of some hydrogen iodide. The analytical results, however, are reliable and consistent when his procedure is employed.

Description of Apparatus

The apparatus is shown in Figure 1. Outdoor air is led to the apparatus through lead tubing, filters through cotton in a tower, and then passes through a 20-cm. length of copper oxide contained in a silica tube 25 mm. in internal diameter heated electrically by a 110-volt current passed through 975 cm. of No. 20 B. and S. Nichrome wire (0.65 ohm per foot) which is wound over a layer of asbestos paper. This winding is covered with magnesia steam-pipe insulation. An external resistance is adjusted to keep the copper oxide at a dull red heat. The copper oxide tube is followed by a bubble counter containing a 30 per cent solution of potassium hydroxide.

The aerator is made from a 50-cc. distilling flask with a neck 8 cm. long and 18 mm. in diameter and sealed to a No. 7 standard-taper ground female joint. The air inlet tube is connected to the first three-way stopcock by a short rubber connector for convenience in introducing the sample. By means of two three-way stopcocks the air flow can either be passed through the aerator during the analysis or by-passed while sweeping the apparatus.

Following the aerator and connected to it by means of the ground-glass joint is a U-trap. The arms of the U, 8 mm. in internal diameter, terminate in three bulblets of 5 cc. each and are filled with pellets of potassium hydroxide. The bulblets, constricted at the bottom, are connected by a 10-cc. bulb which formed the bottom of the U and served to hold any liquefied caustic solution. Care should be taken to eliminate here and throughout the apparatus any "dead spaces" which might act as pockets when sweeping the apparatus.

The iodine pentoxide tubes used in this work were originally of the U form described by Haggard and heated in a Crisco bath. With continued use, however, the oil gave off such objectionable fumes that an electrically heated furnace was made from a 30-cm. length of 5-cm. (2-inch) iron pipe wound with 9.6 meters (32 feet) of No. 20 B. and S. Nichrome wire on a double layer of asbestos paper (see also Haggard and Greenberg, *5*). The furnace is then insulated with wrappings of asbestos paper softened with a sodium silicate solution. An external resistance serves to regulate the temperature. The reaction tube is connected with 2-mm. capillary tubing to the ground-glass joint of the potassium hydroxide U-tube. The reaction tube, 20 cm. long and 20 mm. in diameter, is filled with a 15-cm. length of iodine pentoxide–pumice held in place with two plugs of glass wool. Projecting at the bottom of the furnace is another No. 7 male ground-glass joint. The 8-mm. glass tube leading out of the furnace is wound with heavy copper wire which serves to conduct heat down the tube and thus prevent the deposition of iodine at the outlet of the furnace which is closed with an asbestos-silicate plug.

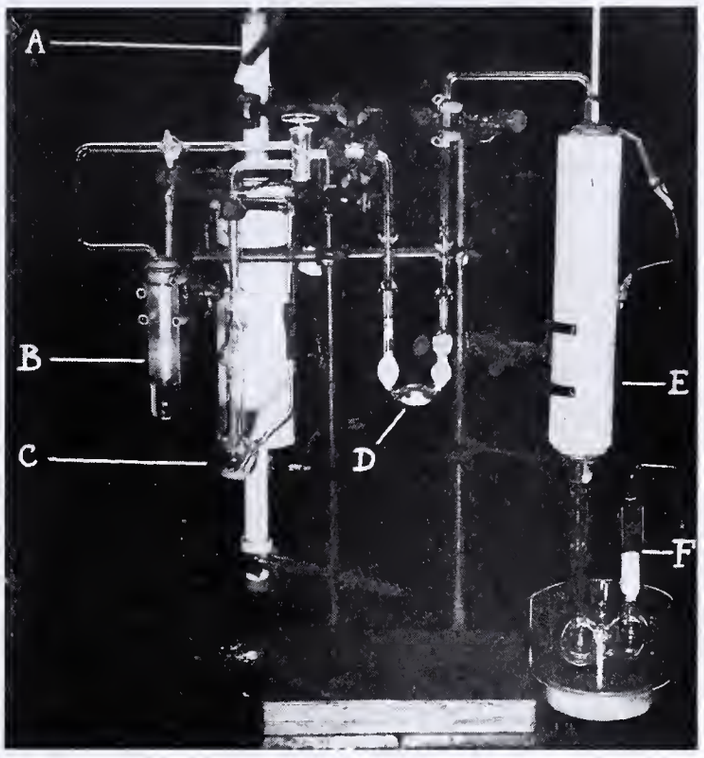

FIGURE 1. APPARATUS FOR DETERMINATION OF DIVINYL ETHER

A. Copper oxide tube and furnace
B. Bubble counter
C. Blood aerator
D. Potassium hydroxide trap
E. Iodine pentoxide tube furnace
F. Iodine absorber

The iodine pentoxide–pumice is prepared as follows: Pumice is broken and sieved to particles 1 to 3 mm. in size. The granular pumice is then treated with aqua regia for 24 hours, thoroughly washed, and ignited in a porcelain crucible for an hour at red heat.

Forty grams of iodine pentoxide (iodic anhydride, Merck) are dissolved in 35 cc. of hot water and the solution is gradually poured with stirring onto 40 grams of the granular pumice in a small casserole. The mixture is then heated over a free flame with constant and rapid stirring until all the water is driven off and iodine vapors begin to be evolved. On cooling, the impregnated granules are ready for the reaction tube. Occasionally, lumps are formed if the stirring is not sufficiently vigorous. The material must then be reworked. The ratio of iodine pentoxide to pumice is not critical and largely depends on the porosity of the pumice. Good tubes have been made with a 1 to 4 as well as the above 1 to 1 ratio of iodine pentoxide to pumice.

The iodine adsorber is made of two 50-cc. bulbs connected at the bottom. The inlet tube, 20 cm. by 8 mm., ended in a No. 7 female standard joint. The outlet, to which suction is applied, is a 10-cm. length of 2-cm. tubing constricted at the bottom packed with glass beads. During the analysis, the bulbs are chilled with ice, leaving the narrow inlet tube free to be heated with a flame to volatilize the condensed iodine.

Manipulative Procedure

With the apparatus assembled, the first three-way stopcock may be greased, but the second stopcock, the aerator joint, and the two No. 7 joints on the potassium hydroxide tube must be lubricated with the glycerol-dextrin-mannitol lubricant described by Meloche and Frederick (*14*). The joint on the potassium iodide bubbler is not lubricated. After testing for leaks, the apparatus is ready for the first operation of conditioning the iodine pentoxide tube.

Suction is applied at the outlet of the pentoxide tube and the flow through the system regulated to 30 cc. per minute. The copper oxide tube is brought to a dull red heat and then the pent-

Table I. Recoveries of Ethyl Ether and Divinyl Ether Added to Water and Blood

Liquid	Ethyl Ether Sample	Ethyl Ether Found		Divinyl Ether Sample	Divinyl Ether Found	
	Mg.	*Mg.*	%	*Mg.*	*Mg.*	%
H_2O	4.86	4.65	95.6	2.61	2.50	96.3
	2.70	3.58	96.7	3.65	3.43	94.0
	2.53	2.38	94.1	2.92	2.84	97.3
	2.99	2.85	95.4	4.96	4.80	97.0
	1.65	1.57	95.3	8.79	8.66	98.6
Blood	4.57	4.41	94.5	1.95	1.89	97.0
	2.10	2.02	96.5	1.13	1.05	93.8
	3.81	3.61	94.9	2.68	2.52	94.0
	2.71	2.53	93.5	9.82	9.32	94.9
	1.48	1.40	95.0	4.42	4.12	93.3
	5.63	5.29	94.0			
			Av. 95.0			Av. 95.6

oxide furnace is gradually raised (about 3 hours) to a temperature of 275°. The continuous evolution of iodine vapors will be noticed at about this temperature. The temperature is lowered to 220° and held there for about 24 hours, then dropped to 200°, and a blank run is made. Ordinarily the blank will now be found to require less than a drop of 0.01 *N* thiosulfate. Not infrequently the blank will fail to give even a blue color on the addition of the starch solution.

In practice, the furnaces are both kept heated overnight with a 30-cc. per minute airstream flowing through the apparatus. The pentoxide furnace remained at 200° ± 5° without the necessity of adjustment of the rheostat for weeks. Before making a run the lower projecting portion of the tube and the ground joint connecting with the iodine absorber are heated with a free flame as a precautionary measure to remove any possible trace of condensed iodine. Twenty-five cubic centimeters of 10 per cent iodide solution are poured into the absorber over the glass beads and the absorber is connected to the apparatus. The absorber bulbs are cooled with ice. The air flow is regulated with a Mariotte bottle to give a rate of flow of about 10 cc. per minute.

The ether sample (1 to 9 mg.) contained in a small capillary tube with long fine sealed ends is carefully placed in the neck of the aerator and washed down with 10 cc. of water or oxalated blood. The rubber connecting tube attached to the side arm is closed with a pinchcock and after chilling the aerator on ice a little of the special lubricant is placed on the joint connected to the apparatus. The capillary sample tube is now broken by sharply striking the bulb of the absorber bulb, which contains a few glass beads, against the palm of the hand. The aerator is immediately connected to the apparatus with the stopcocks turned to divert the air through the aerator.

The rate of air flow is adjusted to about 10 cc. per minute. The limiting factor in the flow rate is the tendency of the blood sample to froth. Careful flaming of the neck of the aerator at intervals helps to prevent the froth from being carried beyond the ground joint. Charring of the blood is, of course, avoided. During the analysis the bulb of the aerator is immersed in a beaker of water maintained at 45°.

Ordinarily an analysis can be completed in 1.5 hours or less, but occasionally some blood samples froth badly and 2 hours are necessary. The total air flow for each analysis is about 950 cc. as measured by the water collected from the Mariotte bottle. About 10 minutes before the completion of the analysis the tube projecting from the furnace, the ground joint, and the neck of the iodine absorber are heated with a free flame sufficiently to volatilize the condensed iodine. The iodine is titrated with 0.01 *N* sodium thiosulfate with starch indicator. The standard solution contains 0.2 gram of sodium carbonate per liter as a preservative and before each titration 2 cc. of *N* acetic acid are used to neutralize the carbonate.

One cubic centimeter of 0.01 *N* thiosulfate is equivalent theoretically to 0.1751 mg. of divinyl ether or 0.1544 mg. of ethyl ether. In actual analyses the result thus obtained is multiplied by 100/95, since the average recovery under the conditions used was 95 per cent. The results obtained were reliable to within 3 per cent. Table I gives the results obtained.

In the analytical determinations of the blood of anesthetized animals, the results of which have already been published (*3*, *17*), the samples were handled in two ways. In most of the animal work the blood sample was withdrawn from the blood vessel in a calibrated hypodermic syringe previously moistened with saturated oxalic acid solution. The blood sample was then immediately transferred to the bulb of the aerator while a gentle current of air was drawn through the apparatus. In this way no loss of divinyl ether could occur. In some cases, the blood sample was transferred to a chilled volumetric flask and made up to volume with water at 0° C. The loss involved was not greater than the error involved in successive determinations of anesthetic concentration in the same animal. The results published in an earlier paper (*3*) are probably somewhat lower than the true anesthetic concentrations, because samples were usually taken with the mask off and the animal coming out of anesthesia.

The rapidity with which the concentration of divinyl ether in the blood changes was not realized until after this work had been published. The rate of elimination of divinyl ether was published separately sometime later (*17*).

Summary

The iodine pentoxide method of analysis for volatile organic substances in blood originated by Henderson and Haggard has been modified and the method applied to divinyl ether. Consistent and reliable results accurate to about 3 per cent can readily be obtained with both ethyl and divinyl ether.

Acknowledgment

The author wishes to express his appreciation for the encouragement and facilities afforded by Samuel Goldschmidt, I. S. Ravdin, and R. T. Major, at whose suggestion this work was initiated, and to D. Wright Wilson for his suggestions in the preparation of this paper. He is also indebted to D. F. Hayman, microanalyst, Merck Research Laboratories, who prepared a number of the sample tubes.

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Determination of Vitamin B_1 by Yeast Fermentation Method

Improvements Related to Use of Sulfite Cleavage and a New Fermentometer

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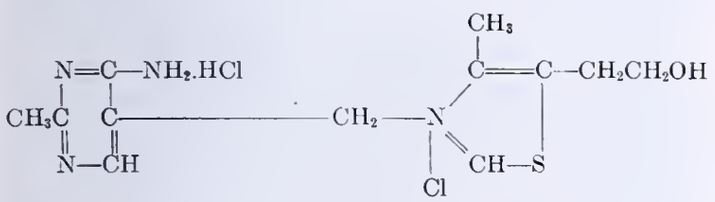
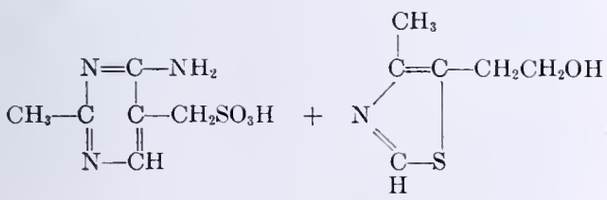
The fermentation activity of thiamin as measured by the method of Schultz, Atkin, and Frey is approximately 99 per cent destroyed by treatment with sulfite at pH 5 to 6 and at 100° C. for 30 minutes. The known interfering substances are not affected by this treatment.

Thiamin assays are based on the measurement of fermentation activity before and after sulfite treatment. This technique has been applied to a wide variety of materials, the thiamin content of which may be of interest. A small compact fermentometer has been designed, to make the yeast fermentation method available for routine use.

THE yeast fermentation method for the determination of thiamin is based on the observation (*2*) that the rate of alcoholic fermentation by living yeast may be increased by the addition of thiamin chloride, and is one of the first microbiological assay methods to be successfully employed. Like most other biological methods, it is highly specific under suitable conditions. The only interfering substance of any significance which has thus far been found (*3*) is 2-methyl-5-ethoxymethyl-6-aminopyrimidine. The corresponding 5-hydroxymethyl compound is a hydrolysis product of thiamin and is also active in the fermentation reaction. Except for its possible existence in urine, this type of substance is not found to any great extent in nature. The interference caused by it may be overcome by oxidation of the vitamin B_1 with alkaline ferricyanide (*4*, *5*) or treatment with sulfite (*6*). Both procedures depend on the chemical inactivation of thiamin by the reagents mentioned and determination of the interfering substance in the residue. The sulfite procedure is preferred because it is more convenient and is applicable to a wider variety of materials. The sulfite cleavage occurs in the following manner (*7*):



Thiamin Chloride



I — 2-Methyl-6-aminopyrimidine-5-methyl Sulfonic Acid

II — 4-Methyl-5-(β-hydroxy)-ethyl Thiazole

The cleavage products, I and II, do not possess fermentation activity. Diphosphothiamin (cocarboxylase) is similarly inactivated by cleavage. The interfering aminopyrimidine, mentioned above, is not affected by sulfite treatment, at least so far as fermentation activity is concerned. It appears likely, therefore, that the thiamin content of an unknown substance is equivalent to that portion of the fermentation activity of the substance which can be destroyed by treatment with sulfite.

Method of Assay

Two preparations are made of each substance to be analyzed—one treated with sulfite and one untreated—and then fermentation activities are determined with the aid of a fermentometer. The difference, expressed in terms of pure thiamin chloride, is taken as a measure of the thiamin content of the unknown.

PREPARATION OF SAMPLES. A water-soluble substance is dissolved in a convenient volume of water (depending upon the potency of the sample). Insoluble materials must be finely divided either in the dry state by grinding or in the wet by means of the Waring Blendor or its equivalent. The latter procedure has been found particularly useful in the preparation of bread and tissue samples. The suspension should be dispersed well enough to permit the measurement of an accurate aliquot by means of a pipet. The solution or suspension is made acid to Congo red paper with sulfuric acid and heated at 100° C. for 20 minutes. This is conveniently done in an Arnold steam sterilizer. When cool the solution is made to volume and a portion is taken for sulfite treatment (described below). The remainder is neutralized to neutral litmus paper by means of sodium hydroxide and is then used for the determination of total fermentation activity—i. e., before sulfite treatment.

TREATMENT WITH SULFITE. In a 50-ml. Pyrex Erlenmeyer flask is placed an aliquot (not exceeding 20 ml. in volume) of the sample prepared as above, and 0.7 ml. of *N* sulfuric acid and 5 ml. of a freshly prepared solution of sodium sulfite containing 0.2 gram of $Na_2SO_3.7H_2O$ per 5 ml. are added. The reaction is adjusted to pH 5.2 to 5.6 with dilute sulfuric acid or sodium hydroxide solutions, using bromocresol purple as an outside indicator to set the pH. The flask is capped with a 30-ml. beaker, heated at 100° C. for 30 minutes, and then cooled. Excess sulfite is destroyed by the addition of the exact quantity of a 3 per cent solution of hydrogen peroxide. The end point of the reaction is determined with the aid of an outside indicator made by mixing one drop of 5 per cent potassium iodide, one drop of 1 per cent soluble starch indicator, and one drop of 50 per cent sulfuric acid. This mixture develops a pink tinge which gets darker with time. If a drop of a sulfite-containing solution is transferred to this indicator by means of a glass rod, the pink color is discharged. As the end point of the titration is approached the pink color fails to disappear and when an excess of peroxide is present a deep violet color appears.

$\xrightarrow{+\ Na_2SO_3}$

When the excess sulfite has been destroyed the solution is neutralized to neutral litmus with sodium hydroxide and made to volume, and an aliquot portion is used for the determination of residual fermentation activity.

Reagents for Fermentation Test

All chemicals used should be of the best grade commercially available.

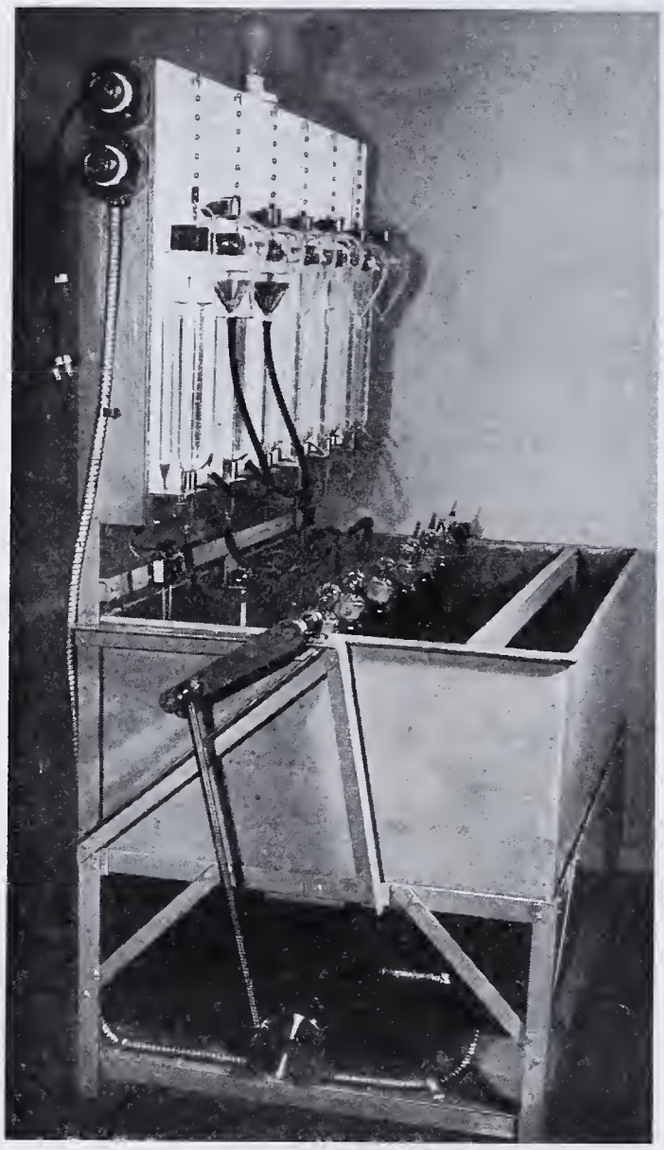


FIGURE 1. ORIGINAL FERMENTOMETER

SOLUTION A. One liter contains 180 grams of ammonium dihydrogen phosphate, 72 grams of diammonium hydrogen phosphate, and 0.2 gram of nicotinic acid. This solution should be sterilized by heating at 100° (Arnold sterilizer) for 30 minutes. If desired, the sterile buffer solution may be transferred to a sterile automatic buret and thereafter kept at room temperature. If such a buret is not available, the solution may be handled in the same manner as solution B.

SOLUTION B. One liter contains 200.0 grams of dextrose (anhydrous), 7.0 grams of magnesium sulfate ($MgSO_4.7H_2O$), 2.2 grams of potassium dihydrogen phosphate, 1.7 grams of potassium chloride, 0.5 gram of calcium chloride ($CaCl_2.2H_2O$), 0.01 gram of ferric chloride ($FeCl_3.6H_2O$), and 0.01 gram of manganese sulfate ($MnSO_4.4H_2O$). Solution B should be distributed in 500-ml. Erlenmeyer flasks, cotton-plugged, and sterilized at 100° on three successive days. Thereafter the flasks should be capped with foil or paper and then may be stored at room temperature. After a flask has been opened (for use) it is kept in a refrigerator at 4° C. until empty.

THIAMIN STANDARD SOLUTION. A quantity of crystalline thiamin chloride is dried over phosphorus pentoxide for 24 hours. A weighed amount is dissolved in distilled water to make a solution containing 0.1 mg. of thiamin chloride per ml. A subdilution is made to contain 1.0 microgram per ml. This solution is distributed in 200-ml. Erlenmeyer flasks (cotton-plugged), sterilized at 100° for 20 minutes on two successive days, and then treated like solution B.

YEAST SUSPENSION. Fleischmann's baker's yeast (10.0 grams) or its equivalent is weighed into a deep weighing scoop or a small beaker. The small foil-wrapped cakes of yeast or the high vitamin B_1 baker's yeast should not be used. Distilled water is added to the yeast, slowly at first, until a smooth, heavy cream has been produced and then enough to make the volume of the suspension 200 ml. This suspension is made just prior to each run.

Fermentometer

The apparatus required for the fermentation method consists of a water thermostat, a shaking device, and a series of gasometers. A combination of these three elements has been called a fermentometer. Figure 1 shows the earlier fermentometer employed until recently in the authors' laboratory. This instrument has provision for numerous other applications and is a relatively large piece of equipment. It has space for six gasometers and is satisfactory for research purposes but was considered too large and expensive for control analytical use. The rapidly expanding program of vitamin enrichment of foods will require routine thiamin assays by a large number of laboratories, many of them small or already crowded. Accordingly, a new and more compact fermentometer was designed (Figure 2) which occupies about 0.19 square meter (2 square feet) of table space and has provision for twelve gasometers. This should not be confused with the Warburg apparatus, which has been employed in an ultramicromodification of the fermentation method (*1*).

The shaking device, or cradle, of the new fermentometer is based on a parallelogram (Figure 3). Four hinges comprise the corners of the parallelogram. The cradle is supported by two additional hinges placed midway on the short sides of the parallelogram. By this arrangement a reciprocal motion applied to one of the corners shakes the reaction bottles which are fixed to the long sides of the parallelogram. By a proper alignment of the six pivotal points the twelve reaction bottles receive a uniform shaking motion. Details of the construction of the cradle are shown in Figure 4. The main supporting bars are of aluminum alloy. Clips of spring-tempered bronze hold the bottles in place.

The drive is a Boston Ratiomotor which produces 96 r. p. m. Temperature control of the water bath (at 30° ± 0.2° C.) is obtained by means of a Fenwal thermoregulator which controls a 200-watt heater. An auxiliary 500-watt heater for rapidly raising the temperature of the bath is manually controlled.

The gasometers, six on each side, are supported on detachable boards. They are of simple design and have a capacity of 260 ml. with graduations at each 2 ml. The gasometer fluid is a 10 per cent solution of calcium chloride colored blue with a small quantity of cupric chloride. The leveling pears are supported by means of bars of brass rack and short lengths of square brass tubing, and are held in place by a small tab at the bottom of the square tube. Since the gasometer boards are discrete units, it is possible to hang one of them on a wall if it is desired to operate the fermentometer from one side only. Connection between the reaction bottles and the gasometers may then be made by means of somewhat longer lengths of rubber tubing.

Procedure

The procedure employs volumes and concentrations designed for use with the new fermentometer—i. e., one half the quantities employed in the original method (*2*, *3*). It uses 0.5 gram of yeast in 50 ml. of solution, compared with 1.0 gram of yeast in 100 ml. of solution in the original method.

The apparatus and technique may be considered to be satisfactory when duplicate fermentation measurements, made simultaneously, differ by less than 2 per cent. Uniform temperature, uniform shaking, freedom from leaks, and accurately calibrated gasometers are necessary if this criterion is to be met. When making these preliminary tests it is also desirable to check the rate of shaking, making certain that the rate is not so slow that the yeast settles out of suspension, thereby causing a supersaturation of carbon dioxide and consequent irregular gas evolution. On the other hand the shaking rate should not be so fast that the reaction mixture is violently thrown against the stopper.

As a further preliminary test a control run similar to that described in Table I should be made. The difference in the 3-hour gas total between 1 and 2 micrograms of thiamin should not be less than 20 ml. and preferably closer to 30. If the increase in fermentation rate is satisfactory, the apparatus and solutions are ready for use. The fermentation activity

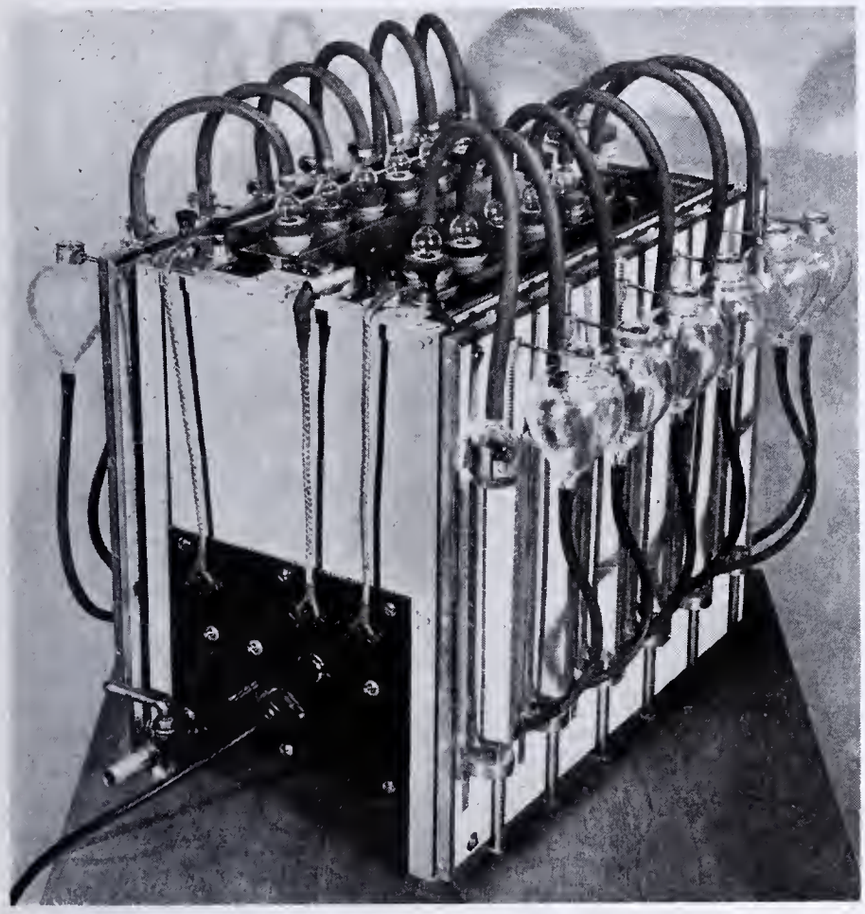


FIGURE 2. OBLIQUE VIEW OF NEW FERMENTOMETER

of an unknown is estimated on the basis of a linearity of response between the stimulations produced by 1 and 2 micrograms of thiamin. If the fermentation activity of a large aliquot of the unknown is still below 1 microgram, as frequently happens after sulfite treatment, one may add 1 microgram of thiamin to the unknown and thus bring it into the proper range.

TYPICAL ASSAY. Table II describes a typical assay run in which two complete determinations were made with the use of six gasometers. It is apparent that the use of twelve gasometers permits the assay of five substances at one time.

In each of six reaction bottles 2.5 ml. of solution A and 7.5 ml. of solution B were placed, followed by the addition of the vitamin-containing solutions—i. e., to No. 1, 0.8 ml. of a 1 per cent suspension of Hi-B_1 baker's yeast (treated as described in Table II); to No. 2, 4.0 ml. of sulfite-treated yeast plus 1.0 ml. of a solution containing 1.0 μg. of thiamin; to No. 3, 5 ml. of a wheat germ suspension; to No. 4, 20 ml. of sulfite-treated wheat germ suspension plus 1 μg. of thiamin; to No. 5, 1 g. of thiamin; to No. 6, 2 μg. of thiamin. Distilled water was then added to each bottle to make the volume to 40 ml. Noting the time, 10 ml. of a yeast suspension containing 0.5 gram of moist compressed yeast were added to each bottle as rapidly as possible. A free-flowing pipet is desirable for this purpose.

With a minimum of wasted time all the reaction bottles were placed in the fermentometer and connected to the gasometers. Shaking was started and after 2 to 3 minutes the initial reading of the gas volumes was made. At the end of 3 hours from the time of adding the yeast suspension, the final reading of the gas volumes was made. The difference represents the gas evolved and Table II shows how the fermentation activity of each test is estimated. The evaluation of these results in terms of the thiamin content of the unknowns is shown in Table III.

TABLE I. CONTROL RUN ON FERMENTOMETER HAVING SIX 260-ML. GASOMETERS

(Solution A, buffer and nicotinic acid, 2.5 ml. Solution B, sugar and salts, 7.5 ml. Yeast suspension, 10 ml.)

Bottle No.	Distilled Water	Thiamin Solution, 1 μg./Ml.	Gas Evolved in 3 Hours
	Ml.	*Ml.*	*Ml.*
1	30	0	120
2	29	1.0	155
3	28	2.0	184
4	30	0	118
5	29	1.0	154
6	28	2.0	184

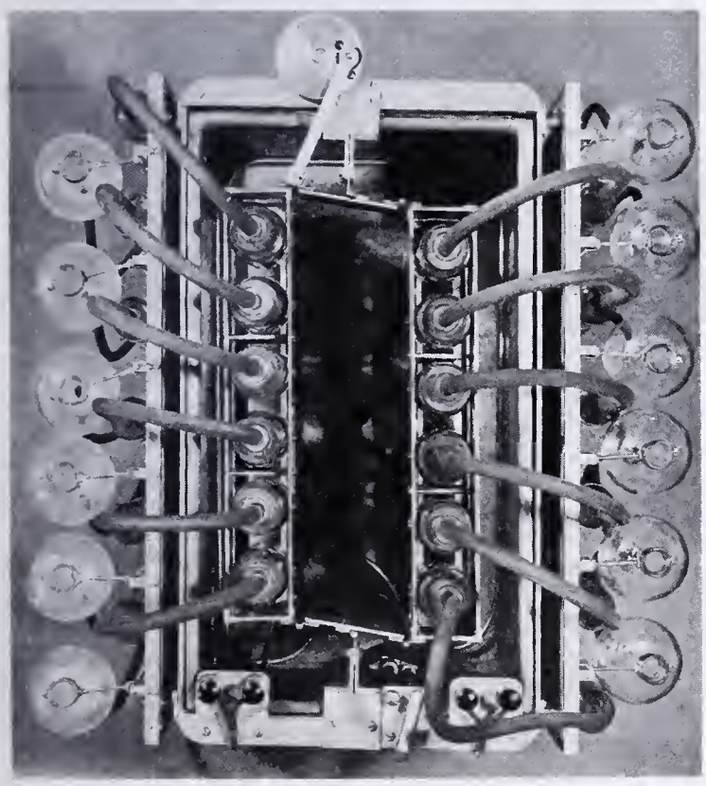


FIGURE 3. TOP VIEW OF FERMENTOMETER

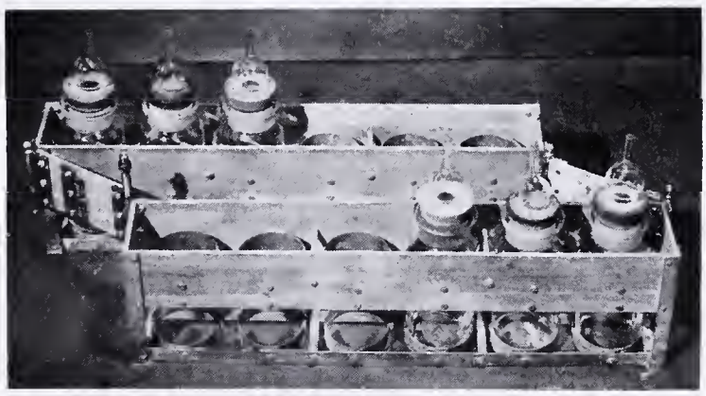


FIGURE 4. CRADLE OF FERMENTOMETER

TABLE II. TYPICAL ASSAY RUN WITH SIX GASOMETERS

(Analysis of two unknowns)

Bottle No.	Addition to Control Reaction Mixture		Gas Evolved in 3 Hours	Fermentation Activity per Test
	Mg.		*Ml.*	*μg. of thiamin*
1	8	Yeast (baker's Hi-B_1)[a]	192	1.75
2	40	Yeast (baker's Hi-B_1)[a], SO_2-treated, plus 1 μg. of thiamin[b]	180	1.32
3	50	Wheat germ[c]	186	1.54
4	200	Wheat germ[c], SO_2-treated[d], plus 1 μg. of thiamin	182	1.39
	μg.			
5	1	Thiamin	171	1.00
6	2	Thiamin	199	2.00

[a] 0.8 ml. of 1% suspension of Hi-B_1 baker's yeast heated at 100°, flowing steam, for 20 minutes at reaction blue to Congo red, cooled, and neutralized.
[b] 4.0 ml. = 40 mg. of sulfite-treated Hi-B_1 baker's yeast. One gram of yeast suspended in 20 ml. of H_2O, 0.2 gram of $Na_2SO_3.7H_2O$ added, reaction adjusted to pH 5.2, and then heated at 100° for 20 minutes. Cooled, excess sulfite destroyed, neutralized, and made to 100-ml. volume.
[c] 5 ml. of 1% suspension treated as in [a].
[d] 20 ml. = 200 mg. of sulfite-treated wheat germ treated as in [b].

TABLE III. EVALUATION OF RESULTS OF TEST DESCRIBED IN TABLE II

	Hi-B_1 Baker's Yeast	Wheat Germ
	Micrograms per gram	
Total fermentation activity	219	30.8
Residual fermentation activity after sulfite treatment	8	1.95
True thiamin content	211	28.85

TABLE IV. INFLUENCE OF TIME AND TEMPERATURE ON SULFITE CLEAVAGE OF THIAMIN

(At pH 5.2, concentration of sulfite 0.25%)

Temperature	Extent of Inactivation	
	30 minutes	120 minutes
° *C.*	%	%
37.5	64	96
55.0	92	98.5
100.0	99	99.2

Influence of Various Factors on Sulfite Cleavage

In order to establish the conditions most suitable for the quantitative cleavage of thiamin, a series of studies was made in which the effects of variations in pH, time, temperature, and sulfite concentration on the reaction were examined.

pH. The effect of pH on the extent of cleavage was studied in the presence of a small quantity of citric acid.

To each of a series of flasks were added 0.2 gram of sodium sulfite ($Na_2SO_3.7H_2O$), 0.1 gram of citric acid, 19 ml. of water, and sufficient 1 *N* sodium hydroxide or sulfuric acid to adjust the pH to the required value. Finally, 1 mg. of freshly dissolved thiamin chloride (contained in 1.0 ml. of solution) was added to each flask. The flasks were then heated at 100° C. for 30 minutes, cooled, and treated as described—i. e., sulfite destroyed by peroxide, neutralized, and tested for residual fermentation activity. The results are shown in Figure 5, where the extent of inactivation in per cent has been plotted as a function of pH. It is apparent that the optimum pH for inactivation (99 per cent) is 5.0 to 6.0.

TIME AND TEMPERATURE. A series of experiments similar to those described above was made, except that the pH was uniform (at 5.2) and the flasks were heated for various periods of time at several temperatures. The results, given in Table IV, show that 30 minutes and 100° C. are sufficient to ensure 99 per cent inactivation of thiamin.

SULFITE CONCENTRATION. A study was made of the inactivation of pure thiamin solutions and of the thiamin contained in a wheat by-product. Table V shows that the inactivation of pure thiamin is 99.3 per cent complete at a sulfite concentration of 0.25 per cent. In the case of the thiamin contained in a wheat by-product the results shown in Table V indicate that the extent of inactivation is not increased by tripling the sulfite concentration.

Application of Sulfite Cleavage to Miscellaneous Substances

The fermentation method, embodying sulfite cleavage, has been applied to a variety of substances with results shown in Table VI. These assays were made as described above.

None of the various materials which have thus far been subjected to sulfite treatment has presented any particular

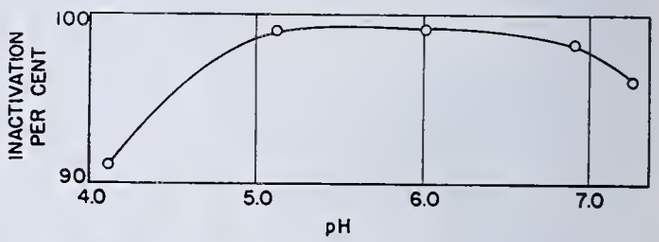


FIGURE 5. CURVE OF pH *vs.* PER CENT INACTIVATION

TABLE V. INFLUENCE OF SULFITE CONCENTRATION ON CLEAVAGE OF THIAMIN

(At pH 5.2, heated for 30 minutes at 100° C.)

	Sulfite Concentration	Extent of Inactivation
	%	%
Pure thiamin	0.25	99.3
	0.50	99.1
	1.00	99.2
Wheat by-product	0.8	94.3
	1.6	94.6
	2.4	94.0

TABLE VI. SULFITE TREATMENT OF MISCELLANEOUS SUBSTANCES[a]

Substance	Amount Treated	Volume during Treatment[b]	Extent of Inactivation	Thiamin Content
	Grams	*Ml.*	%	*μg./g.*
Wheat A	1	25	92	5.5
Wheat B	2	20	96	6.05
Wheat germ	1	20	94	29.0
White bread (Hi-B_1, dry)	2	20	92	4.62
White bread (enriched, dry)	2	20	90	4.62
Baker's yeast (Hi-B_1)	0.2	20	96	211
Dry yeast (90 U.)	0.2	20	87	290.0
Dry yeast (200 U.)	0.2	20	95	700.0
Vitamin extract A	0.5	20	77	615
Vitamin extract B	1.0	50	18	30
Human liver A	3.0	20	76	0.83
Human liver B	3.0	20	82	0.9
Human heart muscle	3.0	20	90	2.2
Human kidney cortex	3.0	20	85	1.1
Human skeletal muscle	3.0	20	91	0.4
	Ml.			*μg./ml.*
Fresh milk (pasteurized)	10.0	20	93	0.38
	Grams			*μg./g.*
Dry milk	2.0	20	92	5.6
	Ml.			*μg./ml.*
Evaporated milk A	10	20	73	0.89
Evaporated milk B	10	20	73	0.89
Orange juice	10	20	94	0.86
Human urine $^3/_{24}$[c]	10	20	40	0.212
Human urine $^3/_{25}$	10	20	36	0.175
Human urine $^3/_{26}$	10	20	41	0.200

[a] Sulfite treatment at pH 5.2, 30 minutes at 100° C.
[b] Sulfite in each case 0.2 grain of $Na_2SO_3.7H_2O$.
[c] 24-hour urine excretion of normal subject on average diet made to 2000-ml. volume.

difficulty. However, sulfite treatment of natural materials seldom results in 99 per cent inactivation of the fermentation stimulatory property, as was obtained with pure thiamin. This must be explained either by the hydrolytic cleavage of thiamin during the reaction—i. e., production of an aminopyrimidine not inactivated by sulfite—or by the previous existence of hydrolysis products in the material itself. In the majority of cases 90 per cent or more of the fermentation activity is removed by sulfite, leaving a "sulfite blank" of 10 per cent or less. Since the experimental error involved in vitamin determinations by animals is seldom less than 10 per cent, and frequently is much more, it is rather difficult to reach a satisfactory conclusion regarding the significance of a small blank. This problem is under investigation.

The results shown in Table VI are in accord with generally held ideas concerning the vitamin B_1 content of the materials assayed. The values obtained for the cereals and yeasts are normal, but the two vitamin extracts are noteworthy because they show, by the lowered extent of inactivation, that during their preparation they had been subjected to conditions which were conducive to hydrolytic cleavage—i. e., elevated pH or temperature or both. The results for evaporated milk are indicative of the same type of treatment. The results with human tissues are merely given as examples of technique—the samples were obtained through the cooperation of J. W. Ferrebee of the College of Physicians and Surgeons, and a discussion of the results awaits the completion of this work. The results with urine are in accord with previous observations (*4*, *5*) that urine contains an interfering aminopyrimidine.

Acknowledgment

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Determination of Choline

A Photometric Modification of Beattie's Method

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THE present wide industrial production and use of vegetable phosphatides have created considerable interest in methods for the determination of lecithin. Since in nature lecithin is always accompanied by other phosphatides, methods for its determination are based on the fact that it yields choline upon hydrolysis.

In general use are the platinum chloride method described by Levene (*4*), the periodide method of Roman (*5*), the gravimetric method of Kapfhammer and Bischoff (*3*), and the colorimetric method of Beattie (*1*). The last two depend upon the precipitation of choline as the reineckate. The platinum chloride method cannot be used if the original material contains cephalin, since the aminoethanol–platinum chloride salt cannot be separated quantitatively from the choline–platinum chloride salt. The periodide method is rather difficult to use, since it involves working with a very unstable compound. It has also been found that this method gives low results when used as described by the author. The gravimetric method of Kapfhammer and Bischoff is long and tedious and involves the drying of a rather unstable compound to constant weight, while the colorimetric method of Beattie is unsatisfactory because of the difficulty of making accurate measurements with a visual colorimeter.

It is apparent from the work of Kapfhammer and Bischoff and of Beattie, that the precipitation of choline as the reineckate is quantitative when carried out under proper conditions. Therefore, the measurement of this choline reineckate should provide an accurate method for the determination of choline. It seemed probable that this measurement could be made with speed and accuracy by use of a photoelectric photometer. Accordingly, an investigation, based on the measurement of the light transmittancy of a solution of the red choline reineckate in the KWSZ photoelectric photometer, was carried out. This resulted in the establishment of an accurate method for the measurement of choline reineckate, which permits the determination of choline with an error of 2 per cent or less, over a concentration range of 0.3 to 6.5 mg. per ml.

Experimental

The KWSZ photoelectric photometer (*6*) was used to measure the light transmittancy of the choline reineckate solution.

By use of a visual spectrophotometer it was found that an acetone solution of choline reineckate exhibits maximum light absorption in the range 500 to 550 mμ. Therefore, in the photoelectric photometer a combination of filters transmitting in the range 400 to 660 mμ with the maximum from 500 to 550 mμ was used: Corning blue No. 428 and Corning amber No. 346. In addition Wilkens-Anderson filters No. 2776-FC were used to replace the usual 5 per cent copper sulfate solutions. The light source was a 50-watt projection bulb.

In order to establish a choline concentration curve for reference, it was necessary to obtain a solution of known choline content. A quantity of choline chloride (Eastman Kodak Co.), which is very hygroscopic, was recrystallized four times from absolute ethanol, introduced into a weighed bulb, and dried at 84° C. *in vacuo* for 48 hours, after which the bulb was sealed and weighed. The sample was then dissolved in water and adjusted to volume. To establish the purity of the choline chloride, the solution was analyzed for nitrogen by the Kjeldahl method and for choline by the Roman and the platinum chloride methods.

The results given in Table I show that the choline calculated from the nitrogen values and that determined by the platinum chloride method agree very closely with the amount of choline known to be present. The results obtained by the Roman method are about 11 per cent lower. From these data it may be assumed that the choline chloride is really of high purity and that the values obtained by the Roman method are too low by approximately 10 per cent. This choline chloride solution was used for preparation of the reference curve.

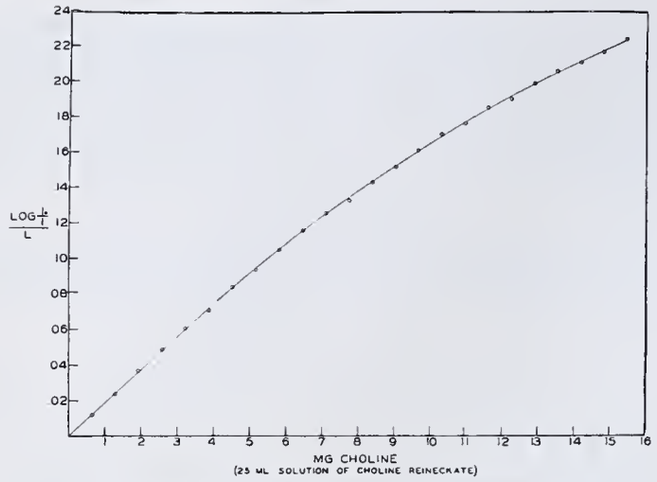

FIGURE 1. PURE CHOLINE CHLORIDE

TABLE I. PURITY OF CHOLINE CHLORIDE

Method	Choline Chloride Weighed	Choline Chloride Found	Recovery
	Mg./ml.	*Mg./ml.*	%
Roman	1.50	1.33	
	1.50	1.33	
	1.50	1.33	88.66
		Av. 1.33	
Platinum chloride	1.50	1.48	
	1.50	1.49	
	1.50	1.50	99.33
		Av. 1.49	
Kjeldahl nitrogen	1.50	1.48	
	1.50	1.49	
	1.50	1.50	99.33
		Av. 1.49	

Preparation of Choline Reineckate Solution

REAGENT. Ammonium reineckate, $NH_4[Cr(NH_3)_2(SCN)_4].H_2O$, may be prepared according to the method of Dakin (*2*) or purchased as the pure salt from biological supply houses. It was stored at low temperature and a fresh, saturated, aqueous solution was prepared each day by stirring a quantity of the salt in water for 15 minutes and then filtering off the undissolved portion.

This saturated solution contained from 25 to 30 mg. per ml., which theoretically should be capable of precipitating about 10 mg. of choline. However, a large excess of reagent was always used.

Preliminary work substantiated Beattie's statement that precipitation of choline reineckate is complete in 10 minutes at room temperature. It also showed that no higher results are obtained by allowing the precipitate to stand overnight in the refrigerator, as suggested by Kapfhammer and Bischoff, provided the concentration of choline is above 0.3 mg. per ml.

PROCEDURE. The procedure used for the preparation of the choline reineckate solution was the same as that described by Beattie (*1*). After addition of the saturated ammonium reineckate solution to the sample of choline, the mixture was allowed to stand at room temperature for at least 10 minutes. The precipitate was then separated by filtering through a No. 4 Jena sintered-glass crucible and was washed until the excess reagent was removed. Usually washing twice with small portions of ice-cold water and once with a small portion of absolute ethanol was sufficient. It was then dissolved and washed through the crucible into a 25-ml. volumetric flask with acetone and adjusted to volume. A portion of this solution was transferred to a 2.5-cm. cell and its transmittancy was determined in the KWSZ photoelectric photometer.

Choline-Concentration Reference Curve

In order to obtain the data necessary for establishing the basic curve for choline concentrations, the transmittancies of choline reineckate solutions, prepared from varying amounts of the known choline chloride solution, were measured. The total choline present in these samples varied from 0.64 to 15.5 mg. and measurements were made at regular intervals of 0.64 mg. throughout this range. The choline concentration of these samples was 1.29 mg. per ml. in all cases. The data obtained from this series of samples are shown numerically in Table II and graphically in Figure 1, where the concentration of choline in milligrams is plotted against the value $\log \frac{I_0}{I}/L$, which is the negative log of the transmittancy of the solution divided by the length of the cell in centimeters. The data in Table II are averages of duplicate or triplicate determinations. The deviation from the average is less than 2 per cent in most cases and dependable results can be obtained throughout this concentration range, which represents transmittancy readings from approximately 87 to 25 per cent.

When these averages are plotted as shown in Figure 1, a straight line does not result, but a curve can be drawn which approaches all points closely. This curve was used in the calculation of the choline content of other samples, such as the hydrolyzates of soybean phosphatides.

Effect of Choline Concentration

In order to determine the effect of concentration on the accuracy of the method, samples of choline chloride solutions having choline concentrations of 0.129, 0.258, 0.322, and 6.45 mg. per ml. were analyzed (Table III).

These values show that low results are obtained when the choline concentration is below 0.3 mg. per ml., even though the total amount present is relatively large. There is also some tendency for the results to be slightly high when the choline concentration is as great as 6.5 mg. per ml. Therefore, there is a rather wide range in which the choline concentration has no appreciable effect on the accuracy of the method and samples of practically all materials can be prepared which will fall within this range.

TABLE II. DATA FOR CHOLINE-CONCENTRATION REFERENCE CURVE

Choline Taken	I_0	I	$\log \frac{I_0}{I} / 2.5$	Maximum Deviation from Average
Mg.	%	%		%
0.64	93.0	86.9	0.0118	±0.0
1.29	93.1	81.3	0.0235	±3.4
1.93	93.3	75.6	0.0366	±0.3
2.58	93.2	70.5	0.0485	±0.2
3.22	93.3	66.0	0.0602	±0.7
3.87	93.1	62.1	0.0703	±1.3
4.51	93.0	57.5	0.0835	±0.6
5.16	93.6	54.7	0.0933	±0.2
5.80	93.3	51.2	0.1042	±1.8
6.44	93.2	48.0	0.1153	±0.4
7.09	93.2	45.4	0.1249	±0.6
7.73	93.0	43.4	0.1324	±0.3
8.38	93.2	41.0	0.1426	±0.1
9.02	93.1	38.9	0.1516	±0.3
9.67	93.6	37.1	0.1608	±0.5
10.31	93.0	34.9	0.1703	±1.2
10.95	93.2	33.8	0.1762	±0.9
11.60	93.7	32.2	0.1855	±0.2
12.24	93.2	31.1	0.1908	±0.7
12.89	93.1	29.6	0.1990	±0.5
13.53	93.7	28.6	0.2061	±0.2
14.18	93.6	27.7	0.2115	±0.7
14.82	93.3	26.7	0.2174	±1.2
15.47	93.8	25.7	0.2249	±0.3

TABLE III. EFFECT OF CHOLINE CONCENTRATION ON ACCURACY OF PHOTOMETRIC METHOD

Choline in Sample		I_0	I	$\log \frac{I_0}{I} / 2.5$	Choline Found		Average	Choline Recovered
Mg.	*Mg./ml.*	%	%		*Mg.*	*Mg./ml.*	*Mg./ml.*	%
2.58	0.129	94.0	72.7	0.0446	2.40	0.120		
		94.5	73.4	0.0439	2.37	0.119		
6.45	0.129	93.4	50.6	0.1064	5.90	0.118	0.119	92.2
2.58	0.258	95.5	73.3	0.0460	2.48	0.248		
		95.0	72.9	0.0460	2.48	0.248		
6.45	0.258	93.7	49.1	0.1122	6.26	0.250		
		93.2	49.1	0.1113	6.21	0.248	0.248	96.1
6.45	0.322	93.9	48.3	0.1155	6.47	0.324		
		93.8	48.9	0.1132	6.33	0.317	0.320	99.7
6.45	6.45	93.0	47.8	0.1156	6.47	6.47		
		92.9	47.0	0.1184	6.66	6.66		
		92.6	47.2	0.1171	6.57	6.57	6.57	101.8

Table IV. Stability of Choline Reineckate in Acetone

Choline in Sample	Elapsed Time	Transmittancy, I
Mg.	*Min.*	%
7.5	1	44.3
	5	44.3
	10	44.2
	15	44.3
	20	44.2
	25	44.3
	30	44.2

Table V. Choline Content of Hydrolyzates of Soybean Phosphatides

Method	Sample Hydrolyzate		I_0	I	$\text{Log}\frac{I_0}{I}$ / 2.5	Choline Found		Choline Recovered by Roman Method
		Ml.	%	%		*Mg.*	*Mg./ml.*	%
Photometric	1	5	92.8	58.9	0.0790	4.26	0.852	
	1	5	92.6	59.1	0.0780	4.22	0.844	..
						Av.	0.848	
Roman	1	1	..	..	....	0.763	0.763	
	1	1	..	..	....	0.769	0.769	90.3
						Av.	0.766	
Photometric	2	5	94.6	56.3	0.0902	4.92	0.985	
	2	5	93.9	56.0	0.0898	4.89	0.978	
	2	5	93.8	55.6	0.0908	4.95	0.990	..
						Av.	0.984	
Roman	2	1	..	..	....	0.863	0.863	
	2	1	..	..	....	0.863	0.863	87.6
						Av.	0.863	

Stability of Choline Reineckate in Acetone

A choline reineckate solution was prepared from a sample containing 7.5 mg. of choline. The transmittancy of this solution was determined as soon as possible after its preparation. It was then allowed to remain in the photometer for 30 minutes and readings were taken at 5-minute intervals throughout that period in order to determine whether or not the color faded.

The results (Table IV) show that the choline reineckate is perfectly stable for at least 30 minutes. This is a much longer time than that actually necessary for the measurement of the transmittancy of a sample.

Application of Photometric Method to Determination of Choline in Lecithin

Two different samples of soybean phosphatides were hydrolyzed for 15 hours with 5 N hydrochloric acid. The fatty acids were separated by extraction with ether and the aqueous residues were neutralized with sodium hydroxide and adjusted to volume. Choline was determined on both solutions by the photometric and Roman methods. Known amounts of choline chloride were then added to these solutions and the total choline was determined by the photometric method.

Hydrolyzate 1 was prepared from a sample of purified lecithin which was isolated from crude soybean phosphatides. This material was practically free from impurities and contained sufficient choline to indicate a lecithin content of more than 90 per cent.

Hydrolyzate 2 was obtained by hydrolyzing a sample of commercial soybean phosphatides. After removal of the fatty acids, the aqueous solution was dark in color and contained many impurities.

The results, which are given in Tables V and VI, indicate that the photometric method can be successfully applied to the determination of choline in such relatively crude materials as commercial soybean phosphatides, since results were easily reproduced and the added choline was recovered quantitatively. The values obtained by the Roman method are lower than those obtained by the photometric method. In the case of hydrolyzate 1 the result obtained by the Roman method was approximately 90 per cent of that given by the photometric method, while in the case of hydrolyzate 2 the value determined by the Roman method was about 88 per cent of that found by the photometric method.

Discussion

The photometric method for the determination of choline, described above, is very rapid and accurate over a fairly wide range of choline concentrations. The data plotted in Figure 1 are for samples with a choline content ranging from 0.64 to 15.5 mg. In the case of the last sample the transmittancy is about 25 per cent with the filters and cells used. An error of less than 2 per cent can be attained throughout this range, with the possible exception of samples having very low choline content.

Even with large samples, the choline concentration should not be less than 0.3 mg. per ml. if the conditions of precipitation described above are used. Apparently, if the concentration is much above 5 to 6 mg. per ml. slightly high results may be expected, probably because of some occlusion of the reagent by the crystalline precipitate.

The recovery of choline from pure choline chloride solutions by the photometric method is quantitative, but that obtained by the Roman method is 10 to 12 per cent too low. The same ratio holds true in the analysis of lecithin hydrolyzates. This fact, together with the fact that the photometric method gives quantitative recovery of known amounts of choline added to lecithin hydrolyzates, indicates that the results obtained by the photometric method represent the real choline content of the sample.

The photometric method can be used on hydrolyzates containing both choline and aminoethanol, since the latter does not form a precipitate with ammonium reineckate and experience shows that it does not interfere with the accuracy of the choline determination.

The colored choline reineckate in acetone solution is stable for at least 30 minutes. This affords ample time for measuring its transmittancy, since the operation is usually completed within 5 minutes after the preparation of the solution.

Table VI. Recovery of Choline Added to Hydrolyzates

Hydrolyzate	Choline: In hydrolyzate	Choline: Added	Choline: Total	I_0	I	$\text{Log}\frac{I_0}{I}$ / 2.5	Choline Found	Recovery
	Mg.	*Mg.*	*Mg.*	%	%		*Mg.*	%
1	1.70	1.29	2.99	93.1	67.4	0.0561	3.02	
1	1.70	1.29	2.99	92.5	67.4	0.0550	2.96	100.0
						Av.	2.99	
2	4.92	2.58	7.50	93.8	44.8	0.1284	7.33	
2	4.92	2.58	7.50	93.8	44.3	0.1303	7.46	98.7
						Av.	7.40	

Summary

By a modification of the Beattie colorimetric method for the determination of choline, based on the measurement of the transmittancy of acetone solutions of choline reineckate by means of a photoelectric photometer, quantities of choline varying from 0.6 to 16.0 mg., in a concentration range from 0.3 to 6.5 mg. per ml., can be estimated with a maximum error of 2 per cent.

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The U. S. Regional Soybean Industrial Products Laboratory is a cooperative organization participated in by the Bureaus of Agricultural Chemistry and Engineering and of Plant Industry of the United States Department of Agriculture and the Agricultural Experiment Stations of the North Central States of Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin.

Determination of Carbonyl Groups in Bile Preparations

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EXPERIMENTAL evidence has been obtained recently which suggests that carbonyl groups determine the choleretic activity of bile salts used in gall bladder therapy (*1*). Various methods have been presented for the quantitative determination of these groups in different types of chemical compounds but few have been applied to the bile constituents. This article presents a comparison of the usefulness of two methods, both of which are based on the reaction between the keto groups and hydroxylamine with the resultant oxime formation.

Table I. Comparative Analyses

Preparation	Gravimetric Method C=O, %	Gravimetric Method Standard deviation	Gravimetric Method No. of determinations	Titrimetric Method, Indicator C=O, %	Titrimetric Method, Indicator Standard deviation	Titrimetric Method, Indicator No. of determinations	Titrimetric Method, Glass Electrode C=O, %	Titrimetric Method, Glass Electrode Standard deviation	Titrimetric Method, Glass Electrode No. of determinations
Triketocholanic acid[a]	21.7	1.87	12	20.22	0.146	7	19.92	0.335	4
Theoretical	20.9								
Ox bile[b]	1.6	2.02	6	0	...	6	0	...	
Turtle bile[b]	−0.5	0.9	6	[c]	...	..	0	...	
Pig bile[b]	4.0	1.66	7	2.24	0.344	6	0	...	2
Sodium pyruvate	25.88	3.98	9	...	...	..	...	...	..
Theoretical	25.45								
Acetone	....	..	..	47.38	0.057	6	45.99	1.92	2
Theoretical				48.23					

[a] Decholin, Riedel-de Haen, Inc., New York, N. Y.
[b] Burroughs Wellcome & Co. (U. S. A.) Inc., New York, N. Y.
[c] Pigment masks indicator.

The titration method of Bryant and Smith (*3*), which determines the hydrochloric acid liberated from the amine salt after the oxime formation with pure aldehydes and ketones, was found satisfactory for bile preparations which contained no pigments to mask the indicator employed. This limitation was overcome by electrometric titration. Gustus (unpublished data reported by Ivy *et al.*, *1*) determined keto groups in bile preparations by a gravimetric method originally indicated by Hirschel and Verhoeff (*4*) which is based on the recovery of excess hydroxylamine. No details were given but the procedure was described as lengthy and tedious. The method herein discussed also entails the reaction with diacetylmonoxime to form dimethylglyoxime and its subsequent precipitation as the nickel salt of dimethylglyoxime. Under the conditions given below fairly accurate values for carbonyl groups in bile preparations can be obtained in a reasonable length of time.

Reagents for Gravimetric Method

Hydroxylamine hydrochloride, anhydrous C. P., 0.6513 gram in 25 cc. of aqueous solution.

Sodium carbonate, anhydrous C. P., 13.25 grams in 100 cc. of aqueous solution.

Nickel acetate tetrahydrate, C. P., 4.665 grams in 25 cc. of aqueous solution.

Diacetylmonoxime, Eastman Kodak Co., 3.79 grams in 25 cc. of aqueous solution (heat).

Ethyl alcohol, 95 per cent.

Ammonium hydroxide, concentrated and dilute.

Acetic acid, concentrated and dilute.

Procedure

The first reaction is carried out in 15 × 125 mm. Pyrex test tubes. Since the conditions of each run determine the recovery of hydroxylamine, two controls of the amine alone are carried along simultaneously with the other ten or more samples in a single batch determination. These controls contain 2 cc. or 0.0521 gram of the amine hydrochloride. Larger quantities should be added at the start of the reaction when there are large amounts of keto groups present in the unknowns, so that approximately the same quantity of the nickel precipitate may be obtained in each reaction. Therefore, in testing recoveries with 0.05-gram samples of triketocholanic acid (made by dissolving 0.5 gram of pure triketocholanic acid in 1 cc. of sodium carbonate solution and diluting to 10 cc.; 1 cc. contains the 0.05-gram sample needed), 3 cc. of the amine solution (0.0781 gram or 9 mole equivalents to react with the 3 mole equivalents of the keto groups) are pipetted into the test tubes.

The unknowns, which in the following cases are low in keto groups, are similar to the controls except for the addition of 0.05 gram of the bile preparation. Each sample is run in duplicate Water is now added to some to equalize the volumes, 0.5 cc. of sodium carbonate solution is added to each, and the tubes are stoppered tightly with rubber stoppers. After a thorough mixing of the contents the tubes are heated in water at 80° C. on a steam bath for 2 hours. Allowing the mixture to stand at room temperature overnight and then heating for a shorter time is equally effective. The oxime of the cholanic acid usually precipitates as the reaction proceeds. After cooling, the tubes are opened, stoppers washed, and the contents acidified with concentrated acetic acid. (Alkacid test ribbon, Fisher Scientific Co., Pittsburgh, Penna., is very convenient.) Ethyl alcohol is used to prevent foaming. When precipitates are present they are filtered off and washed three times with small amounts of dilute acetic acid. The solutions of all are transferred with washing to 30-cc. beakers and should at this point approximate 10 cc. in volume. They are made distinctly alkaline with concentrated ammonium hydroxide, and 1 cc. of diacetylmonoxime (0.1516 gram or 12 mole equivalents) is added to each. The color should be slightly yellow; if not, more ammonium hydroxide should be added. The solutions, covered with watch glasses, are now heated for 2 hours on a steam bath at 80° C. Crystals of dimethylglyoxime which appear at this stage and increase in amount if the solutions are allowed to cool are redissolved by adding 5 cc. of alcohol to each sample.

Into each beaker is pipetted 0.75 cc. of the nickel acetate solution (0.14 gram or 9 mole equivalents) and the solutions are digested on the steam bath for 2 hours longer without covers but with frequent stirring. After cooling or standing overnight each solution is transferred to a tared 14-cc. conical, heavy-walled Pyrex centrifuge tube by means of a rubber policeman, with addition of ethyl alcohol (to prevent creeping) and dilute ammonium hydroxide. After centrifuging, the red precipitate is suspended in 6 cc. of dilute ammonium hydroxide, centrifuged again, and dried to constant weight at 110° C. A very thin layer of alcohol in the tubes permits manipulation without appreciable loss of material.

Calculations

The weight of the nickel dimethylglyoxime multiplied by the factor 0.4812 equals the equivalent weight of hydroxylamine hydrochloride. The recovery factor, usually over 90 per cent, is determined by dividing the weight of the recovered

hydroxylamine hydrochloride in the controls by the original amount added. The weights of the nickel precipitates of the unknowns are divided by this factor to ascertain the actual recovery and likewise converted to the equivalent weight of hydroxylamine hydrochloride. These differences from the original amounts indicate the amounts of amine that reacted with the keto groups and when multiplied by 0.4029 equal the weights of these groups. These weights divided by the sample weights give the per cent value which is converted to per cent of triketocholanic acid when multiplied by 4.79.

Discussion

The titrimetric method investigated, using an indicator, has limited use in determining carbonyl groups in the presence of bile pigments. At times the formation of a precipitate obscures the end point. With the gravimetric method the use of a recovery factor to account for incomplete reactions and losses due to solubility was necessary. These methods may also be applicable to the various keto steroids related to the hormone field. In accordance with existing data in the literature (*2*) it was found that of the natural sources studied, pig bile contains the largest number of carbonyl groups (Table I).

Of the two methods studied for the determination of carbonyl groups in bile preparations, the gravimetric procedure based on the recovery of excess hydroxylamine was found to be the more consistent. However, when pigments were absent the titrimetric procedure was preferred because of its time-saving advantages.

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Volumetric Determination of Bismuth as Caffeine Tetraiodobismuthate (III)

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A NUMBER of organic bases are known to form insoluble iodometallic compounds from which the metal may be determined by titration of the iodine from the complex ion. Several determinations of bismuth have been made by this method. Hexamethyldiiododiaminoisopropyl alcohol (*1*), naphthoquinoline (*2, 8*), 8-hydroxyquinoline (*2, 4, 10, 12*), 8-nitroquinoline (*3*), quinine (*5*), quinoline (*6*), quinaldine (*7*), dithiocyanato diethylenediamine cobaltithiocyanate (*13*), triethylenediamine cobaltichloride (*14*), and antipyrine methyleneamine (*15*) form complex compounds with bismuth and iodine and methods using these bases have been reported for the determination of bismuth.

Preliminary tests with caffeine showed that it may be satisfactorily used to determine bismuth. An insoluble compound of caffeine, bismuth, and iodine is formed containing the atoms of bismuth and iodine in the ratio of 1 to 4. It was also found that mercury formed no insoluble compound and hence it was possible to devise a method for the determination of bismuth in the presence of mercury. So far as it has been possible to determine, similar methods for bismuth previously reported have all required the absence of mercury.

Solutions

Caffeine Sulfate Solution. Dissolve 133 grams of caffeine in 570 ml. of 6 *N* sulfuric acid and dilute to 1 liter; 30 ml. of this solution contain 4 grams of caffeine.

Caffeine Sulfate Wash Solution. Dissolve 1.5 grams of caffeine in 6 ml. of 6 *N* sulfuric acid and dilute to about 900 ml. Then add 1 gram of pure potassium iodide and bring the volume to 1 liter.

Caffeine Nitrate Solution (for use in the presence of barium ion). Dissolve 133 grams of caffeine in 540 ml. of 6 *N* nitric acid and dilute to 1 liter; 30 ml. of this solution contain 4 grams of caffeine.

Bismuth Nitrate Solution. Dissolve 2.79 grams of C. P. bismuth nitrate pentahydrate in 10 ml. of 16 *N* nitric acid and dilute to 1 liter. This was standardized with concordant results by the phosphate and carbonate methods (25 ml. of this solution contain approximately 0.03 gram of bismuth).

Potassium Iodate Solution. Dissolve exactly 2.0480 grams of C. P. potassium iodate to make 1 liter of solution (1.00 ml. of this solution is equivalent to 0.0010 gram of bismuth). This solution was stable in accordance with studies by Jamieson (*9*).

Potassium Cyanide Solution. Dissolve 32.5 grams of C. P. potassium cyanide to make 1 liter of a 0.5 *M* solution.

Sodium Hydroxide Solution. Dissolve 5 per cent by weight.

Potassium Iodide Solution. Dissolve 8.0 grams of U. S. P. potassium iodide to make 100 ml. of solution.

Hydrochloric Acid, 12 *N*.

Dibutyl Ether. This must be free from peroxides, so as not to oxidize the iodine in the precipitate.

Procedure

Dissolve the sample, containing about 0.03 gram of bismuth, in 10 ml. of concentrated sulfuric acid, remove most of any excess acid by evaporation, and dilute with water to 150 ml. in a 400-ml. beaker. (Determinations were made in solutions containing as much as 6.5 per cent acid by volume but 1.5 to 2 per cent is preferable. Since sodium ion does not interfere, the acidity may be regulated, if necessary, by the addition of sodium hydroxide.) Add 30 ml. of caffeine sulfate solution and precipitate the bismuth by the dropwise addition, with vigorous stirring, of 25 ml. of the potassium iodide solution. Let stand for 10 minutes, then filter by suction through asbestos in a Gooch crucible. It is not necessary to remove the precipitate which adheres to the beaker, since the same beaker is to be used for subsequent operations. Wash five times with 15-ml. portions of caffeine sulfate wash solution and then four times with 15-ml. portions of dibutyl ether.

Place the Gooch crucible containing the washed precipitate in the beaker in which the precipitation was made, add 100 ml. of the sodium hydroxide solution, and heat to boiling to decompose the precipitate. Cool and add 23 ml. of 12 *N* hydrochloric acid, then add 8 ml. of the potassium cyanide solution, and titrate with potassium iodate solution to the iodine cyanide end point according to Lang's method (*11*). This involves addition of potassium iodate until the color of the solution changes from the brown first obtained to a faint yellow, then addition of 10 ml. of starch indicator, and titration just to the disappearance of the blue starch-iodide color.

Six determinations on solutions containing 0.0300 gram of bismuth gave 0.0300, 0.0302, 0.0300, 0.0299, 0.0299, and 0.0300 gram by the above procedure.

For very small amounts of bismuth, proportionate amounts of reagents were used. Eight determinations on 0.00117 gram of bismuth gave 0.00116, 0.00118, 0.00116, 0.00120, 0.00118, 0.00119, 0.00120, and 0.00119 gram.

Table I. Determinations in the Presence of Noninterfering Ions

Ions	Ion Added		Bismuth Present (as Nitrate)	Bismuth Found
	Gram		*Gram*	*Gram*
Ca^{++} K^{+} Sr^{++} Mg^{++} Na^{+}	0.02 each	Nitrates	0.0300	0.0299 0.0300 0.0302 0.0300
Be^{++} UO_2^{++}	0.02 each	Nitrates	0.0300	0.0302 0.0303 0.0304 0.0303
Al^{+++} Mn^{++} Fe^{+++} Zn^{++} Cr^{+++} Ni^{++} Co^{++}	0.02 each	Nitrates	0.0300	0.0296 0.0300 0.0300 0.0299
As^{+++}	0.1	Chloride	0.0293	0.0295 0.0292 0.0292 0.0294
VO^{++}	0.04	Sulfate	0.0300	0.0303 0.0299 0.0299 0.0298
Hg^{++} [a]	0.1	Nitrate	0.0300	0.0299 0.0298 0.0301 0.0299 0.0301
Pb^{++} [b] Cd^{++}	0.05 each	Nitrates	0.0300	0.0298 0.0297 0.0303 0.0297
Ba^{++} [c]	0.02	Nitrate	0.0300	0.0300 0.0301 0.0298 0.0300
Sn^{++++} [d]	0.1	Chloride	0.0300	0.0299 0.0299 0.0296 0.0303
Sn^{++} [d]	0.1	Chloride	0.0293	0.0291 0.0294 0.0293 0.0293
Sb^{+++} [e]	0.1	Sulfate	0.0300	0.0301 0.0300 0.0302 0.0298
AsO_4^{---}	0.2	As_2O_5	0.0300	0.0301 0.0300 0.0302 0.0298
MoO_4^{--}	0.05	H_2MoO_4	0.0300	0.0303 0.0304 0.0301 0.0303
PO_4^{---}	0.14	$NaNH_4HPO_4$	0.0293	0.0294 0.0294 0.0294 0.0295

[a] Precipitated by 3.0 instead of 2.0 grams of potassium iodide.
[b] Lead removed by precipitation with sodium sulfate before precipitation.
[c] Caffeine nitrate solution used instead of caffeine sulfate solution.
[d] 2.5 ml. of 12 *N* hydrochloric acid added before precipitation.
[e] 4 grams of ammonium tartrate added before precipitation.

Determination of Bismuth in the Presence of Other Ions

In the majority of cases, the presence of other ions did not interfere with the determination of bismuth by the procedure described. Slight modifications were necessary in a few instances. In the following separations, synthetic samples were prepared by adding known amounts of pure salts to a standard bismuth nitrate solution containing 1 per cent of nitric acid by volume.

Mercury in the bivalent state did not interfere, provided sufficient potassium iodide was present to convert the insoluble mercuric iodide first formed to the tetraiodomercurate (II) ion. It was necessary, therefore, to boil with concentrated nitric acid to ensure complete oxidation of mercury. It was found that 3.0 grams of potassium iodide instead of the usual 2.0 grams were adequate for the separation of 0.03 gram of bismuth from as much as 0.20 gram of mercury.

Lead, because of the formation of insoluble iodide, was precipitated by addition of excess sodium sulfate and the lead sulfate removed by filtration before continuing with the determination of bismuth by the regular procedure.

Barium gave no trouble in a solution free from sulfate ion. Barium sulfate was found to coprecipitate appreciable quantities of bismuth. A solution of caffeine nitrate was therefore used for precipitations instead of caffeine sulfate.

Tin, in either the stannous or stannic state, offered no difficulty when sufficient hydrochloric acid was added to convert the tin largely to the complex chloride ion. For 0.1 gram of tin in either form, 2.5 ml. of 12 *N* hydrochloric acid were adequate.

Antimony was found to present no difficulty if tartrate ion was present. In the following separations, 4 grams of ammonium tartrate were added prior to precipitation of the bismuth.

Phosphate ion may not be present, of course, in sufficient concentration to exceed the solubility product of bismuth phosphate. In the separations made in solutions containing this ion, the sulfuric acid present was never less than 3 per cent by volume.

The results of the various separations are given in Table I.

Interfering Ions

Bismuth could not be determined in the presence of mercurous ion. In addition to this, the determination could not be carried out in the presence of copper or silver because of the insolubility of their iodides. In the case of copper, an insoluble compound is formed with copper, iodine, and caffeine.

Summary

A new method for the determination of bismuth has been developed in which the element is precipitated as caffeine tetraiodobismuthate (III) and subsequently determined by titration of the iodine. For solutions containing bismuth only, the method has been tested for as little as 1.2 mg. of bismuth.

The method makes possible the determination of bismuth in the presence of mercury, a feature which does not apply to other previously described methods for such analyses. Determinations of bismuth, by modified procedures in some cases, can be made in the presence of calcium, sodium, strontium, potassium, magnesium, beryllium, iron, uranyl, aluminum, nickel, cobalt, manganese, chromium, molybdate, zinc, arsenious, lead, cadmium, barium, vanadyl, stannic, antimonous, arsenate, stannous, and phosphate ions.

Silver, copper, and mercurous ions interfere with the determination of bismuth by this method.

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Measurement of Oxidation Stability of Road Asphalts

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Two test methods are described for determining the oxidation stability of asphalts, to enable forecast of their road behavior.

The first method is rapid and can be used for routine testing of asphalt shipments. It uses data obtained by means of the standard loss on heating test.

In the second, more accurate method, an asphalt solution in benzene is subjected to the action of oxygen at an elevated pressure but at a temperature within the range of those found in service. No volatilization of asphalt constituents takes place. This method is designed for testing unknown asphalt.

A number of representative asphalts have been classified by these methods in comparison to their road performance.

THE tests at present commonly used in specifications for road asphalts fall into two classes: (1) those which show the physical character of the asphalt, such as specific gravity, flash point, penetration, softening point, ductility, and solubility in various solvents, and (2) tests intended to indicate the service behavior of the asphalt, such as the Oliensis test, and those determining penetration of residue after heating, fluidity factor, susceptibility factor, etc. It is well recognized that the tests of the second group are more nearly identification tests for crude source or processing methods than measures of the quality of the bitumen (*5*, *7*); thus the need for a test which measures the durability of asphalts directly is apparent.

In this paper two test methods are described—the first, termed resistance to hardening, can be used as a simple routine test, while the second, leading to what is termed the deterioration index, gives more accurate results but takes somewhat longer to carry out.

Resistance to Hardening

Road failures, other than those caused by improper original construction, appear to be largely due to hardening of the asphalts. A number of investigators have established the fact that when the penetration of an asphalt drops below a certain point, the road in which the asphalt is incorporated will fail because of brittleness (*4*, *9*, *10*, *11*, *13*, *19*). The obvious conclusion, which also has been generally accepted in practice, is that a softer asphalt has the advantage over a harder asphalt of similar hardening characteristics, since the former will take longer to reach a critical penetration (*1*, *7*, *9*, *13*, *16*).

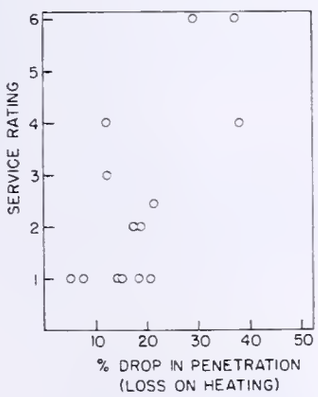

FIGURE 1. CORRELATION OF LOSS ON HEATING TEST WITH SERVICE

While a number of proposed test methods, such as the Shattuck test (*14*), the Raschig and Doyle blowing methods (*12*), and the Lewis oven aging test (*6*) recognize the influence of hardening on the durability of an asphalt, they do not take into account the advantage of the softer material. (These tests have other failings, which are discussed below.) This is because the hardening is expressed as a percentage of the original value, which is fundamentally inadequate, as shown in Figure 1, in which the per cent drop in penetration in the standard loss on heating test (A. S. T. M. Designation D6-39T, Note 2) for some asphalts is plotted against their service rating.

TABLE I. SERVICE RATINGS OF SEVERAL ASPHALTS[a]

Asphalt	Penetration	Observer A	B	C	D
A	171	1	1	1	1
	90	2	2	1	1
	57	2	1	1	1
B + 5% petrolatum	172	1	1	1	1
B + 10% petrolatum	210	1	1	1	1
C	175	1	1	2	1
	84	2	2	2	1−
	52	2−	2	2	1−
D	88	2	2	2−	...
F	81	2	2	3	...
G	84	1	1−	1	...
H	99	2	3	2	...
I	187	3	2−	...	2–3
	89	3	2	4	4
	53	4	4−	4	4
J	87	3	2	4	...
K	118	4	4	3–4	3−
	60	6	6−	6	6
	36	6	6−	6	6
L	91	6	6	...	...

[a] Identification and analytical data for these asphalts are given in Table II.

The service rating is a measure of the performance of an asphalt, and is derived from experimental paving sections in which the asphalts were used under mixing and laying conditions which were as well standardized as possible. These sections were strips of pavement laid in the San Francisco Bay Area which were uniformly subjected to light traffic for the period of one year. The ratings were made visually according to the amount of raveling exhibited by the pavements and cannot lay claim to a precision greater than one unit. Table I gives the ratings made by four observers in order to show the degree of reproducibility to be expected. Grades 1 and 2 signify a very good performance, 4, 5, and 6 poor to very poor performance. The borderline cases are rated as 3.

Figure 1 shows that an asphalt which hardens as little as 12 per cent has a service rating of 4, while another with a penetration drop of 20 per cent performs excellently.

The alternative Nicholson method (*8*) corrects the defect under discussion, but suffers in that high-temperature blowing, as used in this test, is too far removed from the conditions on the road. The latter defect is inherent also in the method proposed by Steinbaugh and Brown (*17*). This point as well as the reasoning which led to the development of the proposed methods are discussed under Deterioration Index from Oxygen Bomb Test.

TABLE II. ANALYTICAL DATA

Designation	Asphalt	Penetration[a]	Ring and Ball Softening Point ° F.	Loss on Heating % Weight	Penetration of Residue	Flash Point[b] ° F.	Sulfur %	Soluble 86° Bé. Naphtha %	Oliensis Spot
A	Mid-continent paving asphalt	190	104.5	0.16	155	625	2.4	89.9	−
A	Mid-continent paving asphalt	92	117.5	0.051	83	610	2.4	82.7	−
A	Mid-continent paving asphalt	60	128	0.04	57	610	2.5	79.3	−
B	California, straight run	68	116.5	0.01	63	600	1.0	91.7	−
	B + 5% petrolatum	182	102.5	0.11	154	530	1.0	91.3	−
	B + 10% petrolatum	206	106	0.137	176	515	0.9	91.5	−
C	Louisiana paving asphalt	177	109	0.29	140	540	4.6	77.1	−
C	Louisiana paving asphalt	84	122	0.316	69.5	560	4.8	74.3	−
C	Louisiana paving asphalt	54	131	0.3	44	575	4.9	72.9	−
D	California paving asphalt A	97	110	0.02	88	580	1.0	91.9	−
E	California paving asphalt B	95	110.5	0.069	87	510	1.0	89.7	−
F	Mexican, straight run	102	119	0.06	90	500	7.25	72.9	−
G	West Texas, straight run	84	115	−0.01	83	675	2.95	87.5	−
H	West Texas, straight run blown	99	114	0.0	93	625	2.7	80.8	−
I	California experimental blend	187	103	0.17	149	480	5.2	77.1	+
I	California experimental blend	92	117	0.081	81	490	5.8	71.8	+
I	California experimental blend	54	125	0.079	47.5	500	6.2	71.2	+
J	West Texas cracked, experimental	87	112	0.46	65	440	3.6	73.3	+
K	California cracked, experimental	115	110.5	0.6	71	420	1.2	73.5	+
K	California cracked, experimental	68	116.5	0.44	43	445	1.1	73.3	+
K	California cracked, experimental	38	125	0.26	27	455	1.3	71.5	+
L	California cracked, experimental	91	112.5	0.48	59	440	1.29	70.6	+

[a] Penetrations were determined on samples used for loss on heating and, being taken from different containers, in some cases differ slightly from those determined on oxygen bomb test samples.
[b] Cleveland open cup.

However, data obtained by means of the loss on heating test, the Lewis test, or other test methods (*8, 12, 14*) can be used in a manner to take into account the initial viscosity of the asphalt. The method may be called resistance to hardening test, because the test results are a rough measure of this property. These results can be plotted against the service ratings of the asphalts (Figure 2), and it is apparent that a critical resistance value of approximately 55 exists, above which no asphalt with a poor service record (4 to 6) is found. This limit effectively excludes poor asphalts. Below 55 there are no asphalts with the good performance record of 1 or 2, so that no good asphalt is unfairly excluded. The procedure of evaluating the results follows:

The asphalt specimen is heated, in the conventional manner, to 163° C. for 5 hours in a standard oven after which the penetration of the sample is taken; this process is then repeated. The logarithms of the penetrations after 5 and after 10 hours are plotted against time in hours. A straight line is drawn through the two points and extended to 10 penetration, which is used as the x axis. The intercept on the x axis gives some indication of the time required by the asphalt to drop to this penetration and is termed the resistance to hardening (Figure 3).

Alternatively, this value can be computed analytically by means of the equation:

$$\text{Resistance to hardening} = 5\left(\frac{y_1 - 1}{y_1 - y_2}\right) + 5$$

y_1 = log penetration after 5 hours' heating
y_2 = log penetration after 10 hours' heating

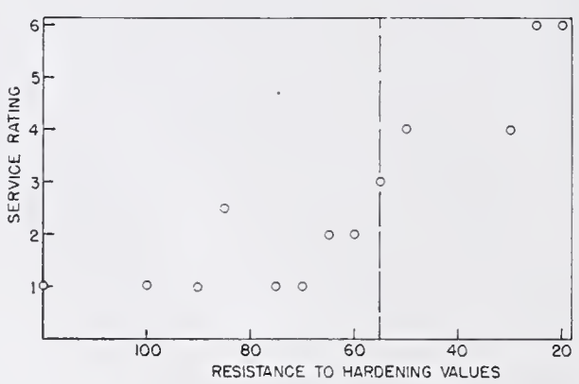

FIGURE 2. CORRELATION OF RESISTANCE TO HARDENING TEST WITH SERVICE

Actually the time to reach 10 penetration is somewhat greater than would be indicated by the simple extrapolation based on the rate of change in penetration in an early stage of the reaction. However, as may be seen from Table III, the extrapolation gives a fair correlation with service ratings according to road performance.

The method of determining resistance to hardening gives but a rough indication of the service to be expected from an asphalt. The reasons for this become evident from a more detailed analysis of the factors influencing the durability of the asphalt, which forms the basis of the development of the more precise method for determining the durability of an asphalt given in the following section.

Deterioration Index from Oxygen Bomb Test

Oxidation and evaporation are generally considered the most important factors that produce the changes known to take place in paving asphalts under the influence of weathering in the road. Attempts in the past to formulate tests for predicting these changes, and hence the service behavior of asphalts, have made no distinction between these two factors (*4, 8, 10, 11, 12, 14, 15*). Moreover, the temperatures used in these tests are high and simulate or exaggerate the conditions of mixing the asphalt with the aggregate rather than those prevailing on the road. Now the hardening during mixing is not negligible but is a constant in the life of any one asphalt, and is generally predictable. On the other hand, it is weathering on the road that governs the change of properties

TABLE III. RESISTANCE TO HARDENING

Asphalt	Original Penetration	Resistance to Hardening	Service Rating[a]
A	190	180	1
	92	120	1
	60	100	1
B + 5% petrolatum	182	75	1
B + 10% petrolatum	206	70	1
C	177	90	1
	84	60	2
	54	65	2
E	95	85	2–3
I	187	85	2–3
	92	55	3
	54	50	4
K	115	30	4
	68	25	6
	38	20	6

[a] Averaged from data of Table I.

TABLE IV. RATE OF HARDENING AT DIFFERENT TEMPERATURES

	Time to Drop from 80 to 70 Penetration[a]	
	At 160° C. *Hours*	At 50° C. *Hours*
Mid-continent asphalt	1.02	257
Mexican (slightly oxidized) asphalt	1.29	112
Western cracked asphalt	0.34	214

[a] From oven aging tests.

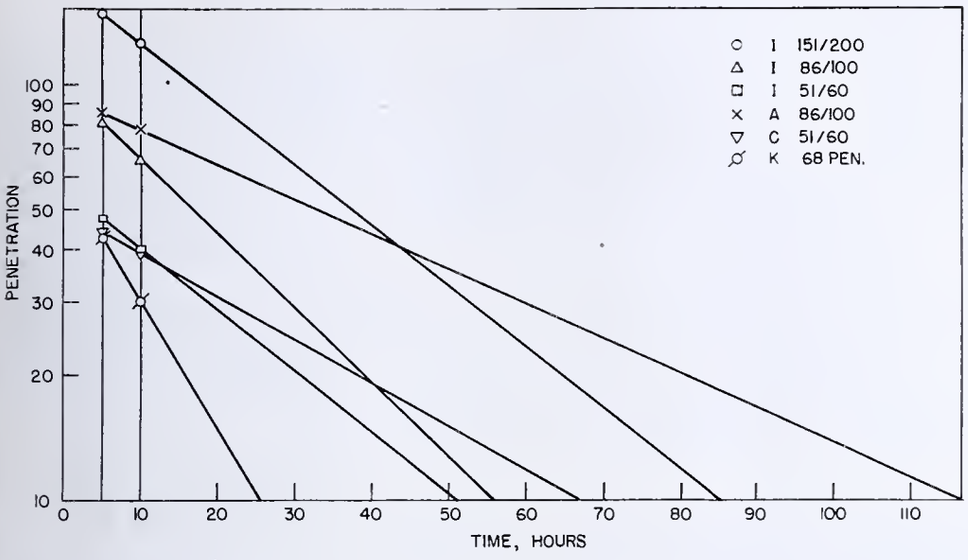

FIGURE 3. RESISTANCE TO HARDENING

of an asphalt. The rate of change is very different from that experienced in the mixer, since the temperature, the relative influence of oxidation and evaporation, and the surface of asphalt exposed are very different. An increase in temperature changes not only the rate, but very frequently also the character of the reaction (Table IV). At 160° C. the cracked asphalt hardens three times as fast, and the Mexican asphalt approximately two-thirds as fast as the mid-continent material. The cracked material at 50° C. hardens only slightly faster but the Mexican asphalt hardens over twice as fast as the mid-continent material. Thus, in the case of the Mexican material, the relative rate of hardening has actually reversed itself.

For the reasons mentioned it is desirable to measure the stability of the asphalt at temperatures within the range of those encountered on the road, and, in the interest of obtaining a more basic knowledge of the reactions, it is desirable to separate the influences of oxidation and evaporation. Since experience and preliminary experiments indicated that oxidation is generally by far the more important factor in the weathering of well-refined asphalt, an effort was made to devise an oxidation test in which volatilization cannot occur, and in which essential conditions, including temperature, approach those prevailing on the road.

If we assume optimum road construction methods, oxidation and evaporation can be considered the principal fundamental causes of eventual failure. The immediate causes are changes in physical properties brought about by these factors, such as the increase in viscosity which eventually results in brittleness, the change in cohesion, the change in adhesion of the asphalt to the mineral aggregate, the change in plasticity, and perhaps others; of these, the increase in viscosity (as measured by drop in penetration) seems to be by far the most important.

Based on the results published in the literature (*4*, *9*, *10*, *11*, *13*, *19*) and the authors' experience, the critical penetration below which an asphalt tends to become brittle was taken to be 20. This represents an average only, for use in calculations. The actual value may fluctuate between 10 and 30, depending on the climate, elasticity and plasticity of the asphalt, grading of the aggregate, and other factors.

As indicated, the time necessary for an asphalt to drop to the critical penetration under standardized oxidizing conditions should be a measure of the durability of an asphalt. Since the attainment of this penetration would in most cases take too long at the low test temperatures stipulated, it is necessary to develop a method of predicting this time from the behavior at shorter times. Direct extrapolation proved impossible, for it was found experimentally that the penetration is not a simple function of time.

The solution found was to combine the oxygen-absorption behavior of the asphalt, in amount and rate at a fixed time, with the hardening caused by a given oxygen absorption.

The oxidation is carried out in a stainless steel bomb similar to that described by Egloff *et al.* (*2*) and modified by Yabroff and Walters (*20*).

The bomb is immersed as directed by Yabroff and Walters (*20*) in an oil bath which can be regulated to 0.1° C. at the working temperature. For observation of the pressure, an automatic recorder, or for more accurate work, a Bourdon-type gage which can be estimated to 0.2 pound per square inch can be used.

One hundred grams of the warm asphalt are poured into a 240-ml. (8-ounce) oil sample bottle containing 67 grams of C. P.

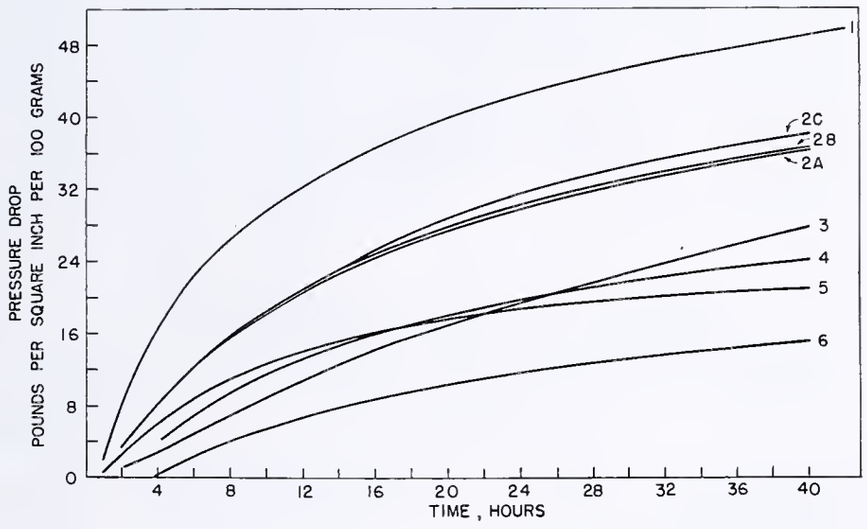

FIGURE 4. PRESSURE DROP WITH TIME

1	K	118 penetration
2A	I	187 penetration
2B	I	89 penetration
2C	I	53 penetration
3	E	97 penetration
4	F	81 penetration
5	C	84 penetration
6	A	90 penetration

benzene. After weighing, the bottle is shaken until the solution is homogeneous; when it has reached room temperature it is placed in the bomb, which is at 25° C. Next, the bomb is sealed and flushed with oxygen by charging it to 100 pounds per square inch gage (70,265 kg. per square meter, 6.8 atmospheres above atmospheric pressure) and releasing it again. The flushing is repeated and the bomb is then charged with oxygen at slightly more than 100 pounds per square inch gage; after a short period (15 minutes) to test for possible leaks, the pressure is adjusted to 100 pounds per square inch, and the temperature is raised to 50° C. The pressure is recorded from now on, either continuously by automatic recorder or manually, as required for the plot described below. For each asphalt a long run of 40 hours (or longer, for greater accuracy), a short run in which the pressure drop should

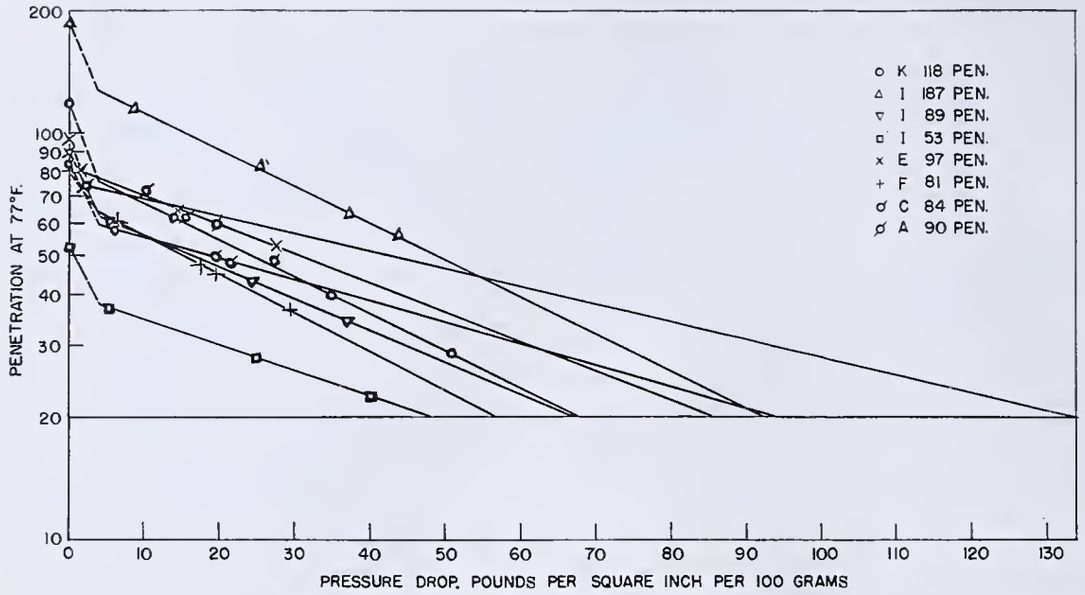
PENETRATION AT 77°F.
PRESSURE DROP, POUNDS PER SQUARE INCH PER 100 GRAMS
o K 118 PEN.
Δ I 187 PEN.
▽ I 89 PEN.
□ I 53 PEN.
× E 97 PEN.
+ F 81 PEN.
σ C 84 PEN.
ø A 90 PEN.

FIGURE 5. HARDENING RATING CURVES

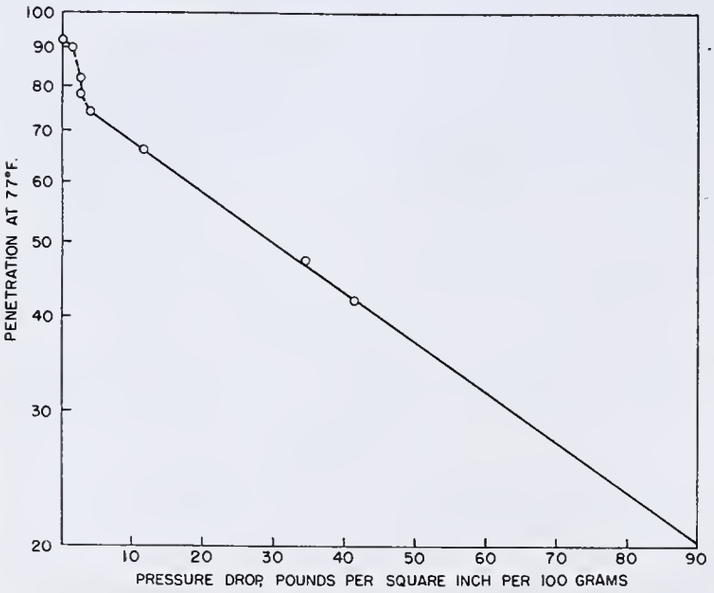

FIGURE 6. HARDENING RATING CURVE FOR ASPHALT E

be at least 4 pounds per square inch, (0.272 atmosphere), and an intermediate check run is made. At the end of each run, the oxygen is released, and the asphalt is recovered as soon as possible to prevent its hardening in the solution. The equipment serving to recover the asphalt from the benzene solution for the penetration measurements is that described by Ford and Arabian (*3*). Better agitation of the hot asphalt is provided by substituting for the single capillary described there a curved tube having several outlets for the gas directed toward the bottom of the flask. The penetration of the asphalt is then measured (A. S. T. M. Designation D5-25). The data so obtained are used in the following manner:

The pressure drop is found by subtracting the observed gage pressure from the pressure of the original system at 50° C. This datum pressure, calculated from the gas law and corrected for the solubility of the oxygen in the solution, the increase in vapor pressure of the benzene, and expansion of the materials, is approximately 108 pounds per square inch gage (7.35 atmospheres) for the system under consideration. This value was corroborated by experiments on inert materials and consequently used in all the calculations.

The pressure drop is plotted against time, for the longest run; the other runs should check this plot within the limits given below. From this graph the pressure drop at 40 hours and the slope of the tangent at this point (in pounds per square inch per hour) are evaluated. The product of the pressure drop and the slope is called the oxidation rating. Several of the asphalts tested are thus plotted in Figure 4.

Next, the logarithm of the penetration after oxidation is plotted against the pressure drop for the three (or more) runs. The amount of oxygen needed to reduce the penetration to the value of 20 is obtained by extrapolation, and is called the hardening rating and expressed in pounds per square inch (Figure 5).

The deterioration index indicative of the service behavior of an asphalt is obtained from the two quantities as follows:

$$\text{Deterioration index} = \frac{\text{oxidation rating}}{\text{hardening rating}} \times 100 \quad (1)$$

The deterioration index has been determined for a number of asphalts (Table V).

Runs should be made in duplicate or triplicate, and should check within 1 pound per square inch of their average; larger differences indicate leaks or other disturbances. The largest errors are introduced in the evaluation of the rate of oxidation and in the extrapolation. The deterioration indices of the duplicate samples should be within 10 per cent of each other. Other types of bombs could be used; however, for expression of the results in terms of deterioration index as here defined these would need to be standardized by means of asphalts that have been rated in the specified apparatus.

The oxidation rating relates the oxygen consumption of the asphalt to time by multiplying the oxygen pressure drop at 40 hours by the rate of oxygen consumption at that time. This procedure permits an over-all characterization of the oxygen-absorption behavior of the asphalt, since it in effect projects the oxidation beyond the limited periods of time permissible in laboratory tests.

The hardening rating was derived from the linear relationship which was found to exist between the logarithm of penetration and the oxygen consumption (except for a short initial period in which the asphalt hardens more for a given oxygen consumption, which prohibits the use of the original penetration as a point on the curve). A typical plot is shown in Figure 6.

The ratio of the two ratings gives the desired relation of critical oxygen consumption to time and, as is evident from Equation 1, is expressed directly in pounds per square inch per hour; it therefore has the dimensions of a rate, and represents the rate at which the asphalts deteriorate.

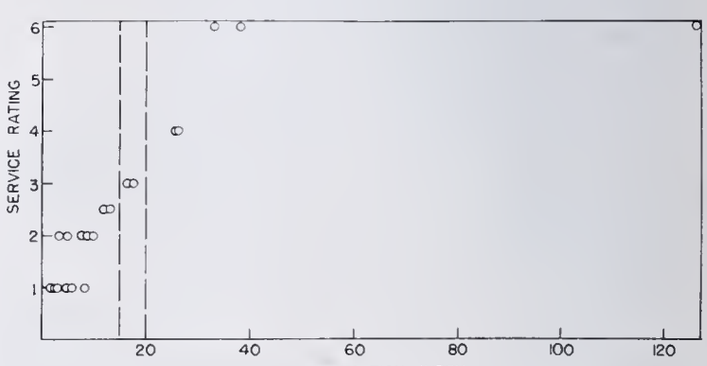

FIGURE 7. CORRELATION OF DETERIORATION INDEX WITH SERVICE

Table V. Deterioration Data and Indices and Service Ratings

Asphalt	Penetration	Pressure Drop at 40 Hours	dP/dt at 40 Hours	Oxidation Rating	Hardening Rating	Deterioration Index	Service Rating
		Lb./sq. in.	*Lb./sq. in./hr.*	*(Lb./sq. in.)²/hr.*	*Lb./sq. in.*	*(Lb./sq. in./hr.) × 100*	
A	171	16.5	0.18	3	200	1.5	1
	90	15.6	0.20	3.1	134	2.3	1
	57	17	0.19	3.2	115	2.8	1
B	68	30.6	0.27	8.3	66	12.7	None laid
B + 5% petrolatum	172	30	0.21	6.3	107	5.9	1
B + 10% petrolatum	210	29.5	0.24	7.1	146	4.9	1
C	175	20.5	0.09	1.9	98	1.9	1
	84	21.5	0.13	2.9	94	3.1	2
	52	22.8	0.19	4.3	90	4.8	2
D	88	33.6	0.23	7.7	89	8.7	2
E	97	28	0.40	11.2	86	13	2–3
F	81	24.6	0.23	5.5	56	9.9	2
G	84	21.5	0.22	4.7	59	8.1	1
H	99	21.2	0.26	5.4	71	7.6	2
I	187	36.5	0.3	11	92	11.9	2–3
	89	37	0.32	11.9	67	17.7	3
	53	38.5	0.32	12.3	48	25.6	4
J	87	33.7	0.28	9.3	57	16.3	3
K	118	49.5	0.36	17.8	67.5	26.4	4
	60	51	0.33	16.8	45	38	6
	36	50.5	0.36	18.2	15	126	6
L	91	45.6	0.42	19.2	59	33	6

Figure 7 shows the correlation found between service rating and deterioration index. It is evident that while a deterioration index lower than 15 indicates fully satisfactory performance, poor durability can definitely be expected with an index above 20. No exceptions have been found to occur for any of the wide variety of types tested.

This method is designed for the testing of new asphalts, the stability of which is unknown; further samples of an asphalt which has been accepted on the basis of this test can be checked by simple routine tests such as the resistance to hardening method described at the beginning of this paper.

Acknowledgment

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New Indicators for Iodate–Iodine Monochloride Andrews Analytical Procedures

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THE use of iodate as oxidant in volumetric analysis at high concentrations of hydrochloric acid was originally developed by Andrews (*1*) and further popularized by Jamieson (*4*). The Andrews iodate–iodine monochloride reactions, while precise and versatile, require a high hydrochloric acid concentration (2.5 to 6 *M*), and the use of carbon tetrachloride or chloroform as immiscible solvent to indicate the completion of the reaction, by the presence or absence of a violet coloration.

The present paper describes conditions under which the Andrews reactions can be applied using dyestuff internal indicators, the color destruction of which corresponds with the completion of the oxidations involved. The iodate–iodine monochloride reactions are simplified and made more rapid and convenient, without appreciable loss of accuracy or versatility.

The Andrews (*1*) procedures as extended by Jamieson (*4*) have been reviewed by Lang (*2*). In order to apply the Andrews reactions to solutions at much lower concentrations of hydrochloric acid, Lang used (*2*) potassium cyanide to cause the formation of iodine cyanide in place of iodine monochloride. By this change starch may be employed as indicator, but this development is burdened by the presence of hydrogen cyanide. The same advantages are attained by the Berg modification, which employs acetone to form iodoacetone in place of iodine monochloride or iodine cyanide (*2*). The applications in this case are limited in number. The determination of iron by the Andrews procedure in the presence of organic matter was described by Heisig (*3*). The dyestuff indicators, some of which are used in the present work, were studied by Smith and Bliss (*5*).

Requirements of Internal Indicators

In the iodate–iodine monochloride reactions using carbon tetrachloride or chloroform as an extraction solvent indicator, the equivalence point is determined by the disappearance of the iodine color on the addition of the first slight excess of iodate. A dye suitable for use as an internal indicator for such

reactions should be stable in the presence of iodine, iodine monochloride, and strong hydrochloric acid. It should be destroyed under the same conditions by the first slight excess of iodate, should have no appreciable blank correction at sufficient concentrations to produce a vivid color change, and should be applicable at room temperature and at slightly increased temperatures.

Accordingly, the dyes studied for use as indicator reagents by Smith and Bliss were examined for use in the present application (Table I).

Reagents. Iodate solution, 0.025 *M*. Indicators, 0.2 per cent aqueous solutions. Iodine monochloride made by dissolving 0.279 gram of potassium iodide plus 0.172 gram of potassium iodate in 250 ml. of water and adding to 250 ml. of hydrochloric acid (sp. gr. 1.19).

Procedure. Ten milliliters of iodine monochloride solution plus 35 ml. of concentrated hydrochloric acid were diluted to 100 ml. Indicator (0.5 ml.) and 0.05 ml. of 0.1 *N* potassium iodate were added. Temperature, 35° C.

Of the sixteen dyes found by Smith and Bliss (*5*) to be applicable to bromate oxidations, only those listed in Table I were found suitable for use in the Andrews reaction. Failure in most cases was due to the lesser oxidation potential provided by iodate as compared to bromate, the higher acidities employed, or the oxidizing properties of iodine or iodine monochloride under the conditions selected. It is possible that similar dyes not found suitable for bromate reactions by Smith and Bliss (*5*) may also be applicable here. The indicators of Table I are sufficiently versatile to cover all practical uses.

Table I. Application of Smith and Bliss Internal Oxidation Indicators

Indicator	British Colour Index No.	Color Change	Remarks
Naphthol Blue Black	246	Green to faint pink	Fairly satisfactory
Brilliant Ponceaux 5R	185	Orange to colorless	Satisfactory
Amaranth	184	Red to colorless	Satisfactory

Effect of Temperature and Acid Concentration

For this purpose 10 ml. of iodine monochloride and 0.5 ml. of indicator were added to sufficient hydrochloric acid to give a concentration of 1 to 7 molar hydrochloric acid upon dilution to 100 ml. One drop (0.05 ml.) of 0.1 *N* iodate solution was added to these solutions at temperatures of 25° to 70° C. and the effect was noted.

At a hydrochloric acid concentration of 1 to 5.0 molar the indicators listed in Table I were not destroyed at temperatures from 25° to 90° C.

Upon addition of iodate, Amaranth is satisfactorily destroyed at acidities of 3.0 to 5.0 *M* at 30° or above. Brilliant Ponceaux 5R is satisfactory at acidities greater than 3.0 *M* at 25° to 30° and at higher temperatures in 4.0 to 5.0 acidity. Naphthol Blue Black is slower in its response to iodate oxidation, but is satisfactory at 3.0 to 5.0 molar acid strength.

Indicator Blank Correction

One milliliter of indicator under the conditions of titration is destroyed by the addition of 0.05 ml. of 0.1 *N* iodate solution. The indicator blank is therefore negligible, since less than 0.5 ml. of indicator is required for each titration. Since the dyes used as indicators are destroyed at the completion of the reaction, the reactions are not reversible. A tendency of the indicators to fade as the equivalence point is reached is counteracted by delaying their addition until the color of the solution being titrated indicates that completion is near. Just before the equivalence point is reached an additional small portion of indicator may be added.

Table II. Determination of Arsenic Using Amaranth as Indicator

As_2O_3 Taken	KIO_3 Required	As_2O_3 Found	Error in As_2O_3 Found
Gram	*Ml.*	*Gram*	*Gram*
0.1995	40.25	0.1993	−0.0002
0.1996	40.29	0.1995	−0.0001
0.1995	40.30	0.1995	0.0000
0.1998	40.32	0.1996	−0.0002
0.1121	22.65	0.1120	−0.0001
0.2019	40.76	0.2018	−0.0001

Determination of Arsenic

An approximately 0.1 *N* solution of sodium arsenite was titrated by the Andrews procedure. A pipet sample of 25 ml. of arsenite was transferred to a 500-ml. glass-stoppered Erlenmeyer flask, and hydrochloric acid and water were added to make a 100-ml. volume, 4.4 *M* in hydrochloric acid. After addition of 10 ml. of chloroform the solution was titrated with approximately 0.1 *N* potassium iodate, the stoppered flask being well shaken after each dropwise addition of iodate in approaching the equivalence point. The end of the reaction is indicated by the change in the chloroform from violet to colorless.

The same procedure was followed for the indicator method, except that the titration was carried out in a 400-ml. beaker and a few drops of the indicator employed were added in place of chloroform after almost all of the iodine color had disappeared from the solution upon the addition of standard iodate.

The titrations were carried out at room temperature and the color changes indicated in Table I were found to be sharp and distinct. The results by the two methods were identical, using the three indicators described above.

Determination of Arsenic in Pure Arsenic Trioxide. Weighed portions of Bureau of Standards arsenic trioxide were transferred to 400-ml. beakers, 5 ml. of water and 0.5 gram of sodium hydroxide were added, and the sample was dissolved. Then 35 ml. of concentrated hydrochloric acid (sp. gr. 1.19) were added, and enough water to make 100 ml. These solutions were titrated, using a solution containing 5.3505 grams of potassium iodate per liter (1 ml. = 0.0049465 gram of arsenic trioxide). After most of the iodine color had disappeared 0.5 ml. of indicator 184 (Amaranth) was added and the titration was completed. The color changed from red to light yellow. Reactions were at room temperature. The results are shown in Table II.

Essentially the same results were obtained with Naphthol Blue Black and Brilliant Ponceaux 5R. The reactions, when Naphthol Blue Black is used as indicator, are slow at the end.

Determination of Antimony

Antimony was determined by the present method and by a bromate procedure described by Smith and May (*7*).

The iodate solution used for the determination of arsenic, reported in Table II, is equivalent to 8.0882 mg. of antimony per ml. An approximately 0.1 solution of potassium bromate was prepared by dissolving 1.3916 grams of pure potassium bromate in enough water to make 1 liter of solution. An approximately 0.1 *N* solution of potassium antimonyl tartrate was prepared by dissolving 16.7 grams of the salt in 1 liter of water.

Samples of 25.00 ml. of the potassium antimonyl tartrate solution were measured into 400-ml. beakers, 50 ml. of concentrated (sp. gr. 1.19) hydrochloric acid were added, and dilution was made to 100 ml. with water (6.2 *N* in hydrochloric acid). The resulting solutions were titrated with potassium iodate, using a potentiometric end-point determination. The same procedure was employed using the indicators described above. Finally the bromate solution was used to standardize the potassium antimonyl tartrate solution and portions equivalent to a known amount of antimony were evaluated, using standard iodate (Table III).

Table III shows that using iodate, the potentiometric and indicator methods for the determination of the equivalence point give the same results. The iodate method using indicators 184, 185, and 246 gives the same results as the bromate method (*6*) using Bordeaux as indicator.

Table III. Determination of Antimony

(Comparison of iodate and bromate procedures using indicator and potentiometric end-point determination)

Iodate Required in Iodate Procedures			
Potentiometric procedure	Indicator 184	Indicator 185	Indicator 246
Ml.	*Ml.*	*Ml.*	*Ml.*
25.10	25.10	25.10	25.13
25.10	25.10	25.07	25.12
25.15	25.10	25.03	25.05
Av. 25.11	25.10	25.06	25.10

(Iodate procedure compared with bromate procedure, $KBrO_3$ as standard, Bordeaux, *6*, as indicator)

KIO_3 Required, Indicator 184	Sb Taken	Sb Found	Error	No. of Determinations
Ml.	*Gram*	*Gram*	*Gram*	
25.55 to 25.60	0.1559	0.1555 to 0.1559	−0.0001 to −0.0003	10

For the antimony determination indicator 184 is most satisfactory, the color change using indicator 185 is not so satisfactory, and indicator 246 is slower in reaction. The Andrews-Jamieson method was found unsuited to the determination of antimony in a tartrate solution because of the failure of the carbon tetrachloride or chloroform to develop a violet color during the progress of the reaction.

Determination of Iron

The Andrews method as developed by Heisig (*3*) was compared with the present method.

The Heisig method was applied by taking a 25.00-ml. portion of a standard ferrous sulfate solution, to which were added 50 ml. of concentrated hydrochloric acid, and diluting the whole to 100 ml. with water. Then 10 ml. of iodine monochloride, prepared as described above, were added and the solution was titrated, using 0.1 *N* potassium iodate after addition of carbon tetrachloride as indicator.

The indicator method was applied in exactly the same manner, except that carbon tetrachloride was replaced by Amaranth, Brilliant Ponceaux 5R, and Naphthol Blue Black, which were added after the iodine liberated in the reaction was almost all oxidized by the iodate added. The Heisig procedure and the present method with all three indicators were found to agree within 0.1 per cent.

Iron was determined in Bureau of Standards ingot iron (Sample 55) containing 99.85 per cent of iron, following the present internal indicator method, and results were compared with the Heisig method using a solution of ferrous sulfate. The latter solution was also evaluated by comparison with standard permanganate and ceric ions with perfectly satisfactory agreement. The solution of iodate was thus found to be 0.1009 *N*. The same iodate solution when standardized using pure arsenic trioxide and the Andrews method or the present method gave a value 0.1000 *N*. It was therefore concluded that the iodate solution for use in the determination of iron must be standardized using iron as reference and that low results are obtained if arsenic trioxide is used as standard of reference.

Samples of the ingot iron were weighed into 250-ml. beakers, 10 ml. of concentrated hydrochloric acid (sp. gr. 1.19) were added, and the beakers were placed on the hot plate until dissolved. The resulting solution was cooled and passed through a Walden silver reductor to reduce the iron, treated with 40 ml. of concentrated hydrochloric acid, and diluted to 100 ml. with water. To this solution 10 ml. of iodine monochloride solution were added and the liberated iodine was titrated with potassium iodate until the iodine color disappeared. A 0.5-ml. portion of indicator 184 was added and the titration was completed. The results are shown in Table IV.

In Presence of Organic Matter. An advantage in the determination of iron by the Heisig method (*3*) or the bromate method of Smith and Bliss (*6*) consists in the fact that most organic material does not interfere. The same can be shown to be true of the present method.

A sample of 5.5761 grams of Bureau of Standards ingot iron 55 was dissolved in 100 ml. of hot concentrated hydrochloric acid, cooled, and passed through a Walden silver reductor. The resulting solution was diluted to 1000 ml. in a volumetric flask. Samples of 25.00 ml. of this standard solution were titrated as described in Table IV in the presence of organic reagents.

Table V shows that the internal indicator procedure is admirably adapted to use in the presence of organic matter.

Table IV. Determination of Iron Using Amaranth as Indicator

Sample Bur. Standards 55 Fe Taken	0.1009 *N* KIO_3	Fe Found	Fe Error
Gram	*Ml.*	*Gram*	*Gram*
0.1458	26.10	0.1457	+0.0001
0.1420	25.40	0.1417	−0.0001
0.1509	26.98	0.1506	−0.0001
0.1469	26.26	0.1466	−0.0001
0.1427	25.55	0.1426	+0.0001
0.1429	25.60	0.1429	+0.0002
0.1456	26.04	0.1455	−0.0001
0.1418	25.36	0.1416	−0.0002
0.1507	26.94	0.1504	−0.0003
0.1467	26.22	0.1464	−0.0003
0.1425	25.51	0.1424	−0.0001
0.1427	25.52	0.1424	−0.0003

Table V. Determination of Iron in Presence of Organic Matter Using Iodate and Amaranth

(Iron taken for each titration, 0.1382 gram)

Organic Matter Present		0.1009 *N* KIO_3	Fe Found	Fe Error
	Grams	*Ml.*	*Gram*	*Gram*
Tartaric acid	2	24.51	0.1381	−0.0001
		24.52	0.1382	±0.0000
		24.50	0.1380	−0.0002
Citric acid	2	24.54	0.1383	+0.0001
		24.55	0.1383	+0.0001
		24.52	0.1382	±0.0000
		24.54	0.1383	+0.0001
95 per cent ethanol	10 ml.	24.53	0.1382	±0.0000
		24.57	0.1384	+0.0002
		24.56	0.1383	+0.0001
Sodium oxalate	1	24.53	0.1382	±0.0000
		24.54	0.1383	+0.0001
		24.53	0.1382	±0.0000
Glycerol	10 ml.	24.54	0.1383	+0.0001
		24.56	0.1384	+0.0002
		24.55	0.1383	+0.0001

Determination of Thallium

An approximately 0.2 *N* thallous nitrate solution was prepared by dissolving 13.3 grams thallous nitrate in 500 ml. of molar perchloric acid. The solution was standardized potentiometrically by titration using standard ceric sulfate. The titration was made at 7.0 *N* strength of hydrochloric acid using the platinum-calomel electrode system, 10.00 ml. of the thallous solution were found to contain 0.19895 gram of thallium as the average of a number of consecutive determinations.

Six 10-ml. portions of the standard thallous nitrate solution were transferred by pipet to 250-ml. beakers, 40 ml. of concentrated hydrochloric acid were added, and the solution was diluted to 100 ml. with water. The resulting solutions were titrated, using standard iodate which had been standardized using pure arsenic trioxide and the Andrews reaction and found to be 0.09996 *N*. As soon as the iodine color had almost disappeared 0.5 ml. of Amaranth was added as indicator coloring and the titration completed to the destruction of the indicator color. The six samples required from 19.46 to 19.48 ml. of iodate which corresponds to 0.1988 and 0.1990 gram of thallium.

The results by the indicator method are thus seen to duplicate the results by the Andrews-Jamieson procedure and the ceric sulfate potentiometric procedure of Willard and Young (*9*).

Determination of Thiosulfate

Comparison was made between the titer obtained following the Andrews-Jamieson method and the internal indicator procedure.

Table VI. Determination of Hydrogen Peroxide

(30.00 ml. of 0.0940 N sodium arsenite plus 10 ml. of 2.5 N NaOH 11.00 ml. of unknown H_2O_2 solution approximately 0.1822 N. Titration of excess arsenite by 0.1000 N KIO_3)

	Potassium Iodate Required		
Andrews-Jamieson method	Indicator 184	Indicator 185	Indicator 246
Ml.	*Ml.*	*Ml.*	*Ml.*
8.23	8.18	8.20	8.17
8.20	8.23	8.22	8.25
8.19	8.23	8.19	8.13
Av. 8.21	8.22	8.20	8.17

A solution of approximately 0.1 N sodium thiosulfate was made by dissolving 12.4 grams of sodium thiosulfate pentahydrate in 1000 ml. of water.

The Andrews-Jamieson method consisted in diluting a 25.00-ml. sample of thiosulfate with 50 ml. of concentrated hydrochloric acid and 25 ml. of water in a 500-ml. glass-stoppered Erlenmeyer flask, to which 5 ml. of chloroform were added as indicator. This solution, which is approximately 5 molar in hydrochloric acid, was titrated using 0.1 N potassium iodate until the last faint trace of violet color in the indicator was dispelled.

The same procedure was applied in the use of internal indicators 184, 185, and 246 except that 0.5 ml. of the indicator was used in place of chloroform. The internal indicators were not added until the liberated iodine was practically all oxidized to iodine monochloride.

Amaranth served best of the three indicators. The results by the new procedure were found to be precisely the same as by the Andrews method.

Determination of Thiocyanate

Three solutions of approximately 0.1 N potassium thiocyanate were prepared by dissolving approximately 1.6 grams of the salt in 1000 ml. of water, and were titrated using approximately 0.1 N potassium iodate solution by the Andrews-Jamieson method and with Amaranth as indicator.

The Andrews-Jamieson method was carried out by transferring 25.00 ml. of potassium thiocyanate solution to a 500-ml. Erlenmeyer flask and adding 50 ml. of concentrated hydrochloric acid, 25 ml. of water, and 5 ml. of chloroform. The titration was begun immediately to prevent air oxidation at this high acidity, adding the solutions of iodate until the last trace of violet color was dispelled from the chloroform.

Using the indicator method, the chloroform was omitted and 0.5 ml. of indicator 184 was added after most of the iodine formed had been oxidized to iodine monochloride. The results using the Andrews procedure and the present method with any of the three indicators described were practically identical in 18 comparison determinations. The thiocyanates must be titrated without delay after the solution is made 5.0 M with hydrochloric acid to prevent oxidation in part by contact with air.

Determination of Sulfurous Acid

Using a saturated solution of sulfur dioxide in water at room temperature as sample for analysis, 25.00-ml. portions were transferred to 500-ml. glass-stoppered Erlenmeyer flasks, 40 ml. of concentrated hydrochloric acid were added, and the samples were diluted to 100 ml. with water. After addition of 5 ml. of chloroform the solutions were titrated with 0.1 N potassium iodate solution until the disappearance of the violet color from the chloroform. The same procedure was followed with internal indicators 184, 185, and 246, except that the chloroform was omitted and 0.5 ml. of indicator solution was added after disappearance of the iodine color with the oxidation to iodine monochloride.

The determination of sulfurous acid was found to give exactly the same results by the Andrews-Jamieson method and the internal indicator procedure. The variation in no case was greater than one part per thousand. Indicator 184 was preferred because of the sharpness of the color change.

Determination of Hydrogen Peroxide

By the Andrews-Jamieson procedure hydrogen peroxide is determined by addition of the peroxide to a sodium hydroxide solution of sodium arsenite. The solution of excess standard arsenite after reaction with hydrogen peroxide is acidified using a large excess of hydrochloric acid, and the unoxidized arsenite is oxidized to arsenate using standard iodate with chloroform as indicator.

A solution of hydrogen peroxide was prepared by diluting 50 ml. of ordinary commercial peroxide to 500 ml. A solution of sodium arsenite was prepared by dissolving 4.9 grams of arsenic trioxide in a slight excess of sodium hydroxide and diluting to 1000 ml. with water. The arsenite solution was standardized using the methods described above and found to be 0.0940 N. A solution of 10 grams of sodium hydroxide pellets in 100 ml. of water was used for the preparation of the arsenite solution for reaction with the peroxide to be determined.

The Andrews-Jamieson method was applied by taking 30.00-ml. portions of the 0.0940 N arsenite, to which were added 10 ml. of 10 per cent sodium hydroxide solution using a 500-ml. glass-stoppered Erlenmeyer flask as container. Samples of 11.00-ml. volume of the unknown hydrogen peroxide solution were slowly added from a buret to the alkaline arsenite solution with constant agitation. After allowing 2 minutes to complete the reaction, 40 ml. of concentrated hydrochloric acid were added and the flask and contents were shaken vigorously. Five milliliters of chloroform were then added and the solution of the excess arsenite was titrated, using 0.1 N potassium iodate until the violet color of the chloroform indicator was dispelled.

The same procedure was applied in the case of internal indicators 184, 185, and 246, except that the chloroform was omitted and 0.5 ml. of the indicator solutions was added after the oxidation of iodine to iodine monochloride was almost complete.

From an examination of Table VI it is observed that hydrogen peroxide can be determined using either the Andrews-Jamieson method or the internal indicator method with concordant results. Indicator 184 is preferred because of the sharpness of the color change involved.

Table VII. Determination of Hydrazine and Phenylhydrazine

(Approximately 0.1 N $N_2H_4.H_2SO_4$ in water, 25.00 ml. taken for analysis. Approximately 0.1 N $C_6H_5.NH.NH_2$ in dilute HCl solution, 25.00 ml. taken for analysis. Oxidant, 0.1000 N potassium iodate solution.)

	Potassium Iodate Required			
Sample	Andrews method	Indicator 184	Indicator 185	Indicator 246
	Ml.	*Ml.*	*Ml.*	*Ml.*
$N_2H_4.H_2SO_4$	22.95	22.90	22.95	22.95
	22.87	22.89	22.95	22.95
	22.85	22.90	22.93	22.97
	Av. 22.92	22.90	22.94	22.95
$C_6H_5.NH.NH_2$	17.50	17.45	17.40	17.50
	17.45	17.50	17.35	17.40
	17.40	17.45	17.35	17.55
	Av. 17.45	17.47	17.36	17.48

Determination of Hydrazine and Phenylhydrazine

The determination of hydrazine has been described by Jamieson (*4*) and there should be no complications in applying the scheme to the determination of phenylhydrazine. However, the phenylhydrazine ordinarily contains some impurity which is extracted by the chloroform used as indicator, which prevents the detection of the presence or absence of a violet coloration at the equivalence point of the reaction.

For determination of hydrazine, a solution of approximately 0.1 N $N_2H_4.H_2SO_4$ in water was used. For the Andrews-Jamieson procedure 25.00-ml. portions of the hydrazine sulfate solution were transferred to 500-ml. glass-stoppered Erlenmeyer flasks and 40 ml. of concentrated hydrochloric acid were added, followed by dilution to 100 ml. with water.

The resulting solutions were titrated using 0.1 N potassium iodate, after the addition of 5 ml. of chloroform, until the color changed from violet to colorless upon the addition of the last drop of iodate solution.

The same procedure was applied in the internal indicator procedure, except that no chloroform was added and 0.5 ml. of indicators 184, 185, and 246 was added after the iodine liberated by the first addition of iodate was oxidized almost completely to iodine monochloride (Table VI).

For the determination of phenylhydrazine an approximately 0.1 N solution was prepared by dissolving 1.2 ml. of the product

in 1000 ml. of hydrochloric acid made by diluting 100 ml. of concentrated acid with water.

The procedure employed in the titration of hydrazine sulfate was employed in the titration of 25.00-ml. portions of phenylhydrazine solution, except that beakers were employed and the iodate oxidation was followed potentiometrically (*8*).

The internal indicator procedure then was applied in the same manner, except that 0.5 ml. of indicators 184, 185, and 246 was added after the reaction of the oxidation of iodine was almost complete. The results of a series of titrations are given in Table VII.

Table VII shows that for the determination of both hydrazine and phenylhydrazine the Andrews-Jamieson procedure and the use of internal oxidation indicators give essentially the same results. Indicators 184 and 185 are preferred in the determination of hydrazine and indicator 185 is preferred in the determination of phenylhydrazine.

Summary

The dyestuffs Amaranth, Brilliant Ponceaux 5R, and Naphthol Blue Black, having British Colour Index numbers 184, 185, and 246, respectively, may be used as oxidation indicators. They are not destroyed by high concentrations of hydrochloric acid in the presence of small amounts of iodine or iodine monochloride, but are readily destroyed by a trace of iodate under the same conditions. No indicator blank is required for sufficient amounts of indicator solution to give vivid end-point determinations.

The determinations by the new procedure follow the established Andrews-Jamieson iodate–iodine monochloride reactions, except that chloroform or carbon tetrachloride is not required for use as immiscible solvent type of indicator.

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Colorimetric Determination of Phenothiazine

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SMALL amounts of phenothiazine, such as may be encountered in the examination of insecticidal spray residues, may be determined colorimetrically by measuring the intensity of the red color produced by addition of bromine to the alcoholic solution of the phenothiazine (*2*). The same colorimetric measurement is employed for the analysis of commercial samples of phenothiazine (*3*). In describing certain modifications and improvements in the method as applied to the analysis of both foliage samples and commercial samples of phenothiazine, Dawsey (*1*) mentions the fact that the color development does not run its usual course with "high" concentrations of phenothiazine in the alcoholic solution, a green compound being formed in place of the desired red compound, but he gives no definite concentration at which the green compound first makes its appearance. Other workers have noted that off-colors are sometimes obtained with concentrations of phenothiazine within the photometrically measurable range.

In applying the bromination method to the determination of phenothiazine in spray residues, the writer encountered considerable difficulty of this nature, and found the variation in visual color to be accompanied by a substantial change in the photometric reading. This was true for measurements with both a visual and a photoelectric photometer. The off-colored solutions had a purple hue and to the eye were darker than the normal-colored ones, but in the photometers they gave lower readings (absorptions). Investigation disclosed that off-colors could be produced at will by adding the bromine water drop by drop, whereas a quick addition tended to produce the normal red color.

Three experimentally controllable factors were found to favor the formation of the normal red color: quick addition of the bromine water with rapid stirring, addition of the phenothiazine solution to an excess of bromine water, and having the solutions warm when developing the color. In addition, where the solution contains plant extractives, it is necessary to allow some time for bromination and to make sure that bromine is present in excess. With the aid of this information it was found possible to standardize the conditions of bromination so that consistent results were obtained with known amounts of phenothiazine.

The procedure adopted was to add 5 cc. of saturated bromine water quickly to 50 cc. of 95 per cent ethyl alcohol containing up to 4500 micrograms of phenothiazine contained in a 100-cc. volumetric flask and previously warmed to 60° C. The stoppered flask was allowed to stand 15 minutes in an oven at 60° C., and then a second 5 cc. of saturated bromine water was quickly added. After the flask had stood 10 minutes longer, the excess bromine was boiled off and the solution was cooled, diluted to definite volume, and filtered through a folded filter, the funnel being covered to minimize evaporation. Photometric measurements were made on the filtrate, after the first 25 cc. had been discarded.

The precision of measurement is approximately one-tenth division at the mid-point of the photometer scale, amounting to ±2 per cent. With absorption cells 1, 3, and 10 cm. in length, satisfactory photometer readings may be made on solutions containing from 100 to 3000 micrograms of phenothiazine per 100 cc. The following data illustrate the accuracy of determination of known amounts of C. P. phenothiazine, as read from the appropriate calibration curves:

Phenothiazine Taken	Phenothiazine Found	Error
Micrograms	*Micrograms*	%
150	140	−6.7
450	430	−4.4
703	730	+3.7
1200	1175	−2.1

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Determination of Menthol in Oil of Peppermint

Acetyl Chloride as a Reagent

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THIS study was undertaken to meet the demand of Oregon State College Experiment Station for a simpler and more rapid method for analysis of the oil of peppermint. In a recent publication (*1*) Brignall cited this same problem, discussed the limitations of the official method, and described a new procedure based on the use of an acetylating mixture of acetic anhydride and *n*-butyl ether.

A great deal of attention has been given to esterification procedures for the assay of alcohols. For this purpose acetic anhydride with and without pyridine has been used almost exclusively. Although acetyl chloride is a much more active acetylating agent, little study (*4*) has been given to the possibilities of using it in quantitative work.

Recently the authors made a rather extensive investigation (*2*) of acetyl chloride as a reagent for determination of the hydroxyl content of organic compounds, in which seventy different alcohols and phenols were studied. Although acetyl chloride gave approximately the same precision, it was apparent that from the standpoint of speed it was far superior to acetic anhydride as an esterifying agent.

Reagents

Eastman practical acetyl chloride was used in this work. The equivalents of silver nitrate required to measure the chlorinity of a measured charge of this reagent was equal to one-half the equivalents of base required to neutralize the same charge.

Sodium hydroxide, 0.3 *N* (carbonate-free), made by diluting 17 ml. of 50 per cent sodium hydroxide to 1 liter with distilled water.

Silver nitrate, 0.3 *N*.

Phenolphthalein indicator, made by dissolving 0.5 gram of indicator in 500 ml. of alcohol and diluting to 1 liter with distilled water.

Potassium chromate indicator, 5 grams dissolved in 100 ml. of water.

Apparatus

The pipet (*3*) and reaction vessel have recently been described (*2*).

Analytical Procedure

A 1-gram sample of dry filtered peppermint oil was weighed in a 10-cm. (4-inch) test tube, a measured charge (0.5 ml.) of acetyl chloride was added by pipet, and the small test tube was placed in a 20-cm. (8-inch) test tube containing 5 to 10 ml. of water. A 15-cm. (6-inch) test tube inverted over the smaller test tube served to make a water seal (*2*, Figure 2). The tube was stoppered and placed in a water bath at 50° C. To ensure the best accuracy the pipet and reagent should be at room temperature, and the acetyl chloride for both the blanks and the regular runs should be measured at the same time.

After 20 to 30 minutes the reaction vessel was removed and then inverted to hydrolyze the excess acetyl chloride, and the contents were transferred to a 250-ml. Erlenmeyer flask. The reaction vessel was finally rinsed with a small amount of alcohol and the last traces of ester were washed into the titration flask. The addition of a small amount of carbon tetrachloride (5 to 10 ml.) to highly discolored solutions removed the interfering materials and greatly aided the titration. Whenever the phenolphthalein end point faded, the base was added in small increments (0.03 ml.) until the color persisted for 30 seconds.

Since the oil of peppermint contains considerable amounts of pinene which absorbs some of the evolved hydrogen chloride, it is necessary to measure the chloride ion in order to determine the actual acetyl chloride involved in the acetylation. This was readily accomplished by a second titration with silver nitrate solution, using potassium chromate as the indicator. An allowance of 0.1 ml. was subtracted from the measured amount of silver nitrate solution to account for the indicator blank (for formation of silver chromate). Since

Equivalent NaOH used = equivalent HCl + equivalent $HC_2H_3O_2$ (after hydrolysis)

Equivalent $AgNO_3$ = equivalent HCl

$$\text{Equivalent HCl absorbed} = \frac{\text{equivalent of blank}}{2} - \text{equivalent } AgNO_3$$

Equivalent of alcohol = equivalent of blank − equivalent of NaOH − equivalent HCl absorbed

$$\text{Equivalent of alcohol} = \text{equivalent of blank} - \text{equivalent of NaOH} - \frac{\text{equivalent of blank}}{2} + \text{equivalent } AgNO_3$$

The per cent of free alcohol will be given by the expression

$$\frac{[1/2 \text{ blank} - \text{ml. of NaOH} + F(\text{ml. of } AgNO_3)] \times N \times \text{molecular weight}}{10 \times \text{weight of sample}} = \% \text{ menthol}$$

where N = normality of NaOH and $F = \frac{\text{normality of } AgNO_3}{\text{normality of NaOH}}$

This equation is valid only for pure acetyl chloride or samples in which the equivalents of chloride per milliliter are equal to half equivalents of base required to titrate 1 ml. of the hydrolyzed reagent. Otherwise it would be necessary to revise the equation and determine both a silver nitrate and a sodium hydroxide blank.

Because of variation of the titer with temperature, etc., blank determinations should be made at the time of the run. These blank runs also serve as a good check on the analyst's precision.

Results and Discussion

Results of the analysis of nine samples of oil of peppermint by this procedure, by the method of Brignall, and by the official method are given in Table I.

Table I. Analysis of Oil of Peppermint

Sample No.	1	2	3	4	5	6	7	8	9
	Per Cent of Menthol								
Authors' method	42.9	50.3	46.1	43.7	44.9	43.0	43.0	42.1	43.3
Brignall's method	42.9	50.3	45.5	43.8	44.9	44.2	43.3	42.8	43.0
Official method[a]	..	..	..	..	43.7	43.4	42.2	42.7	42.1

[a] Analyses furnished by Oregon State College Experiment Station.

All values are averages of three or more determinations, which agreed within 0.5 per cent.

Good agreement was obtained in most cases. Where considerable differences occurred, as in samples 3, 6, and 8, the determinations by the authors' method were repeated. In all cases previous results were duplicated.

To determine the total alcohols in peppermint oil, an ester determination on a second sample is made in the usual manner. The per cent of total alcohol can then be calculated from the formula (*1*):

Table II. Analysis of Essential Oils

Oils	Authors' Method % of alcohol constituent	Authors' Method Average deviation	Brignall's Method % of alcohol constituent	Brignall's Method Average deviation
Cedar oil 1	13.9	1.1	12.7	0.8
Cedar oil 2	6.7	0.3	8.7	0.8
Lavender oil	Negative values	...	16.6	...
Sandalwood	65.1	0.5	63.5	0.7
Rosemary	9.8	0.6	10.1	0.1
Cinnamon	...	...	13.3	1.3
Citronella	23.6	0.4	35.0	1.1
Cloves	72.6	1.4	73.9	0.1

$$\% \text{ total alcohol} = \% \text{ free alcohol} + \left(\frac{\text{molecular weight of alcohol}}{\text{molecular weight of ester}}\right) \% \text{ ester}$$

Analyses of other essential oils containing various alcohols were attempted with this procedure. The results are given in Table II.

As indicated, the results in most cases were not very satisfactory, probably because of the unsaturated nature of the alcohols in question, which apparently absorb the dry hydrogen chloride. During titration this hydrogen chloride is slowly released, giving rise to erroneous results. For this reason the authors do not recommend the procedure as described for determination of free alcohols in oils other than peppermint.

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Acidimetric Orthophosphoric Acid Assay

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KOLTHOFF (*1*) has shown that the presence of sodium chloride in the alkalimetric titration of orthophosphoric acid lowers the pH of both the first and second end points and that the acid may be accurately titrated as a monobasic and dibasic acid by using methyl yellow and phenolphthalein, respectively, as indicators for the two end points. In the titration to the phenolphthalein end point, the solution is half saturated with sodium chloride to suppress the ionization of the dibasic salt, while the methyl yellow titration is conducted without the addition of sodium chloride. Similar procedures are used in the U. S. Pharmacopoeia (*3*) and other standards (*2*). The method, however, cannot readily be applied to solutions of orthophosphoric acid containing unknown amounts of sodium chloride and hydrochloric acid, especially since the titration of the first end point requires almost complete absence of sodium chloride. Furthermore, the authors' experience has shown that the color transition of the indicators at both end points is sluggish and difficult to follow, particularly when the illumination from ordinary incandescent lamps is employed.

In many orthophosphoric acid assays, particularly of pharmaceuticals, detergents, and alkali-metal phosphates, by the magnesium ammonium phosphate method, the solution prior to precipitation has contained only alkali-metal orthophosphates and chlorides or could be adjusted to this composition by a simple process. To avoid the time and manipulation required by the gravimetric assay, a modification of Kolthoff's method has been developed which approximates the magnesium ammonium phosphate method in accuracy.

In the authors' modification, the orthophosphoric acid is assayed by titration from the $H_2PO_4^-$ to the HPO_4^{--} end point in a solution saturated with sodium chloride using cresol red and bromophenol blue indicators, respectively, and a color standard for each of the two end points. The pH of the $H_2PO_4^-$ and HPO_4^{--} end points in a saturated sodium chloride solution, at the assay concentrations, has been found to be approximately 3.15 and 7.65 at 25° C., respectively, by potentiometric titration employing a glass electrode. Because of the salt effect on the two indicators, due to the high concentration of salts in the assay solution, buffer solutions of equivalent pH cannot be used in the preparation of the color standards. Buffer solutions of pH 3.62 and 7.98 at 25° C. have been found to yield the proper end point colors.

Assay Method

The solution must contain only alkali-metal salts of orthophosphoric acid and hydrochloric acid. Small amounts of the other mineral acids will have no appreciable effect upon the end points. Organic acids may be removed by charring; carbonates, by boiling with an excess of hydrochloric acid; silicates, by evaporation to dryness in a solution containing an excess of hydrochloric acid; and meta- and pyrophosphates may be converted to orthophosphates by boiling in a solution made acid with hydrochloric acid. Other ions which must be absent may be removed by conventional methods.

To 100 ml. of solution containing about 1.0 gram of phosphorus pentoxide, add 60 grams of sodium chloride and adjust the temperature of the solution to 25° C. Add 0.4 ml. of cresol red indicator and adjust the pH of the solution to match color standard A by using carbonate-free sodium hydroxide solution or hydrochloric acid. The solution should be approximately 120 ml. in volume at the cresol red end point. When color standard A has been matched, add 2.2 ml. of bromophenol blue indicator and titrate with 0.5 *N* hydrochloric acid until the solution matches color standard B. The solution must be saturated with sodium chloride at all times and vigorous stirring must be used to ensure saturation. The temperature and concentration are not critical. One milliliter of 0.5 *N* hydrochloric acid is equivalent to 0.04902 H_3PO_4 and 0.03551 P_2O_5.

Solutions and Color Standards

Cresol red indicator, 0.04 per cent. Triturate 0.1 gram of cresol red with 13.1 cc. of 0.02 *N* sodium hydroxide. Dilute to 250 cc. with distilled water.

Bromophenol blue indicator, 0.04 per cent. Triturate 0.1 gram of bromophenol blue with 7.45 cc. of 0.02 *N* sodium hydroxide. Dilute to 250 cc. with distilled water.

Boric acid–potassium chloride solution. Dissolve 12.404 grams of recrystallized boric acid and 14.912 grams of recrystallized potassium chloride in sufficient water to make 1000 ml.

Color Standard A. To 50 ml. of the boric acid–potassium chloride solution, add 4.38 ml. of 0.2 *M* sodium hydroxide and sufficient water to make 200 ml. To a definite volume, add an

amount of cresol red indicator to yield the same concentration as in the assay solution at the pH 7.65 end point.

Color Standard B. To 50 ml. of 0.2 *M* acid potassium phthalate solution add 5.75 ml. of 0.2 *M* hydrochloric acid and dilute to 200 ml. with water. To a quantity of the solution, add sufficient cresol red and bromophenol blue indicator to yield approximately the same concentration of both indicators as in the assay solution at the pH 3.15 end point.

Discussion

The method has been applied to the assay of reagent grade orthophosphoric acid and alkali-metal orthophosphates and the results obtained have been checked by gravimetric assay and by potentiometric titration. In addition, numerous pharmaceuticals and detergents have also been assayed by both the gravimetric and the authors' method. In all of the assays, no deviation greater than 0.2 per cent from the potentiometric or gravimetric method has occurred.

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Anchor-Type Laboratory Stirrer for Viscous or Foamy Materials

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In the course of an investigation made in this laboratory it became necessary to reflux a troublesome reaction mixture with stirring. The mixture foamed at first, became viscous and pasty as the reaction proceeded, and finally became hard and adhered to the inside of the flask. Several well-known types of stirrer were found to be useless.

Various stirrers, different from the ordinary propeller, have been described—e. g., the chain type (*1*), the bicycle-chain type (*2*), and a gas-tight stirrer for pasty materials (*3*). It was thought that an adaptation of the anchor type of stirrer, well known in industry, might be effective here, because of the scraping action.

The stirrer was to be used in a 500-ml. Pyrex 3-necked flask. Measurement of the circumference of the flask with a flexible steel tape gave a value of about 32 cm. (12.625 inches). Accordingly, a circle with a 5.1-cm. (2-inch) radius was drawn on a sheet of cardboard. Since the inside diameter of either side neck of the flask was 1.6 cm. (0.625 inch), a second circle was drawn, concentric with the first, having a radius of 3.5 cm. (1.375 inches). A diameter was drawn, and with a pair of scissors the cardboard was cut along the diameter and along both circles, producing two semiannular templates.

Before a template would pass freely into the flask by way of a side neck, it was necessary to reduce the width of the cardboard strip somewhat. The radius of the outside edge also had to be reduced, in order to make the edge of the template exactly fit the curvature of the glass.

After the dimensions of the template had been established by trial, the curves were laid out on No. 25 gage (or slightly heavier) Alleghany stainless-steel sheet. They were very carefully cut out with a pair of heavy tinsmith's shears, a trifle oversize, so that the outside edge could afterward be smoothed with a file to the exact radius determined by the template. (Any suitable sheet metal may be used instead of stainless steel.)

As no two flasks have identical inside diameters, it is necessary to make an individual sheet-metal blade for each flask used.

For the shaft a 30.5-cm. (12-inch) length of 0.635-cm. (0.25-inch) stainless-steel rod was used. This was slotted along a diameter with a hacksaw, the slot being 0.635 cm. (0.25 inch) deep (*A*, Figure 1). At the center of the sheet-steel blade a corresponding slot was filed, 0.635 cm. (0.25 inch) wide by 0.32 cm. (0.125 inch) deep or slightly deeper (*B*, Figure 1).

To set up the stirrer, the blade is introduced into the clean flask through a side neck, from the position shown in dotted lines (Figure 2) by rotating it about its center of curvature. The shaft is next introduced through the center neck, and the slots in the blade and shaft are caused to engage one another. The flask is then charged with the desired materials, and a stuffing box (or a perforated cork) is slipped over the shaft and seated in the neck of the flask. The whole is clamped into position on a ring stand and the upper end of the shaft engaged in the chuck of a variable-speed stirring motor (or a pulley for leather belting may be attached by means of its setscrew).

During all the operations subsequent to the engagement of the shaft with the blade, care must be exercised not to raise the shaft at all, for otherwise the shaft may become disengaged from the blade.

The rate of stirring must be fairly slow (250 to 300 r. p. m.) to avoid splashing into the side necks of the flask, and for the same reason not more than 200 ml. of liquid may be placed in a 500-ml. flask.

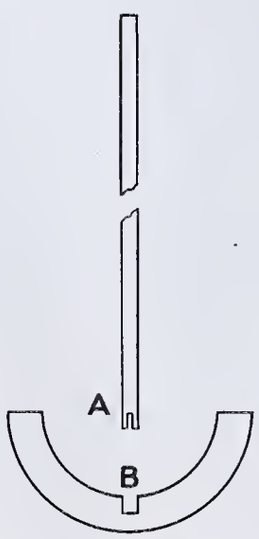

FIGURE 1

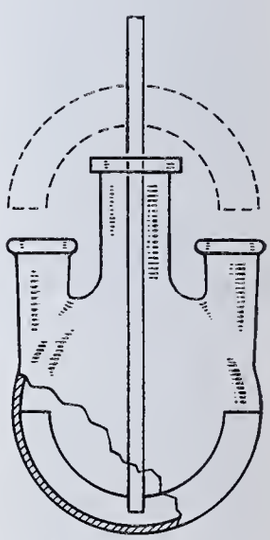

FIGURE 2

For a stirrer to fit a 1-liter flask, the same shaft may be used, but the blade must be cut from heavier sheet metal, about No. 19 gage (or slightly heavier). The radii in this case are approximately 6.2 cm. (2.4375 inches) and 5.1 cm. (2 inches). The blade should be sufficiently flexible to permit of some deformation, in order to be able to introduce it into the flask without difficulty, especially in the case of the 1-liter flask.

When this type of stirrer was used with the reaction mixture mentioned above, the liquid was thrown against the wall of the flask, forming a hollow sphere. This suppressed foaming over. Finally, when solid material was precipitated, the scraping action of the blade proved effective to prevent the solid from adhering to the inside of the flask.

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Magnesium Acetate as an Ashing Agent in Fluorine Analysis

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IN SEARCHING for a suitable reagent to use in ashing organic materials for the determination of small amounts of fluorine it became apparent that any compound to be acceptable must have certain properties: It should have a greater alkalinity than pH 11.00, to prevent the loss of fluorine compounds that are volatile in acid solution; it must form a basic matrix to forestall "fluffing" and loss of ash through handling; and it must be capable of forming completely homogeneous mixtures to ensure even suspension in such semisolid substances as may be encountered—e. g., macerated food samples.

TABLE I. RECOVERY OF ADDED FLUORINE

Urine Sample	F Present in Sample	F Added as NaF_2	Total F Recovered
	Mg.	*Mg.*	*Mg.*
1	0.260	0.020	0.282
2	0.197	0.020	0.218
3	0.143	0.060	0.201
4	0.185	0.060	0.244
5	0.241	0.100	0.343
6	0.138	0.100	0.238

The literature suggests several compounds which are unquestionably satisfactory for use when the minimum amount of fluorine encountered is in excess of 0.005 gram (*1*). Unfortunately, however, all such compounds were found to contain fluorine in concentrations ranging from 12 to 60 micrograms per gram of material. Since the total quantity of fluorine in samples such as those representing a 24-hour fluid intake, 24-hour food intake, or 24-hour urine output is often less than 100 micrograms, in many instances the blank values for these ashing agents would contribute a larger amount of fluorine than that present in the sample, and in 25 per cent of the cases there would be a possible source of error in excess of 40 per cent (Tables III and IV). This is particularly true in the case of aliquot samples of food that contain less than 0.1 mg. of fluorine.

Magnesium peroxide (Mallinckrodt's analytical reagent grade, Control Series JHTI) was used as reagent with fair success for some 2000 analyses. Samples representing a 24-hour fluid intake, 24-hour urine output, and 24-hour fecal excretion gave analytical results which checked excellently. Difficulty was encountered, however, in the analysis of food samples by the aliquanting method reported by Largent (*2*). It was found on occasion that identical quantities of material showed variations amounting to as much as 40 per cent (Table III), and in many instances from two to five rechecks were needed to establish the amount of fluorine actually present.

Because it conformed to the requirements for an ashing agent of this type, magnesium acetate was suggested as a substitute for magnesium peroxide (*3, 6*). Preliminary analyses proved that C. P. magnesium acetate, prepared from electrolytic magnesium and obtained from one source (Coleman and Bell, Cincinnati, Ohio) was practically free from fluorine. Other sources of this chemical have not been investigated.

For the purpose of simulating experimental conditions as nearly as possible, solutions of magnesium acetate were prepared over a range of concentrations sufficient to cover anticipated requirements and the calcined yield of magnesium oxide was determined to be 20 ± 2 per cent. Using the amounts of magnesium oxide derived from magnesium peroxide and magnesium acetate tetrahydrate as a basis, the amount of magnesium acetate necessary to fix all the fluorine present was established. Comparative studies were then undertaken on identical samples.

In order to establish the efficacy of recovery of known amounts of fluorine with magnesium acetate, six urine samples were selected, the fluorine present was determined, and varying amounts of fluorine were added to each sample. Although the added amounts of fluorine varied from 20 to 100 micrograms, 100 per cent recovery was secured in every case (Table I).

Since these results were promising, 20 food samples were selected and duplicate analyses performed on each; the technique used was the same as for the urine samples except that 7.5 ml. of 60 per cent magnesium acetate tetrahydrate were substituted for 1.5 grams of magnesium peroxide per 150 grams of macerated mixed food samples (Table II).

To check the reproducibility of duplicate analyses, 44 food samples were selected and divided into two sets of 22 samples each; one set was analyzed in duplicate with magnesium per-

TABLE II. RESULTS OF DUPLICATE ANALYSES

Food Sample	F Found with MgO_2	F Found with MgAc
	Mg.	*Mg.*
1	0.147	0.125
2	0.104	0.108
3	0.081	0.094
4	0.058	0.073
5	0.093	0.082
6	0.129	0.112
7	0.084	0.068
8	0.156	0.142
9	0.139	0.132
10	0.136	0.147
11	0.154	0.174
12	0.068	0.067
13	0.211	0.193
14	0.119	0.124
15	0.147	0.129
16	0.099	0.083
17	0.121	0.135
18	0.091	0.101
19	0.198	0.176
20	0.183	0.203

TABLE III. DUPLICATE FLUORINE DETERMINATIONS

(MgO_2 as ashing agent)

Food Sample No.	Fluorine Found		Average	Variation	
	Aliquant I	Aliquant II			
	Mg.	*Mg.*	*Mg.*	*Mg.*	%
1	0.180	0.148	0.164	0.032	19.5
2	0.144	0.166	0.155	0.022	14.4
3	0.180	0.147	0.163	0.033	20.1
4	0.166	0.260	0.213	0.094	44.2
5	0.096	0.066	0.081	0.030	27.0
6	0.197	0.167	0.182	0.030	16.6
7	0.152	0.176	0.164	0.024	14.2
8	0.102	0.107	0.104	0.005	4.8
9	0.178	0.159	0.168	0.019	11.3
10	0.320	0.227	0.273	0.093	34.2
11	0.170	0.202	0.186	0.032	17.2
12	0.157	0.116	0.177	0.041	23.1
13	0.189	0.169	0.179	0.020	11.2
14	0.189	0.168	0.179	0.021	11.7
15	0.119	0.158	0.138	0.039	28.3
16	0.071	0.056	0.063	0.015	23.9
17	0.060	0.161	0.110	0.101	91.5
18	0.079	0.116	0.096	0.037	38.4
19	0.146	0.116	0.131	0.030	22.9
20	0.090	0.078	0.084	0.012	14.3
21	0.254	0.184	0.219	0.070	31.9
22	0.250	0.182	0.216	0.068	31.4
				Av. 0.040	26.0

TABLE IV. DUPLICATE FLUORINE DETERMINATIONS

[$Mg(C_2H_3O_2)_2.4H_2O$ as ashing agent]

Food Sample No.	Fluorine Found Aliquant I	Aliquant II	Average	Variation	
	Mg.	*Mg.*	*Mg.*	*Mg.*	%
1	0.129	0.112	0.120	0.017	14.1
2	0.084	0.064	0.074	0.020	36.9
3	0.156	0.142	0.149	0.014	9.4
4	0.139	0.132	0.135	0.007	5.1
5	0.136	0.140	0.138	0.004	2.9
6	0.164	0.174	0.169	0.010	5.9
7	0.068	0.067	0.067	0.001	1.4
8	0.107	0.107	0.107	0.000	0.0
9	0.050	0.047	0.048	0.003	6.3
10	0.139	0.139	0.139	0.000	0.0
11	0.075	0.090	0.082	0.015	18.3
12	0.056	0.053	0.054	0.003	5.6
13	0.087	0.084	0.085	0.003	3.5
14	0.075	0.073	0.074	0.002	2.7
15	0.092	0.106	0.099	0.014	14.1
16	0.066	0.070	0.068	0.004	5.9
17	0.048	0.047	0.047	0.001	2.1
18	0.062	0.076	0.069	0.014	20.0
19	0.117	0.094	0.106	0.023	21.5
20	0.116	0.129	0.122	0.013	10.6
21	0.092	0.100	0.096	0.008	8.3
22	0.083	0.082	0.082	0.001	1.2
Av.	0.097	0.097	0.097	0.008	8.2

oxide as the ashing agent (Table III), and the other likewise with magnesium acetate (Table IV). The results might give the unwarranted inference that determinations made with magnesium peroxide gave higher fluorine values in comparable samples. Actually the two sets of samples differed in their fluorine content, their variability in this respect being in no wise unusual. (Experience has shown that considerable variability occurs from week to week as an expression both of dietary selection and of differences in the fluorine content of the same articles obtained at different seasons and from different localities.)

It is obvious from Tables III and IV that the use of magnesium acetate has increased the reproducibility of the results not only on samples of low fluorine content but also over the entire range of quantities dealt with.

Method

A duplicate of the food consumed in each 24-hour period is collected in glass-topped, quart-size, fruit jars. The composite sample is ground in a household electric mixer with grinding attachment, the wet weight is determined, and the ashing agent is added. If magnesium peroxide is employed, 1.0 gram per 100 grams of wet food is added; if magnesium acetate (60 per cent aqueous solution), 5 ml. per 100 grams. Mixing is continued for 0.5 to 1.5 hours, depending upon the size and character of the sample. Two 150-gram aliquants are weighed into 200-ml. nickel evaporating dishes, dried on an electric burner, and then placed in the automatically controlled electric muffle furnace, set at 570° C. After complete combustion, the ash is weighed and prepared for distillation.

The fluoride is separated from the ash by distillation with perchloric acid according to MacIntire and Hammond's (*4*) modification of the method of Willard and Winter (*5*). Silver sulfate, as recommended by McClure (*3*), is added to the ash to prevent volatilization of the fluorides.

The back-titration method of Dahle, Bonnar, and Wichman (*1*) is employed from this point on; the unconcentrated distillate is used.

Conclusions

Comparative results indicate that magnesium acetate can be used advantageously in ashing organic materials in preparation for fluorine analysis. Since the blank is negligible, good results can be obtained when fluorine is present in either high or low concentrations. The reagent can be handled in solution, thus saving the time required to weigh solid agents.

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Determination of Hydrazine

A Rapid Ferricyanide-Ceriometric Method

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CERIC sulfate as a standard oxidant in titrimetry has been used more and more in the last few years, partly because of the availability of an increasing number of good reversible redox indicators (*9*). The many advantages of this reagent—its extreme stability, simplicity of chemical change, and use in hydrochloric acid solution—make it far superior to potassium permanganate, and in many respects to potassium dichromate.

There are many different methods in the literature for the determination of hydrazine.

Ray and Sen (*10*) were the first to determine the quantitative relationship between hydrazine and alkaline ferricyanide solutions. The reaction proceeds as follows:

$$N_2H_4 + 4K_3Fe(CN)_6 + 4KOH \longrightarrow N_2 + 4K_4Fe(CN)_6 + 4H_2O$$

They made this reaction the basis for a gasometric method for the determination of hydrazine. The reaction was found to take place without the formation of ammonia, as is the case when many oxidizing agents react with hydrazine. Cuy and Bray (*5*) showed that the oxygen error is negligible if the alkali is added after the ferricyanide. They determined conclusively that in alkaline solution it is the atmospheric oxidation of the hydrazine that produces a weakening of the solution and not the decomposition of the hydrazine. They also suggested a titrimetric method for hydrazine, in which excess of standard potassium ferricyanide solution is added to the alkaline hydrazine solution and the excess ferricyanide determined iodometrically. However, since several more rapid methods were available, they did not recommend this procedure for the determination of hydrazine. Benrath and Ruland (*2*) state that hydrazine is oxidized by ceric sulfate to nitrogen and ammonia according to the following equation:

$$2Ce(SO_4)_2 + 2N_2H_4 \longrightarrow N_2 + (NH_4)_2SO_4 + Ce_2(SO_4)_3$$

However, Lang (*8*) determined hydrazine and hydrazoic acid together by adding an excess of standard ceric sulfate solution, treating the excess with an excess of standard arsenious acid, and completing the titration with standard ceric sulfate. The hydrazine was then determined in a separate sample by the iodine cyanide procedure.

The purpose of the present work was to develop a simple method for the determination of hydrazine by making use of potassium ferricyanide and ceric sulfate. The method con-

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sists in the oxidation of hydrazine with an alkaline ferricyanide solution, and the titration with a standard ceric sulfate solution of the ferrocyanide which is produced. Potassium ferrocyanide is readily titrated with standard ceric sulfate, either potentiometrically (*1*, *6*, *12*) or with indicators (*3*, *6*, *13*) such as *o*-phenanthroline ferrous complex and diphenylamine.

Preparation of Solutions

Hydrazine Sulfate. An approximately 0.1 *N* solution was prepared by dissolving about 3.3 grams per liter, and was standardized by the well-known iodate-thiosulfate method of Bray and Cuy (*4*).

Potassium Ferricyanide, 0.5 *M* solution.

Ceric Sulfate. An approximately 0.1 *N* solution was prepared by dissolving 80 grams of ceric ammonium sulfate in 500 ml. of 2 *N* sulfuric acid and diluting to 1 liter. This solution was standardized against pure potassium ferrocyanide, following the directions of Furman and Evans (*6*).

Sodium Hydroxide, 6 *M* solution.

Hydrochloric Acid, 6 *M* solution.

Table I. Effect of Ferricyanide Concentration

(10 ml. of 6 *M* NaOH, time 2 minutes, 30 ml. of 6 *N* HCl, final volume 100 ml.)

No.	Hydrazine Sulfate	0.5 *M* $K_3Fe(CN)_6$	0.1054 *N* $Ce(SO_4)_2$	Normality of Hydrazine
	Ml.	*Ml.*	*Ml.*	
1	19.84	5	19.12	0.1016
2	19.84	6	19.80	0.1052
3	19.84	7	19.96	0.1062
4	19.84	8	19.98	0.1063
5	19.84	10	19.98	0.1063
6	19.84	15	...	....

Procedure

The procedure consisted of adding to a measured volume of hydrazine sulfate solution an excess of potassium ferricyanide, making alkaline with sodium hydroxide, shaking for 0.5 minute, and letting stand for 2 minutes. The mixture was then acidified with hydrochloric acid, diluted to 100 ml., and titrated with standard ceric sulfate solution.

There is sufficient ferric iron present in the ceric ammonium sulfate, and most other sources of ceric ions, so that colloidal ferric ferrocyanide forms during titration of the ferrocyanide. At the end point the green color, caused by the colloidal ferric ferrocyanide, sharply disappears and the solution has the brownish color of the ferricyanide ions. The writers prefer to add a few drops of 0.5 *M* ferric chloride near the end of the titration to give a sharper end point, especially in the presence of excess ferricyanide ions. Hexanitrato ammonium cerate may also be used as standard oxidant as indicated by Smith, Sullivan, and Frank (*11*). However, since there is not enough iron present in this highly purified substance, it will be necessary to add a few drops of ferric chloride near the end point in order to form the green colloidal ferric ferrocyanide. If the ferric chloride is added too soon, a blue precipitate of ferric ferrocyanide is formed and thus some ferrocyanide is lost. Diphenylamine may also be used as indicator (*3*), but the solution must be diluted to about 0.01 *N* ferrocyanide concentration before titration with ceric sulfate. The writers found some difficulty in obtaining a sharp end point with diphenylamine in the presence of a large excess of ferricyanide.

Effect of Alkalinity

Experiments showed that the oxidation of hydrazine by ferricyanide was complete in solutions of either low or high alkalinity.

Effect of Excess Potassium Ferricyanide

Theoretically 20 ml. of 0.1 *N* hydrazine sulfate solution should require 4 ml. of 0.5 *M* potassium ferricyanide solution for complete oxidation. Table I shows results obtained using varying amounts of ferricyanide, other conditions remaining constant. In Nos. 1 and 2, containing 1 and 2 ml. more than the theoretical amount of potassium ferricyanide, the oxidation was incomplete in the 2-minute interval, and even after a 5-minute interval, on a run containing 5 ml. of ferricyanide solution. In No. 6 the color change was not sufficiently sharp to indicate the end point. Too large an excess of ferricyanide imparts a dark red-brown coloration, and the transition from green to red-brown is not distinct enough to serve as the end point. However, in a potentiometric titration, this excess would not interfere with the end point.

The normality of the hydrazine sulfate solution obtained by the Bray and Cuy iodate-thiosulfate method (*4*) was 0.1062 *N*.

Effect of Time

Table II shows the effect of time on the reaction. With 10 ml. of 6 *M* sodium hydroxide solution in 30 ml. of solution, oxidation is complete if the solution is shaken gently for 0.5 minute after mixing the reagents with the hydrazine and then allowed to stand for another 1.5 minutes. Prolonged standing before acidification produces no error. The same results were obtained on a run which stood for 35 minutes as on one that stood for 6 minutes.

Effect of Final Acidity on Titration

Furman and Evans (*6*) state that the acid concentration should be between 0.5 and 2.0 *M* when ferrocyanide is titrated with ceric sulfate. The writers found that when the free hydrochloric acid in the final solution was more than 30 ml. of 6 *M* acid per 100 ml. of solution the visual end point was not very decisive, as there was not a sharp change from green to brown. However, 15 to 25 ml. of 6 *M* hydrochloric acid per 100 ml. of solution gave good results.

Recommended Procedure

To 25 to 35 ml. of approximately 0.1 *N* hydrazine sulfate solution or approximately 0.1 gram of hydrazine sulfate accurately weighed and dissolved in 25 ml. of water in a 250-ml. Erlenmeyer flask, add 10 ml. of 0.5 *M* potassium ferricyanide solution, followed by 10 ml. of 6 *M* sodium hydroxide solution. Shake gently for 0.5 minute and let stand for at least another 2 minutes. Add 30 ml. of 6 *M* hydrochloric acid and dilute to 100 ml. Titrate with 0.1 *N* ceric ammonium sulfate solution until the green color in the solution just disappears and the solution has a brownish color. For a sharper color change, 2 or 3 drops of 0.5 *M* ferric chloride solution may be added within 4 or 5 ml. of the end point.

Results

Table III gives the results obtained on another hydrazine sulfate solution by both the recommended procedure and the iodate-thiosulfate method (*4*). Table IV gives the results obtained with a sample of Eastman hydrazine sulfate which had been dried for 3 hours at 140° C. A different standard ceric ammonium sulfate solution was used for these determinations.

Table II. Effect of Time

[10 ml. of 6 *M* NaOH, 10 ml. of 0.5 *M* $K_3Fe(CN)_6$, 30 ml. of 6 *M* HCl, final volume 100 ml.]

No.	Hydrazine Sulfate	Time	0.1054 *N* $Ce(SO_4)_2$	Normality of Hydrazine
	Ml.	*Min.*	*Ml.*	
1	19.84	1	19.86	0.1055
2	19.84	1½	19.93	0.1061
3	19.84	2	19.96	0.1062
4	19.84	6	19.98	0.1063
5	19.84	35	19.98	0.1063

Table III. Determination of Normality of Hydrazine Solution

No.	Hydrazine Sulfate	0.1054 *N* Ceric Sulfate	Normality of Hydrazine Solution	
			By ceric sulfate	By iodate-thiosulfate
	Ml.	*Ml.*		
1	21.86	21.76	0.1049	0.1047
2	27.55	27.39	0.1048	0.1046
3	22.87	22.75	0.1049	0.1048
4	24.19	24.04	0.1048	....
			Av. 0.1048	0.1047

Table IV. Per Cent Purity of Hydrazine Sulfate

No.	Hydrazine Sulfate	0.09858 N Ceric Sulfate	Purity By ceric sulfate	Purity By iodate-thiosulfate
	Gram	*Ml.*	%	%
1	0.1055	32.85	99.85	99.81
2	0.1072	33.39	99.88	99.85
3	0.1020	31.75	99.82	99.80
4	0.1021	31.77	99.78	99.88
			Av. 99.84	99.83

Summary

A new titrimetric method for the determination of hydrazine, in which ceric sulfate is the standard oxidant, gives results which check very closely with those obtained by the Bray-Cuy iodate-thiosulfate method (*4*), but it is much more rapid and the cost of reagents per determination is less than a third as much.

The titration is much more easily carried out than the titration in the Jamieson iodate method (*7*), in which the end point is dependent upon the disappearance of the purple iodine color in the chloroform layer. This necessitates closing the titration flask and shaking vigorously after the addition of each drop of iodate solution near the end point, a slow, tedious process.

The main disadvantage of the method lies in the regulation of the amount of ferricyanide to be used. If the excess over the theoretical amount is too small, the reaction with hydrazine will be incomplete, and if the excess is too great, the end point of the titration cannot easily be seen. Ferricyanide should not be added beyond the point at which it just begins to cause a reddish-brown coloration.

The solution during the final titration should be not more than 1.8 *M* in hydrochloric acid.

The method, when properly carried out, is capable of giving results reproducible within less than 0.1 per cent, with a percentage error of about 0.02 to 0.11 per cent compared with the iodate-thiosulfate method (*4*).

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A Sodium-Hydrogen Geissler Tube

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THE need for special glass, preheated electrodes, and a vacuum jacket places the construction of the common sodium vapor arcs (*1*) beyond the resources of the average worker. Such an arc requires a separate installation in addition to the equipment needed for the operation of Geissler tubes.

In connection with the problem of supplying a suitable light source for a Pulfrich refractometer a very satisfactory Geissler tube was developed in this laboratory. It gives a good sodium line as seen through the telescope of the instrument and, under certain conditions, permits focusing on the C and F lines of hydrogen. Since these three spectral lines are frequently encountered in tabulations of refractive index (*3*), this tube serves as a valuable addition to the Geissler tubes used with the refractometer.

The design of the tube is such that it may be constructed at a nominal cost by anyone who has mastered the art of making a small ring seal. Pyrex glass is used and no elaborate technique is required in introducing the sodium because of the presence of an inner shield which effectively prevents surface impurities on the metal from reaching the glass walls that must maintain a pressure difference. The introduction of a stopcock permits the tube to be used while it is undergoing preliminary pressure fluctuations, thus eliminating the need for extreme care in the initial construction.

Construction of Tube

Figure 1 shows the tube with the sodium electrodes, *S* and *S'*, in place. With the exception of these electrodes, the glass-wool plugs, *G*, and the rod, *D*, the diagram represents the tube as it leaves the glass-blowing table. A mercury manometer (not shown) was also included as an integral part of several of the tubes studied. The glass blowing involved is simple. Each arm of the tube was made as a separate unit, the tungsten electrodes, *W* and *W'*, being introduced after the ring seals required to secure the shields, *F* and *F'*, were finished. The tungsten wire was prepared by a method suggested by W. D. Kumler. It was outgassed by heating to a high temperature in the oxygen-gas flame, cleaned by applying a film of sodium nitrite to the hot wire, and washed with water. The bright wire was re-oxidized in the Bunsen flame before fusing the sleeve of Pyrex tubing around it to give the characteristic orange-colored seal. The two completed arms were then connected with a short length of approximately 1-mm. capillary as shown, and the stopcock and manometer were added.

Charging and Filling

The sodium electrodes, *S* and *S'*, are prepared by forcing commercial metallic sodium into lengths of Pyrex tubing. This operation is most conveniently carried out by pressing one end of the tubing, lubricated with a drop of kerosene, into a freshly cut surface of a large piece of sodium. The completed electrode is immediately lowered into place with the aid of the glass rod, *D*, which is prepared for this purpose. The point of the rod is so shaped that it will hold the sodium cylinder but will not break the small tubing when pressure is exerted to force the other end of the sodium cylinder over the tungsten wire. The rod is removed by twisting while pulling very gently. The upper Pyrex-wool plugs, *G*, are added and the tubes, *E* and *E'*, sealed off in the glass-blowing torch under ordinary conditions. The tube is evacuated, gently flamed, and filled with hydrogen several times before use. The hydrogen inlet is designed to permit displacement of air in tube *A*, by passing hydrogen through the capillary, *B*, before the rubber tubing, *C*, is forced into place and the stopcock opened. Commercial electrolytic hydrogen was drawn from a tank equipped with a reduction valve.

Operation

The tubes were operated from the secondary of a transformer capable of delivering 23 milliamperes at 10,000 volts. A current between 15 and 20 milliamperes has been found to give satisfactory intensity of sodium light. Control is accomplished by inserting a variable resistor in the primary circuit or one or two 100,000-ohm, 50-watt resistors in series with the tube in the secondary circuit, or by choosing a working pressure of hydrogen that produces a good light intensity without serious overheating. If the tubes are to be used only as a source of sodium light they will operate successfully at any pressure between 2 and 50 mm. of mercury. However, to make use of the C and F lines of hydrogen as well as the sodium D line, a low pressure of hydrogen (2 to 5 mm. of mercury) must be established and the current controlled by means of resistors. The exact values of the current or pressure are not critical factors, except that the current must produce sufficient light intensity without dangerous overheating, and the pressure must be low if the hydrogen lines are wanted.

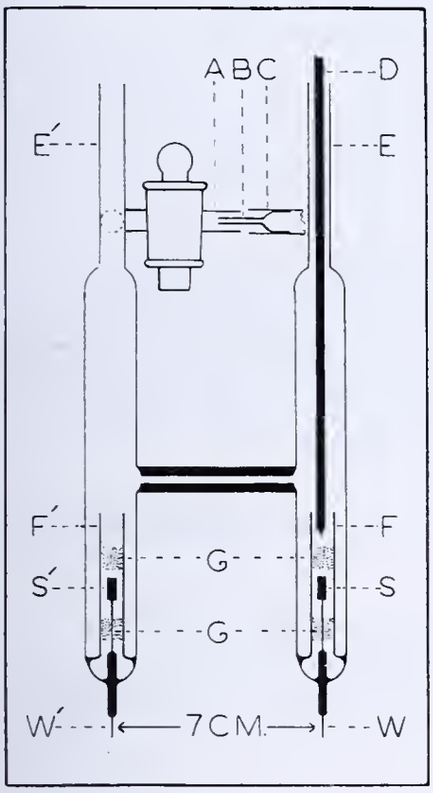

FIGURE 1

A new tube is filled to a pressure between 10 and 20 mm. of mercury and operated with a current of about 20 mm. for 30 minutes, or until the discharge in the capillary takes on the color of the sodium D line. The tube is then evacuated and refilled with hydrogen to whatever pressure seems to be indicated by the previous behavior or by the type of discharge desired. After the preliminary heating has once produced a strong sodium light in the capillary, the characteristics of the tube change. The same current that produced the first sodium light in 20 or 30 minutes will accomplish the same result in one tenth of the time. A deposit gradually forms on the cooler surfaces, and the glass of the inner shield darkens and shows signs of erosion. This change does not affect the usefulness of the tube if the glass-wool plugs are well packed; the inner shields are not required to maintain a vacuum, and the light intensity is sufficient to permit focusing on the side of the capillary. During the early life of the tube the pressure fluctuates to such an extent that it is often necessary to evacuate the tube and refill it with hydrogen. Later, however, the pressure becomes constant, and, after several hours of stable operation with maximum current, the stopcock may be removed with the torch to avoid the possibility of slow leaks.

Tubes built to the specifications given above have been used in this laboratory for several years as a light source for the Pulfrich refractometer. The identity of the three bright lines observed in tubes operating at low pressures has been established by measuring the refractive index of distilled water at 20° C. and comparing the results with the values tabulated in the literature (*2*) and with readings taken when a commercial sodium arc was used as the light source. The agreement was well within the accuracy of the instrument and indicates that the red C line of hydrogen (6563 Å.), the yellow D line of sodium (5893 Å.), and the blue F line of hydrogen (4861 Å.) are produced. Other lines in the green and orange and a continuous spectrum in the violet are too faint to cause confusion.

The intensity of the sodium line developed in seasoned tubes after 2 or 3 minutes of operation is equal to or greater than that of hydrogen lines observed in ordinary Geissler tubes. Although the useful life of these tubes is not so long as that of ordinary Geissler tubes, one tube containing 10 mg. of sodium operated continuously as a source of the sodium line for 50 hours. The capillary was badly etched at the end of this period but again emitted sodium light for a few minutes after the deposit inside the tube had been heated in a gas flame, indicating that failure had been due, not to the condition of the glass, but to the loss of sodium from the electrode region. The condition of the glass is of greater importance when the hydrogen lines are sought. Because of this fact, and also because tubes operating at low pressures tend to overheat if allowed to carry normal current continuously, the life of a tube as a simultaneous source of hydrogen and sodium lines is short unless the current is turned off and the tube allowed to cool at frequent intervals.

Discussion

Aside from the mechanical design which permits the use of sodium that has been briefly exposed to ordinary air, the successful operation of the tube is associated with the action of hydrogen on sodium at high temperatures (*4*, *5*). The observed facts which support this conclusion are: The time required for a satisfactory emission of the sodium line is much less on the second and subsequent heatings than on the first; a deposit forms on the cooler portions of the tube; under certain conditions there is a sharp decrease in pressure inside the tube during the first few hours of operation; when the tube is operated at low pressures the deposit may consist of bands of different colors, principally blue, gray, black, and orange and sometimes metallic, but, with careful heating at higher pressures of hydrogen, a gray or white nonmetallic deposit is observed; this nonmetallic substance may be sublimed from one portion of the tube to another without discoloring the glass; and when the tube is broken open and water added this deposit dissolves with the evolution of a gas.

It is concluded that sodium hydride forms during operation of the tube and that this substance is responsible for the rapid development of the sodium line after the tube is lighted.

Summary

A Geissler tube of Pyrex glass capable of emitting a strong sodium D line is easily constructed and charged with commercial metallic sodium and tank hydrogen and, under controlled conditions, emits the C and F lines of hydrogen and the D line of sodium simultaneously. As a source of the sodium line the tube operates efficiently over a wide pressure range, has a long life, and, after the first few hours of operation, requires no more care than any other Geissler tube.

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A Flask for Separation of Serum from Blood

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THE centrifugation of relatively large amounts of whole blood in the ordinary equipment involves time-consuming operations. In freeing the clot from the sides of the centrifuge bottles in order to obtain a better separation of serum from the clot, considerable hemolysis may unavoidably be produced. The simpler method of allowing the blood to clot, undisturbed, in a plain vessel, is much used in obtaining serum free of red blood cells and products of hemolysis.

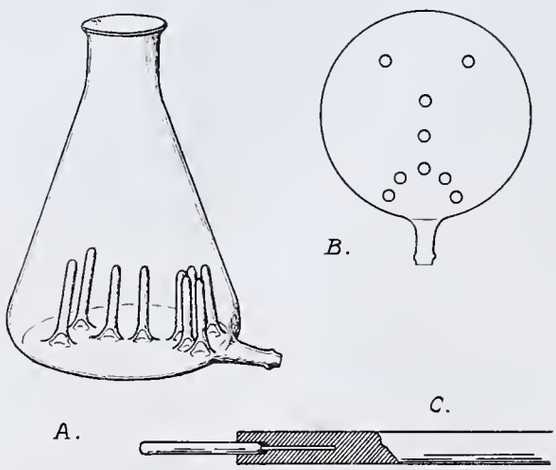

FIGURE 1. FLASK FOR SEPARATION OF SERUM FROM BLOOD

A. Normal view
B. Plan view of bottom, showing arrangement of glass pegs and their relation to outlet tube
C. Wooden dowel, partly in section, with glass rod in place ready for fusion to bottom of flask

The contracting clot retains the red cells and extrudes clean serum. However, it is difficult to recover the last portion of the serum, either by drawing it off through a tube or by pouring it off from the clot, without bruising the clot and liberating small amounts of cells or hemoglobin. In the vessel shown in Figure 1, this difficulty is overcome and the yield is increased by the simple expedient of placing a number of pegs in the bottom of the flask. The clot forms and contracts on the pegs, which hold it without bruising while the serum drains off completely through the outlet tube. The flask has been used routinely in this laboratory for several years for the recovery of goat serum.

During filling, the flask is tipped away from the outlet tube, and the blood is delivered through a clean gum-rubber tube to the bottom of the flask until the pegs are covered and the edge of the pool of blood reaches to just below the outlet tube. The vessel is held in this position on a suitable support until a firm clot has formed and some of the serum has begun to separate from it. The flask is then tipped back to or slightly beyond the upright position and held thus until the serum has all been extruded and has drained through the outlet tube into a receiving vessel.

An assembly of apparatus including a needle, the flask, a receiver for the serum, and connecting rubber tubing, can be sterilized as a unit and used to obtain sterile serum from blood taken directly from the vein of the animal. For this purpose only standard devices and familiar bacteriological technique are required.

In making the vessel, the outlet tube is first fused onto the side of a 1-liter Erlenmeyer flask near the bottom, and then the glass rods are fused in place. The rods are conveniently held, during fusing, in a hole of slightly smaller diameter than the rod in the end of a wooden dowel which is split to a distance of about 50 mm. from the end (*C*). The bottom of the flask is heated strongly at the point where the rod touches it. While this region of the glass is soft, the rod is lifted about 5 mm. to form an inwardly directed cone at the base of the rod to decrease the internal stresses in the glass. The glass is then allowed to cool slightly, so that the wooden dowel can be pulled away from the rod. During the entire process, the lower half of the flask must be kept hot by frequent heating in a bushy flame, and at the end, the finished vessel must be placed immediately in an annealing oven at between 550° and 570° C. If it is not annealed, the vessel will inevitably crack, but it remains strong indefinitely if well annealed. The outlet tube is of 7-mm. (outside diameter) glass tubing. The rods used for pegs are of about 6-mm. diameter, and are between 35 and 40 mm. long near the outlet and between 50 and 60 mm. long at the opposite side. The pattern of placement of the pegs (*B*) promotes contraction of the clot toward the center and provides a weir to prevent blocking of the outlet by the sagging clot.

The design of the 1-liter flask described can, of course, be used in both smaller and larger vessels, and the use of pegs may be applicable in vessels of sufficient size for commercial use. Flasks of the type described should be especially useful for the recovery of maximum yields from unavoidably limited sources—for example, for the separation of convalescent serum for immunization.

An Ether-Insoluble Stopcock Lubricant

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AND

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THE conventional type of stopcock grease was found unsatisfactory for the lubrication of separatory funnels in which lipids were extracted from an aqueous suspension with ethyl and petroleum ethers. The greases were sufficiently soluble in the ethers to cause, in certain cases, a significant increase in the weight of the extract. Omission of the lubricant was impractical because of leakage, and because the ungreased stopcock would stick and not allow the precise adjustment needed for the exact separation of the two layers.

In order to overcome these difficulties, a starch-glycerol gel was tried as lubricant and found very satisfactory. Although it is slowly soluble in water, one application proved sufficient for fifteen successive extractions. It is not dissolved by the ether layer; no increase in weight of extract could be detected even when some of the lubricant was placed directly in the funnel.

This particular gel was prepared by suspending 9 grams of soluble starch in 22 grams of glycerol and heating to 140° C. After standing a short time, the clear solution was decanted from the sediment in the bottom and allowed to cool. The next morning, this mixture had the consistency of a heavy grease. It has proved very satisfactory for the authors' purpose, and may prove useful to others who need a lubricant which will not dissolve in nonpolar compounds.

Aniline Point Determinations with Dark-Colored Oils

A Circulating Test Tube

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ANILINE point determinations of transparent oils are commonly made in an air-jacketed test tube as recommended by Tizard and Marshall (*4*) and prescribed by the American Society for Testing Materials (*1*). When dark oils must be tested, visual observation fails to detect turbidity in layers of liquid 20 to 25 mm. thick, and various other schemes have been proposed and used.

Donn (*2*) proposes determining the temperature at which a break occurs in the viscosity-temperature curve. Van Wijk and Boelhouwer (*5*) and Matteson, Zeitfuchs, and Eldredge (*3*) detect changes in transparency to infrared radiation. Makeshift methods, such as decolorizing the dark oil with adsorbents or diluting with water-white distillates of known aniline point and calculating on the basis of assumed additivity, have also been used.

The writers have been favorably impressed with the method of Matteson, Zeitfuchs, and Eldredge, but note that it does not permit direct measurement of the temperature of the thin layer of oil that is under observation. The circulating test tube developed by the authors, prior to the appearance of the publication by Matteson and his associates, avoids this disadvantage and it might be practical to modify their apparatus to permit its use.

The circulating test tube described below was devised primarily to permit testing oils which were too dark for the conventional apparatus and procedure but not absolutely opaque when dissolved in an equal volume of aniline and observed in layers approximately 2 mm. thick. It has proved entirely adequate for existing needs with the simple accessories that are described herein and is considered more convenient and accurate for light-colored oils than the apparatus prescribed by the A. S. T. M.

Apparatus

The tube is in the form of a U, with a cross tube connecting the two arms about halfway up. The cross tube is flattened in the middle to form a "cell" about 1 to 2 mm. thick and about 20 mm. wide. A tube of proper dimensions to hold 18 to 20 ml. of a mixture of equal volumes of aniline and oil is shown in Figure 1.

A piece of sheet metal with the edges rolled and an aperture 10 mm. in diameter in the center, as shown in Figure 2, is fitted to the U-tube with the aperture directly back of the flattened part of the cross tube. The tube could, if desired, be insulated or en-

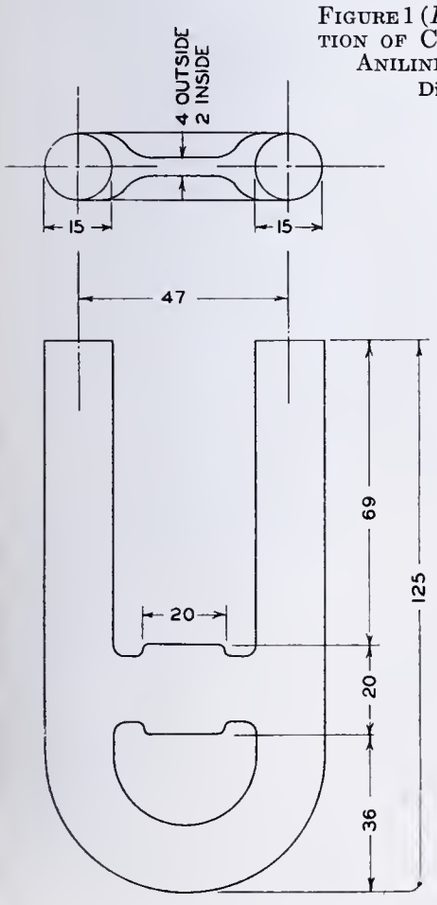

FIGURE 1 (*Left*). DESIGN AND CONSTRUCTION OF CIRCULATING TEST TUBE FOR ANILINE POINT DETERMINATIONS
Dimensions in millimeters

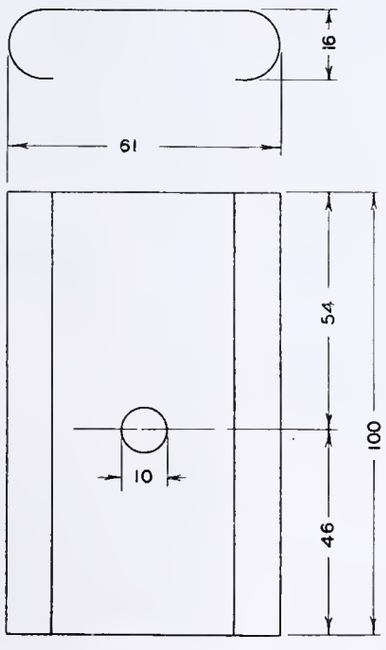

FIGURE 2. DESIGN AND CONSTRUCTION OF METAL SHIELD HOLDING GLASS TEST TUBE
Dimensions in millimeters

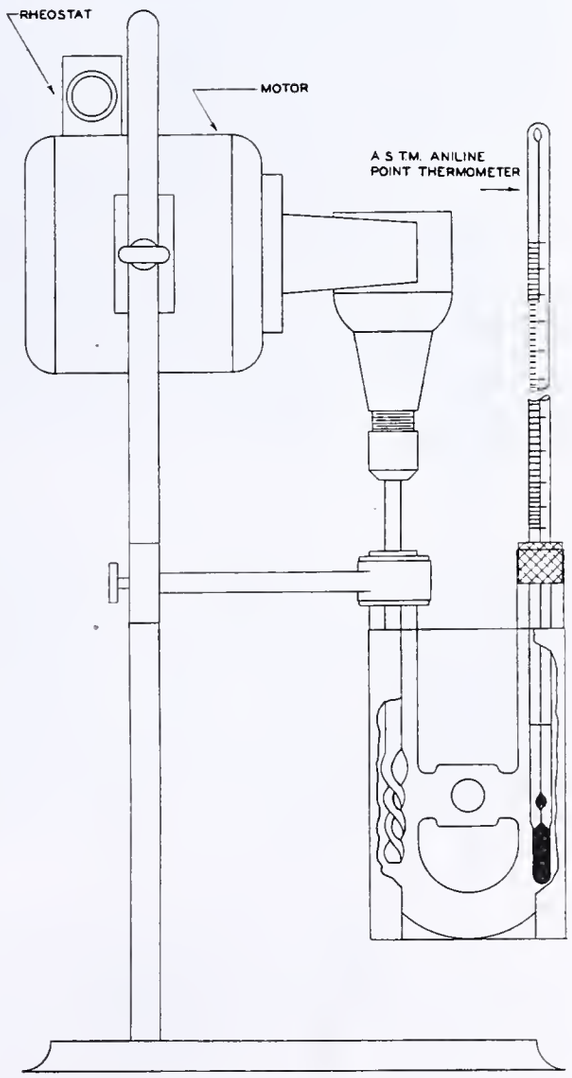

FIGURE 3. ASSEMBLY OF CIRCULATING TEST TUBE, METAL SHIELD, AND ACCESSORIES

Table I. Agreement between Aniline Points as Determined with Circulating Test Tube and A. S. T. M. Method and Apparatus

Product	Color[a]	Aniline Points A. S. T. M. ° C.	Aniline Points New method ° C.
A. S. T. M. precipitation naphtha	25 + S	58.6	58.4
Stoddard solvent	25 + S	57.2	57.5
V. M. & P. naphtha	25 + S	54.5	54.6
Kerosene	25 + S	64.7	65.0
Kerosene	25 S	71.1	71.0
Motor gasoline	17 S	45.2	45.0
Pale Diesel fuel oil	1.5 U	67.2	67.1
No. 2 domestic fuel oil	2.0 U	58.0	58.2
Pale Diesel fuel oil	2.5 U	62.2	62.4
Pale Diesel fuel oil	3.5 to 4.0 U	58.5	58.8
Dark Diesel fuel oil	1/2 T-R	53.6	53.7
Filtered cylinder oil	1/2 T-R	124.0	124.0
Hydraulic oil	3/8 T-R	Too opaque	97.0
Dark Diesel fuel oil	3/8 T-R	Too opaque	57.8
Dark Diesel fuel oil	1/4 T-R	Too opaque	56.0
Dark Diesel fuel oil	About 1/4 T-R	Too opaque	55.6
Coastal black lubricating oil	Darker than 1/4 T-R	Too opaque	82.5
Steam cylinder stock (unfiltered	Darker than 1/4 T-R	Too opaque	131.5

[a] S, Saybolt color; U, A. S. T. M.-Union color; T-R, Tag-Robinson color; 8 U is approximately the same as 1 T-R.

closed to ensure a more gradual change of temperature, but the results obtained have been so satisfactory that there is a doubt as to the justification for more complicated apparatus.

Method of Operation

In operation, the tube is clamped to an ordinary ring support. An auger-type glass stirrer of the type suggested for the A. S. T. M. method is inserted in one side of the U with the helical section located at the junction of the cross tube and the U. The stirrer is connected to a variable-speed motor. A suitable device is a fractional horsepower motor equipped with a speed-reducing gear and a rheostat. A thermometer is inserted in the opposite side of the U-tube with its bulb immersed in the liquid. The volume of liquid should be such that its surface is about 2 mm. above the level of the thin part of the cell, when the thermometer and the stirrer are in position. A microscope lamp is located with the spot of light directed on the aperture in the metal shield. The aniline-oil mixture may be conveniently heated by directing a small flame against the bend of the tube, or other means of heating the mixture may be employed, if desired. Figure 3 is an illustration of the apparatus, assembled, ready for use. In testing very transparent products the turbidity point can be observed in the body of the tube or in the cell without the aid of artificial light, and for dark products the observation is made in the cell with the aid of a spot light.

Scope

The tubes at present available have cells about 2 mm. thick and permit the testing of oils which are considerably too dark for color determinations with either the A. S. T. M.-Union or the Tag-Robinson colorimeter. The results shown in Table I indicate, in a general way, the scope of these tubes when turbidity is observed visually. Their range can undoubtedly be increased by employing infrared radiation, and some additional latitude may even be attainable with visual observation by decreasing the thickness of the cells. It is obvious that the necessity of keeping the liquid in rapid circulation limits this possibility.

Accuracy

The figures in Table I indicate that the apparatus and procedure, described herein, give results that do not differ from those of the A. S. T. M. method by appreciably more than the 0.2° C. which is considered normal reproducibility for the latter.

The writers usually defer the publication of papers describing analytical methods and apparatus until they have been tried out, over a considerable period of time, in routine service in their own and several associated laboratories. In the present case this has not been done, as it is felt that information should be released promptly regarding the basic principle of the circulating test tube, and that improvements in the details of construction and operation will result from its broad use. There is a widespread need for an aniline point method which retains the simplicity and convenience of the A. S. T. M. apparatus and procedure but which is applicable to moderately dark colored oils.

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Electronic Relays

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SEVERAL electronic relay circuits have been described in the chemical literature (*1–5*), but all have certain undesirable features which have been largely eliminated in the circuit here described.

Electronic relay circuits are useful in all cases where it is desirable to use a small controlling current of the order of a few microamperes. The use of such a small current prevents arcing at the control contacts and consequent fouling. The sensitivity of the control can usually be increased, especially with bimetallic type thermoregulators. If, in addition, the control contacts are subject to mechanical vibration it may also be desirable to introduce a time-delay into the circuit in order to prevent chattering of the relay contacts. Only one of the circuits (*4*) referred to makes any provision for a time-delay. This circuit uses the heating time of the tube filament to produce a time-delay but is not a true electronic relay circuit, in that the circuit uses a rectifier tube instead of an amplifier tube, and the entire filament current, 75 milliamperes, passes through the control contacts. The usual condenser connected across the relay coil really acts as a filter condenser and not as a delay condenser.

The circuit herein described uses a diode beam-power amplifier tube with a separate, independent, rectified grid-bias voltage. The importance of an independent grid voltage supply has not, in general, been appreciated. In the usual circuits such as those of Hawes and Waddle and Saeman, the controlling grid voltage is obtained by the cathode-biasing method. This has two disadvantages in relays. (1) The grid-bias voltage is subtracted from the total voltage available to supply the plate voltage, which may then be too low to derive much power output; (2) this method makes it impossible to cut the plate current off completely, since some current must flow at all times in order to produce a negative grid-bias. This continuous current flowing through the relay may cause the relay to stick or fail to open. Higher-priced, low-differential relays can be used but are unnecessary.

The advantage of a beam-power amplifier tube of the type 117L7GT is its ability to provide high-power output at low plate voltages—for example, the 117L7GT will deliver 45 milliamperes at a plate voltage of 110 volts. In contrast, Waddle and Saeman used a triode amplifier, 6C5, whose maximum plate current is not over 15 milliamperes. Rudy and Fugassi state that an advantage of the cold cathode, starter anode, gas-filled tube, OA4G, is its ability to supply a higher plate current at 110 volts than a high vacuum tube. This statement is erroneous because almost any beam-power amplifier tube will supply a maximum plate current of 40 to 50 milliamperes, whereas the maximum plate current of the OA4G is 25 milliamperes. With large plate currents available almost any relay on hand may be used. Successful operation has been obtained from a Struthers and Dunn relay designed for a coil voltage of 220 volts alternating current, with a direct current resistance of 500 ohms without the use of an intermediate, highly sensitive relay. Such a relay will handle a 30-ampere load.

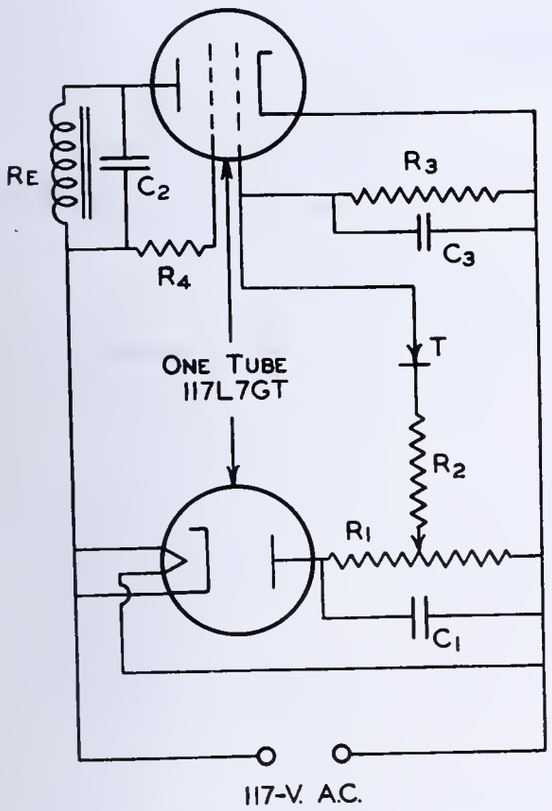

FIGURE 1. RELAY CIRCUIT

C_1, C_2.	4-mfd., 150-volt electrolytic condensers
C_3.	0.05–1.0 mfd., 150-volt paper condenser
R_1.	10,000-ohm, 10-watt potentiometer
R_2.	1-megohm, 0.5-watt resistor
R_3.	5-megohm, 0.5-watt resistor
R_4.	Resistor
R_E.	Relay
T.	Control contacts

The relay circuit shown in Figure 1 utilizes the 117L7GT tube which was introduced recently. Serfass (*3*) has also recently described a circuit which utilizes this tube but has used it in such a manner that the grid-bias voltage is not completely independent of the plate current. The filament of this new tube is designed to be operated directly from the 117-volt alternating current line, eliminating a filament dropping resistor with its resulting inconvenience and power loss.

For clarity the 117L7GT is shown as if it were two tubes. The diode rectifier section furnishes a fixed, rectified, grid-bias voltage. The beam-power amplifier section uses plate rectification of the alternating current which is filtered by means of condenser C_2 and the relay inductance. As shown, the circuit is connected for use with a mercury thermoregulator which closes on rise of temperature. Potentiometer R_1 provides a variable negative grid-bias. It is adjusted by closing the control terminals and adjusting R_1 until the relay just opens. Too fine an adjustment should not be made, as the line voltage may vary during use. Resistor R_2 reduces the current controlled by the relay contacts to a few microamperes. Condenser C_3, with resistors R_2 and R_3, forms a conventional resistance-capacitance time-delay network.

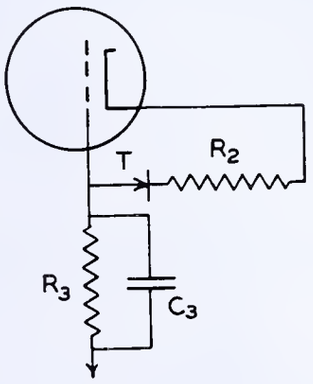

FIGURE 2

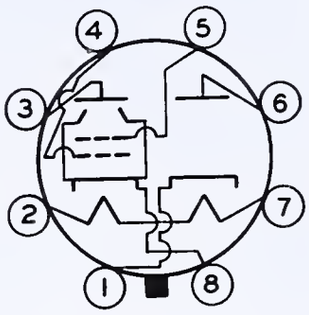

FIGURE 3

Without C_3 no time lag is present, but with C_3 present there is a definite delay of the relay on both making and breaking the control contacts. Suitable values of C_3 have been found to lie between 0.05 and 1 mfd. It is suggested that as a start a 0.05-mfd. condenser be tried. If this does not give sufficient delay to prevent chattering then 0.1 mfd. or larger should be tried. The delay is proportional to the product of R_3C_3 but is also dependent on the value of R_2 and the adjustment of R_1. Of course, if no delay is needed this condenser may be omitted.

Resistor R_4 is a screen voltage dropping resistor whose exact value will depend on the resistance of the relay. The value of R_4 is chosen so that the maximum rated plate and screen currents of the tube will not be exceeded. This is especially necessary when a relay with a low resistance is used. The correct value of R_4 is easily determined by connecting a milliammeter in the plate circuit and changing R_4 until the plate current is not over 45 milliamperes. In some cases R_4 may be omitted and the screen either connected before the relay or tied to the plate, whichever position gives the lowest necessary plate current. These two positions should be tried before using a resistor. If the relay contacts are to open when the control contacts open, Figure 2 shows the necessary circuit changes. For convenience, Figure 3 shows the bottom socket view of the 117L7GT. A very compact apparatus can be constructed because of the use of only one tube. This circuit can be constructed at a cost of approximately two dollars, exclusive of the relay. Several of these relays have been in service for a considerable time and have given very satisfactory performance.

This circuit, which can be used only on alternating current, is not isolated from the supply voltage and there is some danger of accidental shocks when the contact points are touched. However, a 5-megohm resistor in the control circuit greatly reduces this danger. It is, of course, possible to use a transformer to supply the plate voltage and thus eliminate any danger of shocks, but this increases the cost and the weight of the apparatus.

Acknowledgment

The author wishes to express his appreciation to Quick Landis for his assistance in the design of this circuit.

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A Recording Automatic Balance

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THIS paper describes the design of a recording, automatic balance. The instrument, composed essentially of an analytical balance and an old Model R. Leeds & Northrup automatic recording mechanism, employs a motor-driven chain for restoring equilibrium of the balance arm when a change in weight occurs. A recording balance employing a motor-driven chain, but using an electronic balancing system, has been described by Müller and Garman (*1*). Possible advantages of the balance described in this paper over the electronic balance are ease of construction, simplicity of operation, and stability. The sensitivity of the balance could be made equal to that of the electronic balance by use of a more modern balancing mechanism, such as the Leeds & Northrup Micromax.

The balance was constructed for use in rock phosphate sedimentation studies, but other applications suggested by the nature of the instrument involve studies of weight changes, such as dehydration, hygroscopicity, rapid corrosion, and other chemical transitions, and changes in specific gravity.

Figure 1 shows the assembled apparatus. The balance is mounted on a shelf, the recorder mechanism is placed above it, and the sedimentation tube and temperature control are mounted below. The mechanism of the instrument is shown diagrammatically in Figure 2. The material to be weighed is suspended from one end of the balance arm and a chain is suspended either directly from the other end or from the auxiliary weight pan, as shown in Figure 1. The chain is wound on a drum connected to the restoring mechanism. A light-weight A-frame mounted on top of the balance arm engages the galvanometer needle of the recorder. When the balance arm is tilted by an increase of weight, the galvanometer needle is moved to one side, the chopper bar acts on the restoring mechanism, and the drum unwinds the chain to restore equilibrium. When the weight decreases, the needle moves in the opposite direction, the restoring action is brought into play, and the drum winds the chain until

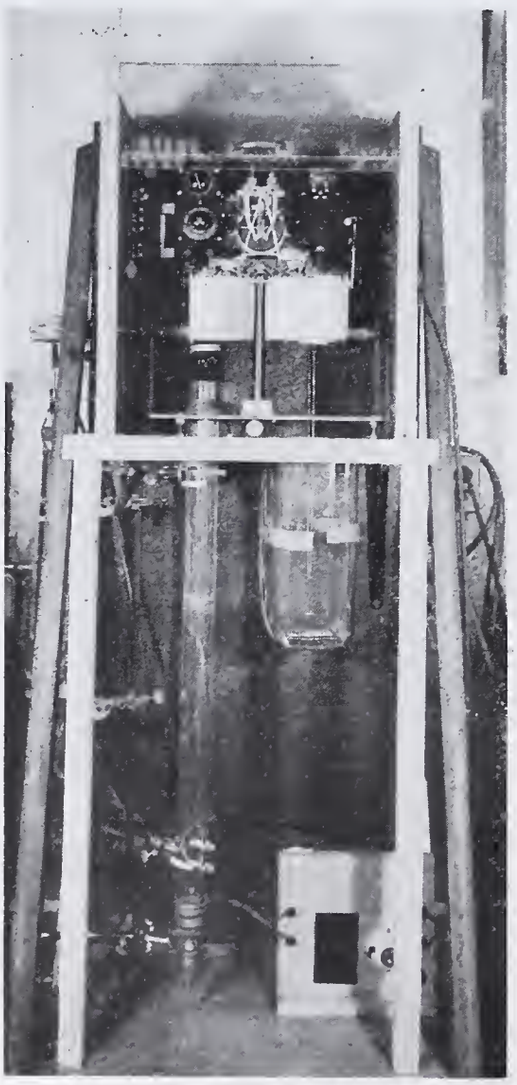

FIGURE 1. ASSEMBLED APPARATUS

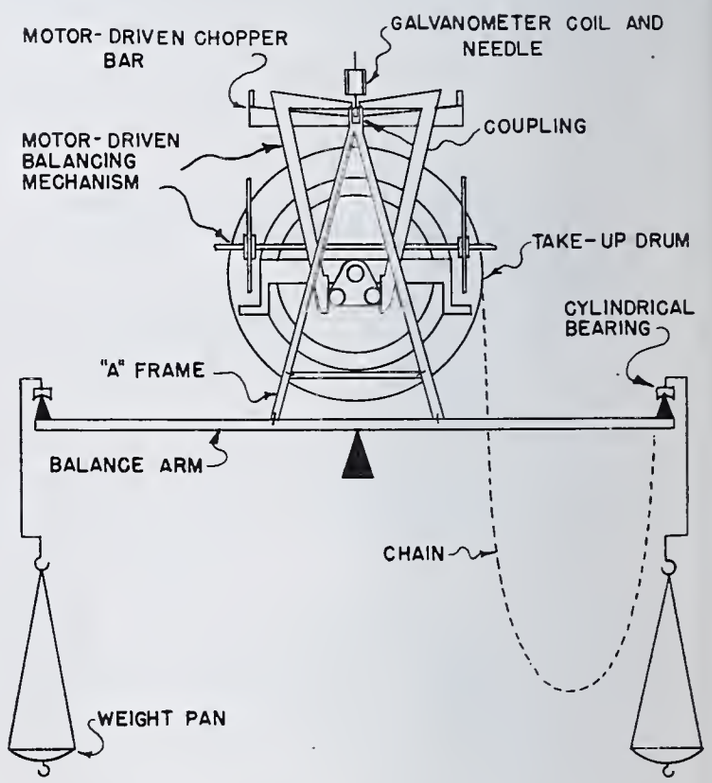

FIGURE 2. AUTOMATIC BALANCING MECHANISM

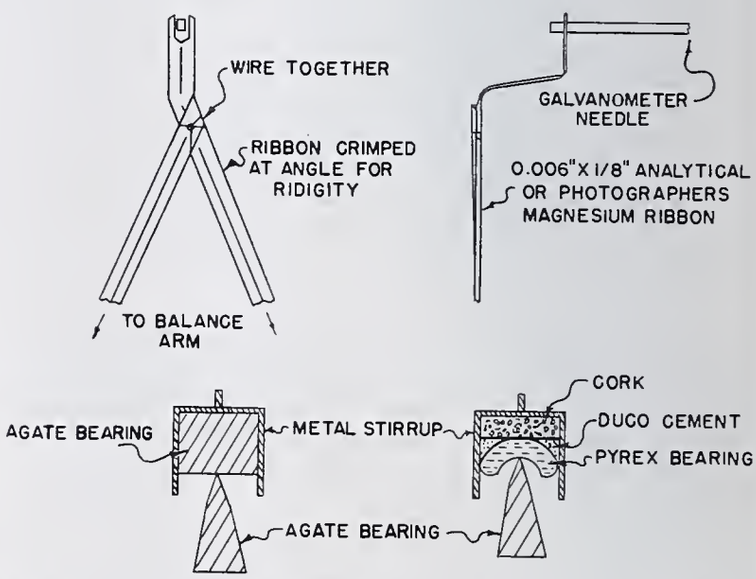

FIGURE 3. BALANCE NEEDLE COUPLING (*above*) AND PYREX BEARING MOUNTING (*below*)

equilibrium is restored. The recorder mechanism is designed so that restoring action is proportional to displacement, which eliminates hunting.

When constructing the apparatus, no permanent change is made in either the balance or the recorder. Both instruments are restored easily to their original condition. The slide-wire of the recorder mechanism is replaced by a drum with flanges. The brass drum has a diameter of 16.9 cm. (6.75 inches) and a width of 2.5 cm. (1 inch), and is provided with 90 cm. (3 feet) of chain made by splicing a cheap necklace chain. The irregularities of the chain are offset by its length.

The A-frame is made of magnesium ribbon, crimped into a V shape for rigidity, and is attached to the balance arm with fine copper wire. Maximum sensitivity is obtained by making the slot that engages the galvanometer needle as narrow as possible. Jarring of the balance arm, which is caused by the chopper-bar action, is eliminated by coupling the balance arm with the galvanometer, as shown in Figure 2. The galvanometer coil is short-circuited to produce the necessary damping effect.

The recorder mechanism is mounted above the balance in a position where the galvanometer needle is directly above the center line of the balance. Because the chain and recorder mechanism form a loop around the chart roll, it is necessary to mount a chart take-up within the chain loop to prevent the unrolling chart from fouling the chain. An ordinary window-shade roller is used for this purpose. The roller spring supplies the necessary tension.

Laboratory vibrations cause the agate pan bearings of the balance arm to rotate on the flat agate plates when equilibrium is maintained for a long period. This trouble is eliminated by substituting glass bearings made of sections of Pyrex tubing for the flat agate plates (Figure 3). The instrument is enclosed in glass to avoid the effects of air currents.

The weight-change range and sensitivity of the balance depend upon the drum size and chain used. In the model described, this range is 1 gram over a chart width of 25 cm. (10 inches). With this spread, a 1-mg. weight change can be detected. The sensitivity of the instrument can be increased up to about half the limit of sensitivity of the balance by moving the attachment point of the chain on the balance arm toward the center. Thus, if the analytical balance is sensitive to 0.1 mg., the instrument can be adjusted to record weight changes of 0.25 to 0.20 mg. Initial balancing is accomplished by adding weights to the auxiliary pan. Weights also must be added from time to time when the weight change to be measured exceeds the automatic range of the instrument.

Acknowledgment

The writer wishes to acknowledge the interest of J. W. Lefforge, who suggested the possibility of using a Leeds & Northrup recording mechanism in the construction of the recording automatic balance.

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Glass Electrode as a Reference Electrode in Electrometric Titrations

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THE use of the glass electrode as a reference electrode was suggested by Heintze (*1*) in 1934. In the following year, Wynd (*6*) discussed the use of the glass reference electrode in determining the oxidation-reduction potential of a solution. Later, Stewart and Carruth (*4*) found that a glass tube containing mercury was a satisfactory reference electrode for redox titrations. Several years ago, while working with the argentimetric method of Tamele and Ryland (*5*), the authors tried using the glass electrode as a reference electrode in precipitation titrations. This application was very successful and it was subsequently extended to other precipitation and redox titrations in which the hydrogen-ion concentration was not a function of the titrating reagent. In these trials, the older fragile electrode as well as the modern durable and shielded electrode was used.

The glass electrode serves as a satisfactory reference in any potentiometric titration in which the hydrogen-ion activity remains practically constant throughout the titration. Therefore, for most titrations, the glass electrode will function as a useful reference electrode in solutions that contain excess base, excess acid, or a sufficient amount of an effective buffer, especially when the titrant is chemically inert so far as changing the hydrogen-ion activity is concerned. If the true hydrogen-ion activity, or pH, of the solution is known or can be readily determined, the glass electrode may also be used as a reference when measuring accurate half-cell potentials.

The glass reference electrode has three important advantages over conventional reference electrodes. (1) It requires very little preparation and is therefore readily available for use. (2) It is chemically inert and therefore does not contaminate a solution that can be safely handled in glass. The glass electrode is especially useful in argentimetric titrations, as it

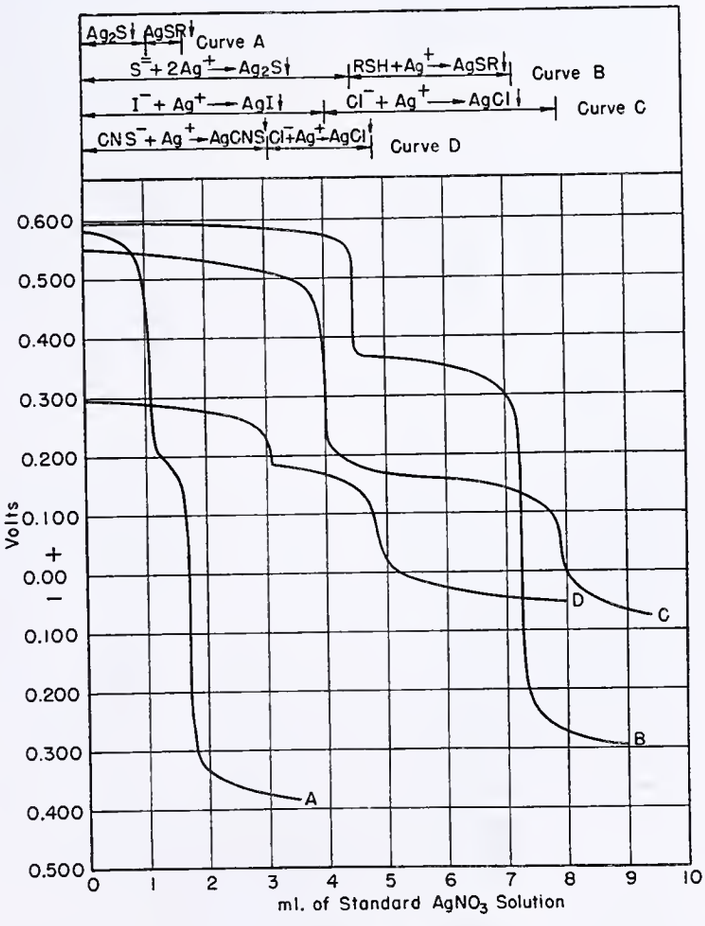

FIGURE 1. TYPICAL ARGENTIMETRIC POTENTIAL-VOLUME TITRATION CURVES
Obtained by using a shielded glass reference electrode

TABLE I. EXPERIMENTAL CONDITIONS IN PRODUCTION OF TITRATION CURVES OF FIGURES 1 AND 2

Curve	Titrated Constituents	Titrant	Titration Medium	Volume Ml.	Indicating Electrode	Temperature ° C.
A	$H_2S + C_2H_5SH$	0.01 *N* $AgNO_3$ in C_2H_5OH	0.1 *N* CH_3COONa in C_2H_5OH	100	Ag_2S, Ag	Room
B	$S^{--} + C_2H_5S^-$	0.1 *N* $AgNO_3$ in water	1.0 *N* NaOH plus 0.05 *N* NH_4OH	100	Ag_2S, Ag	Room
C	$I^- + Cl^-$	0.1 *N* $AgNO_3$ in water	0.05 *N* HNO_3 in water	100	Ag (polished)	Room
D	$CNS^- + Cl^-$	0.1 *N* $AgNO_3$ in water	0.05 *N* HNO_3 in water	100	Ag (polished)	Room
E	$Sn^{++} + Fe^{++}$	0.1 *N* $Ce(SO_4)_2$ in water	3 *N* HCl in water	100	Pt (aged in Ce^{++++})	Room
F	$MnO_4^- + VO_4^{---}$	0.1 *N* $FeSO_4$ in dilute H_2SO_4	6 *N* H_2SO_4 in water	200	Pt (aged in Ce^{++++})	0–5
G	VO_4^{---} (steel)	0.05 *N* $FeSO_4$ in dilute H_2SO_4	6 *N* H_2SO_4 in water	200	Pt (aged in Ce^{++++})	0–5
H	Fe^{++}	0.05 *N* $Ce(SO_4)_2$ in water	6 *N* H_2SO_4 in water	100	Pt (aged in Ce^{++++})	Room
I	$Cr_2O_7^{--}$ (steel)	0.08 *N* $FeSO_4$ in dilute H_2SO_4	6 *N* H_2SO_4 in water	200	Pt (aged in Ce^{++++})	0–5

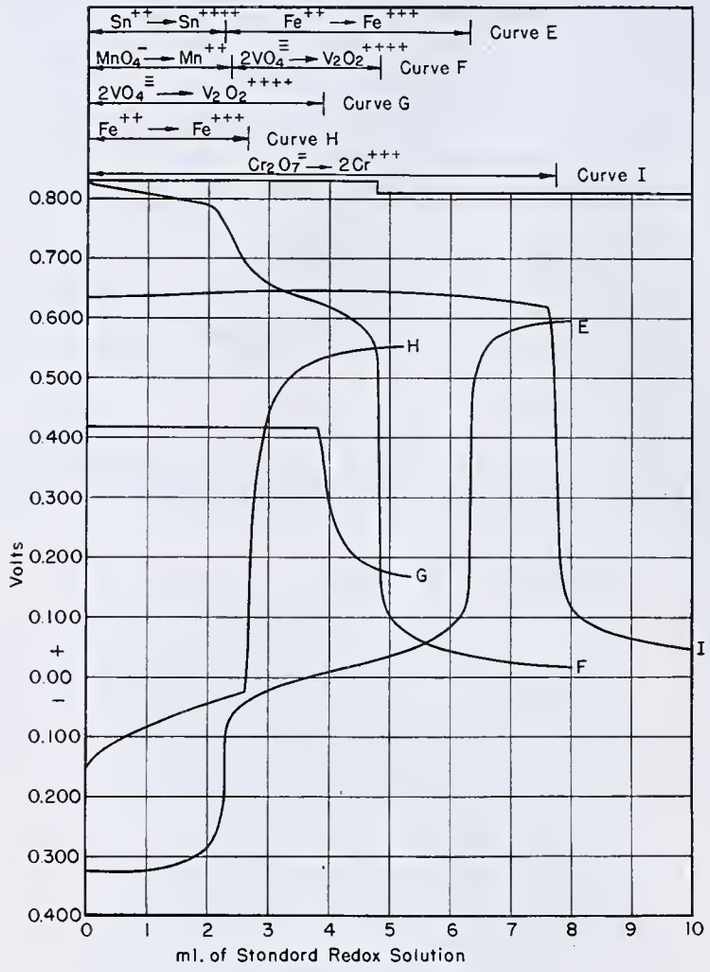

FIGURE 2. TYPICAL REDOX POTENTIAL-VOLUME TITRATION CURVES

Obtained by using a shielded glass reference electrode

avoids the difficulties resulting from the contamination of the test solution with potassium chloride from the usual salt-bridge. (3) The glass electrode (particularly in the newer commercial forms) establishes a definite reproducible potential that does not vary appreciably over short periods of time as long as the electrode is immersed in a solution of constant hydrogen-ion activity. This is especially important when considering the difficulty encountered when the common salt-bridge reference cell is used with concentrated or nonaqueous solutions. In these cases the glass electrode has a boundary potential that is more certain and more dependable than the liquid-liquid potential of the salt bridge. Several years' experience indicates that the glass-metal electrode system is less erratic and is influenced less by methods of treatment before use than the usual bimetallic electrode system.

The chief disadvantage of the use of the glass reference electrode is that a special meter, which functions accurately on input resistances of 100 to 1000 megohms, is required to measure the potential of the electrode system. This difficulty is no longer serious and has largely been dissipated by the increased availability of pH meters, electrometers, and electronic voltmeters which operate satisfactorily in conjunction with high resistance electrode systems.

Most glass electrodes are designed to operate at 0° to 50° C. and consequently should not be used at the boiling temperature of water or at subzero temperatures. This objection has been minimized by the introduction of commercially available glass electrodes that function well at 50° to 100° C. and at ordinary steam pressures.

Titration curves obtained using a glass reference electrode in similar titrations will be relatively the same from one sample to another, but the absolute position of all points on the curve will be shifted if the hydrogen-ion activity of the titrated solutions changes from time to time. This fact does not minimize the usefulness of the glass reference electrode

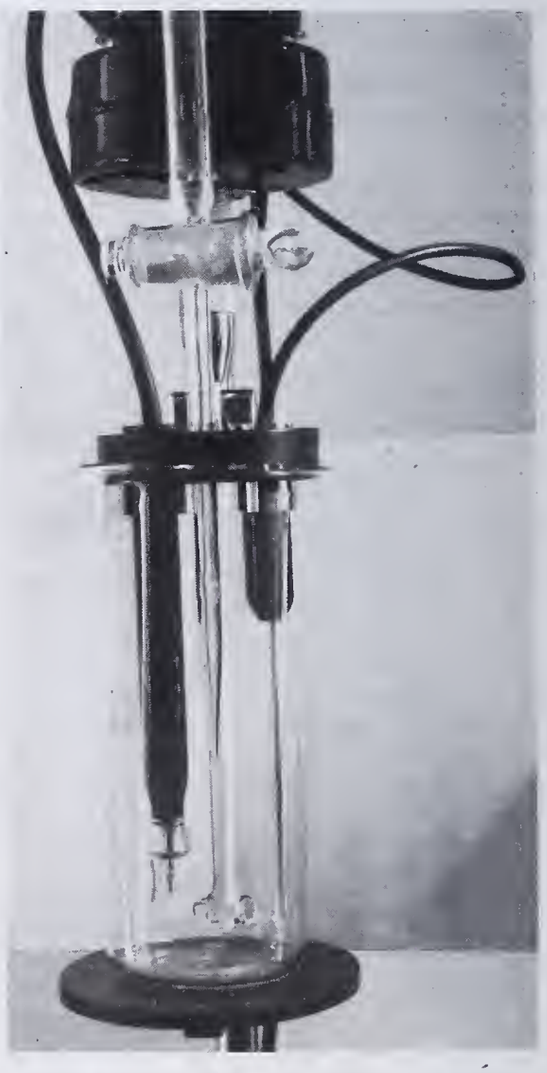

FIGURE 3. ELECTRODE SYSTEM FOR PRECIPITATION OR OXIDATION-REDUCTION TITRATIONS

because the end points are usually indicated by the position of a very obvious inflection point in the volume-potential titration curve and not by the realization of a definite, predetermined potential difference between the electrodes.

Typical potential-volume titration curves resulting from the use of a glass reference electrode in redox and precipitation titrations are given in Figures 1 and 2. The conditions under which the titrations were made are given in Table I. The Beckman shielded glass electrode was used in all the titrations. The titration apparatus is similar to that described by Lykken and Rolfson (*2*); a close-up of the electrode system discussed in the present paper is shown in Figure 3. The titration cell potential was measured with a continuous-reading electronic voltmeter (*3*).

The authors recommend the use of a glass electrode as a reference electrode for all redox and precipitation titrations that yield potential-volume titration curves with realizable inflection points and that can be made at 0° to 50° C. This conviction is based on two years of experience with the glass reference electrode in routine potentiometric titrations.

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Laboratory Flowmeter with Interchangeable Precision-Bore Capillaries

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THE development of the laboratory gas flowmeter, particularly toward ruggedness and ease of calibration, has not been rapid. A number of devices for altering the useful range have been described but only one appears to be adaptable to reproducibility of calibration in mass production.

The capillary tube–manometer combination for measuring gas flow rates, described by Riesenfeld (*6*), had no provision for use in widely different ranges. Later, however, the same investigator (*7*) described a flowmeter in which the capillaries were attached by means of rubber tubing and thus were interchangeable. Yoe (*9*) cemented capillaries in disks which were clamped between flanges when in use. Sharp (*8*) used a slotted stopcock as the constriction in a flowmeter, obtaining in that way a wide range of adjustments. Instead of a capillary tube or an orifice, Hofsäss (*4*) used a rod inserted into a glass tube. The longer the annular space between rod and tube the greater the pressure drop at a given rate of gas flow. Yuster (*10*) selected one from a group of capillaries, sealed in place, by means of one or more three-way stopcocks. He also described a variation employing a large, tapered joint. As an adjustable orifice in noncorrosive applications, Marks (*5*) adapted the iris diaphragm to a flowmeter. Bradford (*2*) measured flow rates at constant pressure drop across a long-taper needle valve with micrometer adjustment. Recently the Ace Glass Company has offered flowmeters in which the capillaries are mounted on interchangeable ground joints and the Corning Glass Company lists one having a stopcock with four orifices of different sizes. For the fundamentals of laboratory flowmeters reference should be made to the excellent discussion by Benton (*1*). Although the interchangeable capillaries or orifices now available extend the range of a single instrument, the conventional U-tube manometer still presents a breakage hazard and is rather difficult to read.

In order to eliminate these disadvantages a flowmeter was designed having the following merits which, it is believed, have not been described before in this connection: (1) The manometer is completely enclosed in a single glass tube 2 cm. in diameter which provides strength and ease of mounting. (2) The tube is graduated so that liquid levels may be read quickly and easily. (3) The interchangeable capillaries are of precision bore and length and thus a series covering a wide range of gas flows is available.

The construction of the flowmeter is shown in Figure 1.

The body, *A*, is graduated in millimeters over a length of 15 cm. Inside, the manometer tube, *B*, with a bore of 2 mm., joins the lower chamber with the upper one, *C*. These chambers are also connected by the inner half of a $^{7}/_{15}$ interchangeable ground joint, *D*, on which the capillary tubes, *E*, fit. The cap, *F*, fits the main portion of the flowmeter by means of a $^{19}/_{10}$ interchangeable ground joint. Gas inlet to the lower chamber is at *G*. The gas outlet from the upper chamber is at *H*. The pressure drop across capillary *E* is read on manometer *B*. Since, as a result of capillarity, the height of the liquid in *B* above the main body of liquid will be greater than that corresponding to the true pressure drop, it is desirable to use tube *J*, open at both ends and of the same bore as *B*, for reference.

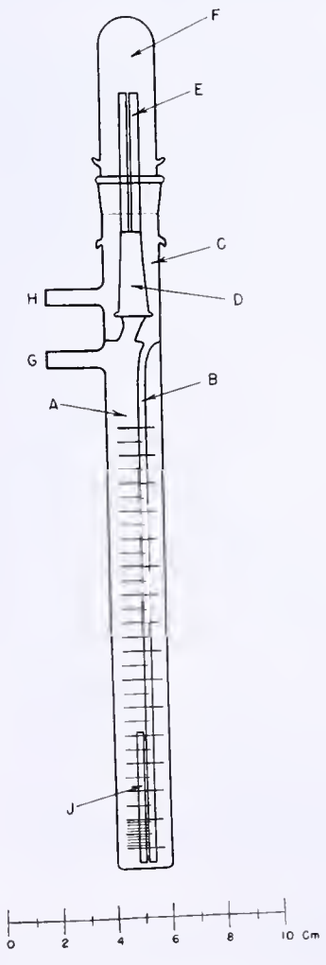

FIGURE 1. GAS FLOWMETER

In event of surges during the operation of the flowmeter, part of the manometer liquid may be forced into the upper chamber. Tube *B* is sealed on at the lowest point, however, and drainage back into the reservoir is practically complete. Furthermore, the gas outlet tube is sealed on at such a height that chances of entraining liquid in the gas stream are slight. With sacrifice of some ease of manipulation, the outlet connection may be placed at the top of the cap, coaxial with the graduated tube. A third 2-mm. tube may be

Table I. Flowmeter Characteristics

(Triplicate capillaries. Bore = 0.676 mm. Length = 50.00 ± 0.03 mm. Air at 20° C. and 760 mm.)

Capillary No.	Manometer Reading, Mm. H_2O	Observed Air Rate, Ml./Min.	Calculated Air Rate[a], Ml./Min.
1	51.7	117.5	118.9
	98.7	210.4	211.2
	139.6	281.2	281.9
2	53.2	123.7	122.1
	101.2	217.2	215.7
	139.6	283.2	281.9
3	50.1	115.3	115.5
	100.0	213.2	213.5
	144.1	288.8	289.2

[a] Calculated from equation of best curve through points. $V = 2.505h - 4.24 \times 10^{-3}h^2 + 5.44 \times 10^{-6}h^3$

included within the envelope with its upper end sealed through the wall at about the level of *G*. Vented to the atmosphere in this way, it will read upstream pressure above atmospheric.

When water is used as the manometer liquid, the addition of a small amount of a selected wetting agent will improve drainage both during normal operation and after a surge. Foster (*3*) suggested methyl salicylate as a manometer liquid in order to avoid the drainage difficulties characteristic of water. It possesses certain disadvantages, however, which make it less desirable than water containing a trace of surface-active material. Agents which have been found suitable include Wetsit, Duponol W A, and Tergitol 7, but many others are probably equally effective. One drop of the wetting agent is sufficient since, in most cases, the total amount of manometer liquid will be approximately 3 ml. This is not enough to alter specific gravity significantly but will improve wetting properties considerably.

The outer half of the $^7/_{15}$ joint is sealed to the capillary in such a way as to leave the capillary end unchanged and unobstructed. In addition, considerable care is taken in the sealing process to avoid altering the bore of the capillary. To determine what degree of reproducibility could be attained in the construction of these capillaries, a set of three, 50 mm. in length, was prepared from Pyrex precision-bore tubing of 0.676-mm. internal diameter. Lengths of slightly more than 50 mm. were ground true on both ends in a Thompson surface grinder, using a 2 per cent aqueous solution of soluble oil as coolant. No deviation greater than 0.03 mm. from 50.00 mm. was permitted in the finished capillaries. Examination revealed that the ends of the bores were completely free from appreciable chipping or fusing as a result of grinding.

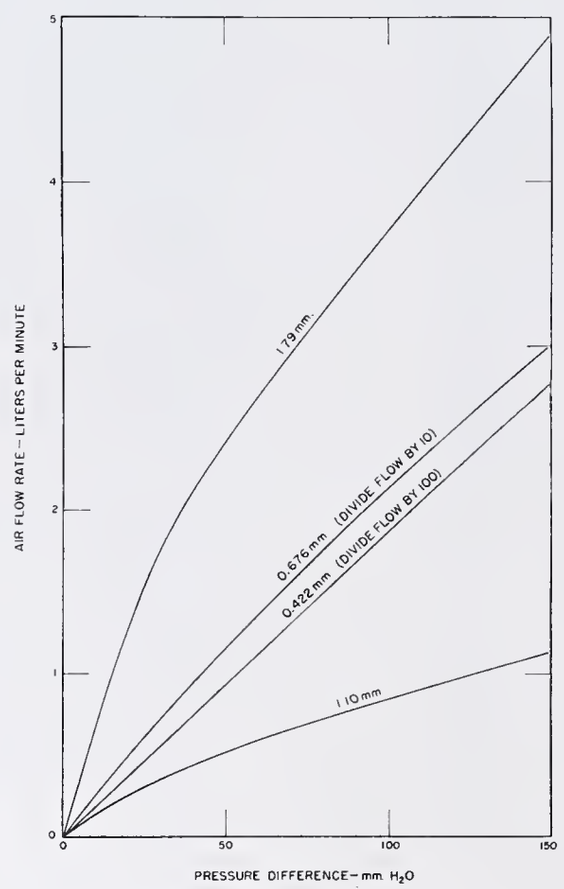

Figure 2. Flowmeter Calibrations

Air at 20° C. and 760 mm.

Table I gives the manometer readings corresponding to several rates of flow for each. The average deviation of the measurements from the best curve through the group is but ±0.53 per cent. This is satisfactorily low and indicates that dimensional reproducibility is easily attainable to the desired accuracy. In its practical aspects this means that a given capillary might be duplicated whenever necessary and the original calibration figures would apply to the new tube as well as to the old one.

With the question of reproducibility of capillaries clarified, a series of tubes covering reasonable gas flow rates was designed. It was assumed that the usable portion of the manometer scale would be 10 to 150 mm. and the liquid would be water. Capillary lengths were established at 50.00 ± 0.03 mm.

Table II shows the bore for each member of the series and the minimum and maximum flow rates.

The calibration curves for the four capillaries are shown in Figure 2. A range of 0.002 to 4.9 liters of air per minute at 20° C. and 760 mm. is covered and may be extended upward with either larger capillaries or manometer liquids of higher specific gravities.

Further work should lead to complete calibrations at higher pressure differences and with empirical corrections for specific gravity and viscosity of gases. There will then be available a rugged and compact flowmeter with but four interchangeable, standard capillaries which will cover the range of laboratory flow rates most used and whose calibration curves will be accurately characteristic within the limits of experimental error, thus allowing replacement or duplication without confusion.

Table II. Flowmeter Capillary Bores

Flowmeter	Capillary Bore	Flow Rate, Air at 20° C. and 760 Mm. Pressure difference, 10 mm. H_2O	Pressure difference, 150 mm. H_2O
	Mm.	*Liters per minute*	
A	0.422	0.002	0.027
B	0.676	0.025	0.30
C	1.10	0.11	1.1
D	1.79	0.70	4.9

Acknowledgment

The author wishes to thank R. Shutt for his continued interest during the course of this work. Grateful acknowledgment is made to the Battelle Memorial Institute for permission to publish this material.

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Immersion Still Head for Low-Pressure Distillation of Organic Mixtures

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A LOW-PRESSURE still head was designed and used successfully on exceedingly refractory organic reaction mixtures. These included high boiling liquids and tarry and resinous materials and were characterized by an excessive tendency to froth, bump, spatter, and form a leathery skin on the surface. The success of this still head in promoting smooth distillation suggested that it would be equally valuable in other laboratories.

Design of Still Head

The usual minimum pressure at which the still head operates is of the order of 1 mm. of mercury, or with many solids, a somewhat lower value, approximating the action of a crude molecular still. The details of construction of the still head are apparent by reference to Figure 1.

If the distillate was liquid, the still head functioned as a condenser and vapor-lift pump; the drops collecting at the bottom of the condenser were lifted through the small central tube by the difference in pressure and allowed to run down through the liquid-vapor separator into the sample bottle. In actual operation, the drops were lifted smoothly and uniformly, chiefly as a bubble-film in the tube. With high melting solids it was necessary to allow the condenser to run hot to keep the distillate liquid. By grinding off the ends of the delivery tubes to an acute angle, drops were discharged uniformly from the extreme ends of the acute tips without a tendency of the liquid to back up in the tube, bridge the walls, and block the tube with liquid.

The still head, drawn accurately to scale in Figure 1, fitted either a 2-liter (as shown) or a 1-liter standard Pyrex flask. Smaller sizes of still heads in smaller flasks were used with equal success. Some adapters accommodated two or more sample bottles at the same time, permitting taking several fractions without breaking the vacuum. A small loss of pressure occurred in lifting the liquid through the small tube out of the flask. By using a small tube, the liquid bridged before a large drop collected, and ascended as a single bubble-film. It was usual to have only one bubble-film in the tube at a time; the pressure drop from lifting was less than 1 mm. of mercury. There was no other drawback to using the same tube as vacuum line and distillate line. If the distillate was solid, it was deposited on the condenser, and periodically removed with a solvent.

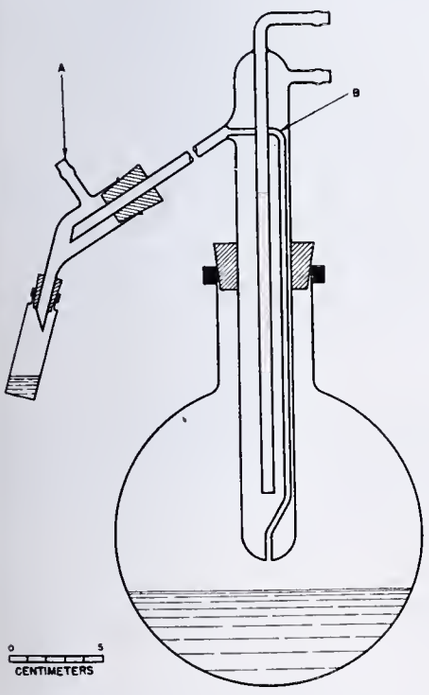

FIGURE 1. IMMERSION STILL HEAD
A, Vacuum line
B, Vapor-lift tube, 3 mm. in outside diameter

Operation

This type of still was developed to effect separation of experimental mixtures which resisted all attempts at separation in other types of apparatus. These mixtures were characterized by high boiling point (100° to 300° at 1 mm. of mercury), viscous or tarry consistency, and an extreme tendency to bump, froth, and spatter. Frequently, the formation of a leathery skin on the surface added to the other difficulties, while antibump devices such as an immersed wire coil heater could not be used because of the viscous medium. This immersion still head was used successfully on these mixtures, even those with a skin on the surface merely required a longer time of distillation. The explanation appeared to be that the flask did not have to be heated so strongly and hence the lower temperature differential caused less superheating.

Inability conveniently to trap all vapors of some mixtures led to the substitution of special mercury pumps and a water aspirator for the usual large-throated mercury pump and mechanical pump, thus avoiding fouling the oil in the latter. Mercury diffusion pumps which require a fore-pressure of 25 to 30 mm. and produce a pressure of 25 to 50 microns were described by Kraus (*1*), Munch (*2*), and others. Two of these mercury pumps, of slightly different size, were used in series with an aspirator as the forepump. This pumping system gave extremely satisfactory service in conjunction with the immersion still head described above. Attempts at trapping all organic vapors were successful only when elaborate apparatus was used; the aspirator and mercury pumps provided a simpler and more satisfactory solution, since the pumps were easily disconnected and cleaned.

Effectiveness of Still Head

All viscous, tarry organic reaction mixtures encountered in this laboratory have been successfully distilled with immersion still heads. Previous attempts at separation with other devices invariably failed. Still heads of this type operate in the important gap between vacuum distillation and molecular distillation. Prior to the use of the immersion still head, it was customary to remove all volatile material in ordinary vacuum distillation apparatus, then put it directly into a molecular still. It was found, however, that high-boiling oils invariably remained in the material and volatilized in the low-pressure atmosphere of the molecular still, in spite of meticulous graduation and adjustment of temperature and pressure, to such an extent that the entire tubing and pumping assembly condensed a film of the volatilized material and limited the pressure of the entire system to the vapor pressure of this condensed film. With the immersion still head, however, these residual, high boiling fractions were easily removed, so that they did not foul the molecular still system. Probably the most important advantage of the immersion still head over standard vacuum distillation apparatus is that the latter, even at the end of distillation, are still refluxing high-boiling fractions on the walls of the flask. The immersion still head, on the other hand, by bringing the condenser to within a few millimeters of the heated material, easily removes these fractions which usually reflux on the walls. That this difference is important is emphasized and best illustrated by the facile and clean-cut separation of the extremely refractory tarry and resinous mixtures noted above.

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An Improved Distilling Column Head

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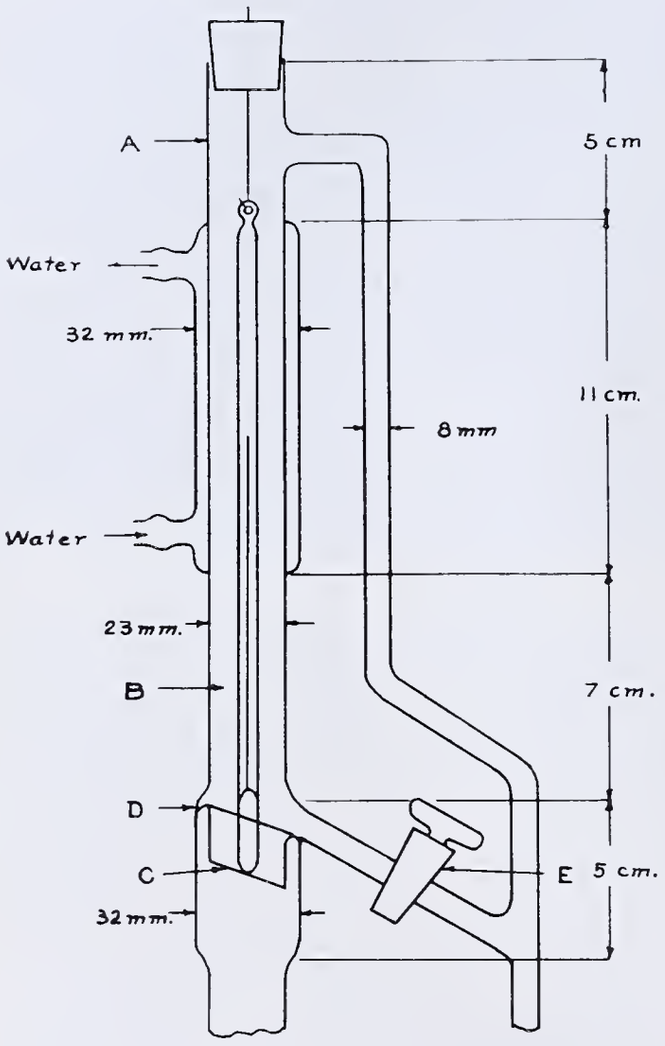
MANY types of column heads commonly used for total reflux–variable take-off distillation, although efficient in operation, possess disadvantages due to inconvenient bulkiness of construction, difficulty in glass blowing, ease of breakage, and inaccessibility to cleaning. In this laboratory a column head has been developed which has all the operating advantages of the more elaborate designs, without the aforementioned disadvantages.



The column head is pictured in the accompanying diagram. The thermometer well, condenser, reflux counter, and variable take-off valve are all constructed around a single tube, *A*, which extends vertically from the column shaft. The thermometer, suspended as shown, comes in direct contact with the vapors from the column; the arrangement is such that there are no dead spaces that would materially disturb equilibrium conditions. When an Anschutz thermometer is used, the uncooled portion, *B*, provides for complete immersion of the mercury stem in the hot vapor. The drop counter, *C*, and take-off devices are combined in one simple rigid unit. The condenser reflux is trapped around the rim of the drop counter at *D*, and sufficient condensate is thereby deflected to the stopcock, *E*.

This system of arrangement is such that the entire apparatus is a short direct continuation of the column shaft itself, and thus any strains or breakage danger caused by bulkiness or dispersed distribution of weight of the parts are eliminated. Furthermore, the apparatus can be cleaned very easily without dismantling; the use of a test-tube brush will usually be sufficient. If some water accumulates in the cooled parts of the tube during distillation, the thermometer can be temporarily removed and the tube wiped out with cotton suspended on a wire; this can be done within a few seconds without interrupting operation. The glass-blowing construction is also of a simple and strain-free nature.

For making the reflux counter, a short section of tubing of the same diameter as that used for the main shaft of the column head is cut and its ends are beveled. The upper end is prepared for sealing by flanging it and then bending the lip slightly downward. In this way a sloping gutter is formed around the tube at *D* when the seal is made. After sealing in the reflux counter, the side tube for the take-off valve may be attached.

This type of column head has been in use on about thirty columns of various types in this laboratory for 2 years, with compounds boiling from 36° to 168° C., and has given uniformly satisfactory service.

Rapid Detection of Nickel in Alloy Steel

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THE need for a rapid and nondestructive method for differentiating steel gears containing 1 to 2 per cent of nickel from gears made of plain carbon and molybdenum-alloy steels resulted in a spot-test procedure using dimethylglyoxime as the indicating reagent. Of prime importance in the test procedure is the use of an acid mixture consisting of sulfuric, phosphoric, nitric, and citric acids, which eliminates the need for preliminary separations of interfering ions and the grievances generally associated therewith.

Previously described spot-test procedures (*1*, *2*, *3*, *5*, *6*, *7*), although using dimethylglyoxime as the indicating reagent, have never given to the conditions of initial reaction the degree of importance that is demanded in a test of this type. In order to obtain unmistakable results, the acid solution of the elements of the steel alloy had to fulfill two principal requirements, which are met by the acid mixture described below.

1. The solution obtained in the initial reaction of acid and steel must be colorless, in order to prevent the possibility of masking the color indication of nickel. Acids such as hydrochloric and nitric used individually produced intensely colored solutions which made impossible any discernment of color indication. Phosphoric acid, suggested by Griffin (*4*), showed no sign of reaction with any of the types of alloy steels that were investigated.
2. The reaction of ferrous ions and dimethylglyoxime must be suppressed. This reaction is characterized by a deep red coloration (*5*), which is easily mistaken for the nickel salt of dimethylglyoxime.

Alpha-benzildioxime was tried as an indicating reagent, but the results obtained were unreliable and too easily misinterpreted.

Reagents and Procedure

Acid Mixture: 25 ml. of distilled water; 10 ml. of sulfuric acid, specific gravity 1.84; 10 ml. of nitric acid, specific gravity 1.42; 10 ml. of phosphoric acid, 85 per cent; and 10 grams of citric acid crystals.

Potassium Hydroxide: 30 grams of potassium hydroxide and 90 ml. of distilled water.

Dimethylglyoxime, 1 per cent, in isopropanol.

Test Papers: Strips of filter paper dipped in a solution containing 10 grams of citric acid, 25 ml. of distilled water, and 10 ml. of 1 per cent dimethylglyoxime in isopropanol. Strips are thoroughly dried and can be kept in stoppered bottles.

One drop of the acid mixture is placed on the steel and allowed to react for 15 to 30 seconds. This solution is then absorbed on a paper test strip and 2 to 3 drops of potassium hydroxide solution are poured over the spot.

If nickel is present an unmistakable red coloration will appear, its intensity increasing with an increase in percentage of nickel present.

The absence of nickel is indicated by an almost total absence of color on the test strip. Any noticeable color that might occur will be distinctly brown [$Fe(OH)_3$] and cannot be confused with the red nickel glyoxime precipitate.

Summary

The rapid nondestructive spot-test procedure described is specific and positive in the identification of nickel in nickel-bearing alloy steels. Positive tests were obtained on a series of typical heat-resistant and corrosion-resistant steels ranging in nickel content from 0.50 to 35 per cent No questionable or interfering color reactions from elements other than nickel have been observed.

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Attachment for Pipets for Precise Transfer of Dangerous Fluids

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A DEVICE illustrated in Figure 1 has been found useful in the laboratories of this division not only for dangerous chemicals, such as combustion fluids, but for precise work with bacterial and virus suspensions and has some advantages over other available devices in simplicity of construction and convenience.

It consists of three essential parts. The first is a suitably sized glass tube constricted at one end, *T*, to a minute opening, with a wide opening at the other end, *Q*, and a small vent, *V*, in the side. The second is a short piece of heavy pressure tubing placed at *T* for attachment to the pipet, *P*. The third is a rubber bulb, *B*, with holes at opposite ends fitted around the glass tube with *V* inside—a single-holed bulb may be bored, stretched over a stopper-borer inserted through the first hole, and bored upon a piece of cardboard or wood till a small second hole is neatly cut, which should be placed next to the pressure tube at *T* for added strength.

The glass part, preferably Pyrex, may be made as follows: Cut a convenient length of tubing, about 5 mm. in outside diameter for small bulbs, 10 ml. or less. Attach a large bulb (about 50 ml., capable of reducing pressure about one-tenth atmosphere) at one end and rotate the other in a flame, hot enough for fusing, but allow contraction to leave a small hole at the end. Test its size after removal from the flame, by squeezing the bulb and noting the speed of refilling—about 1 ml. per second or less should be satisfactory. The slower this is, the greater the control in later use. Then heat a small spot on the side for piercing in the usual manner, but blow out by means of the attached bulb. Fire-polish this opening to form the vent, *V*. This may be made almost as small as that at *T*, if desired, by reheating and gently allowing compression—e. g., letting the tube bend slightly around the flame at the pierce hole and later straightening it. Use of a small vent at *V* or a small bulb, *B*, promotes precise control of outflow, but a compromise must be made to give satisfactory facility in filling. At the time it is made, *V* may be tested as was the first vent at *T*.

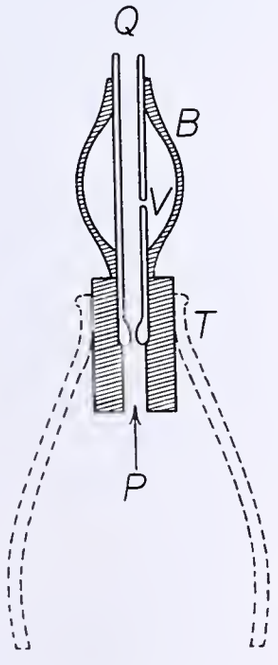

FIGURE 1

In use, pipet *P* is inserted into the pressure tube to butt against the glass tube at *T*. To fill the pipet, the bulb is compressed with second finger and thumb, the index finger is placed over *Q*, the bulb is released, and the thumb is transferred to the rubber tube at *T* opposite the other fingers. When the fluid has risen slightly above the initial mark, the index finger is taken from *Q* momentarily to stop upward flow and returned to restrict downward flow, as in ordinary use of a pipet. Blow-out technique may be used subsequently by closing *Q* and compressing the bulb as required.

For use with combustion fluid, as in the Van Slyke-Folch (*1*) technique, a glass bell may be attached at *T* around the rubber tube (as shown in dotted lines in Figure 1), and the pipet may be left attached to the safety filler, and inserted in a glass tube with closed bottom (test-tube form) of suitable size to permit the glass bell to rest upon and cover the top, suspending the pipet above the bottom or any collected fluid. The tube may slope about 45° from horizontal. This provides protection from dust in intervals between use.

It is convenient also to use a filler with stopcock pipets, and to leave one attached to any pipet used frequently by different workers.

The symmetrical form of the filler permits its rotation with the attached pipet in use—e. g., to facilitate reading, to start flow, etc. However, with large bulbs (50-ml. or greater capacity) it is sometimes preferable to use a T-joint instead of the vent at *V* and attach a single-holed bulb to the side tube.

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Indirect Polarographic Determination of Phosphorus in Biological Material

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POLAROGRAPHIC methods of analysis possess attributes which extend their value far beyond the direct determination of chemical elements in various materials. Especially in routine laboratory procedure, polarographic methods in combination with chemical methods result in shorter time requirements for analysis and the reduction of errors, owing to the elimination of filtering, drying, and weighing procedures. In addition to developing polarographic methods for direct determination of mineral elements in biological material (*4*), an indirect method for the determination of phosphate ions in biological material has been devised.

Phosphate ions are not electroreducible at the dropping mercury cathode. Uhl (*6*) has described an indirect polarographic method for the determination of phosphate ions in pure solutions by precipitating the ions with ammonium heptamolybdate. The resulting ammonium phosphomolybdate precipitate is of definite composition. Addition of identical quantities of a standard molybdate solution to solutions of different phosphate-ion concentrations reduces the concentration of molybdate in the supernatant liquid by the amount of molybdate combined with the phosphate ions precipitated. By polarographic determination of the molybdate ions in the standard solution and the molybdate ions remaining after the precipitation, it is possible to measure, indirectly, the phosphate ions precipitated. It was also proved by Uhl that small amounts of iron, aluminum, calcium, and magnesium added to the pure solutions do not interfere.

Measurements

The current voltage curves were registered with a Leybold-type Heyrovsky-Shikata polarograph (*1*, *2*), equipped with a highly sensitive galvanometer (2.4×10^{-9} ampere per mm. per meter). The adjustment of the instrument was kept constant for all determinations. The wire resistance coil of the potentiometer was connected with a storage battery of 4 volts. Solutions to be tested were placed in 25-ml. glass cups above a layer of mercury 4 mm. in depth, which served as the anode. The mercury reservoir of the cathode was maintained within ±0.1 cm. of 80.0 cm. Emission of mercury droplets from the cathode, into the regulating solution used for the phosphate determination, was 5 drops per 10 seconds, with the dropping mercury cathode disconnected from the polarizing e. m. f. This drop rate was kept constant. After the determinations the capillary was allowed to release mercury droplets in air for about 15 minutes, then dipped into mercury and the connection to the mercury reservoir closed. With a satisfactory electrode it is possible to perform routine analysis for months without difficulty.

The determinations were performed at 26.0° C. The instrument was situated in a room, the temperature of which was very constant. When the temperature changed more than 0.5° C. a new calibration was performed immediately. Thus, in the course of the analyses calibration curves were obtained for various temperatures (range, 26.0° ± 3° C. within one year). These curves could be used whenever the room temperature corresponded to the temperature at which a calibration had been made. Use of a thermostat thus can be avoided for practical analysis.

Leybold's Handwriter (*1*), which may be attached to the polarograph, was used for recording the current voltage curves. This device is useful in routine analysis and its recording of the current voltage curves is as accurate as the photographic method. With an adequate electrode and a galvanometer sensitivity as low as $^1/_{1000}$, oscillations are not noticeable. When higher sensitivities are employed, galvanometer oscillations can be practically eliminated by slight changes in the concentration of the colloid, the neutral salt concentration of the regulating solution, or the rate of droplet emission from the cathode. In routine analysis, it is advisable to check the calibration curve every day, before starting series of analyses, by determining the concentration of a known standard solution.

The diffusion current was determined by measuring the wave heights of the current voltage curves, with the intersection points method frequently used in practical polarography (*1*). The molybdate waves are very well shaped and the correction for the residual current by this method is sufficiently accurate.

Determination of Molybdate Ions in Pure Solutions

Molybdate ions are electroreducible at the dropping mercury electrode and give a "polarographic wave". The wave appears in neutral and acid solutions, whereas in strongly alkaline solutions no wave can be observed, as Uhl (*6*) and Thanheiser and Willems (*5*) stated. Thanheiser and Willems found that the molybdate waves in neutral and acid solutions are difficult to measure and they preferred, therefore, the determination of molybdate in steel by polarometric titration with lead. Uhl found that the height of the characteristic wave of molybdate ions in acid solution is proportional to the concentration only in the presence of free lactic acid and worked out a special procedure to adjust the acidity of the solutions so that the wave heights were proportional to the concentrations.

In developing the indirect method for the determination of phosphate ions in biological materials, the most simple procedure for the determination of molybdate ions in pure solutions was the primary requirement. Investigation of the behavior, under different conditions, of the polarographic wave

due to molybdate ions showed that in a 3 *N* nitric acid solution containing 2 *N* ammonium nitrate the waves have a rounded maximum which makes quantitative determinations impossible. This maximum cannot be suppressed with gelatin because the rather high acidity of the solution causes precipitation of the colloid. In neutral solutions of 2 *N* ammonium nitrate containing some gelatin or methylcellulose, however, the molybdate wave shows the regular steplike form which may be used for quantitative determinations, the diffusion current being proportional to the concentration (Figure 1).

The half-wave potential of the molybdate wave in 2 *N* ammonium nitrate solution containing 3 grams of methylcellulose per liter is −0.82 volt *vs.* the normal calomel electrode. The space requirement of the wave is rather large. Best results were obtained with a regulating solution which contained 3 grams of methylcellulose (or 1.0 gram of gelatin) in 1 liter of 2 *N* ammonium nitrate solution.

By determining the wave heights for different molybdate concentrations and plotting them against the concentrations, a calibration curve is obtained which permits reading for unknown concentrations of molybdate solutions. Thus, molybdate ions can be determined within a concentration range of 10^{-4} to 10^{-6} gram per milliliter with an accuracy of ±2 per cent, by using a suitable galvanometer sensitivity. Oxygen must be removed from the solution with nitrogen.

The wave heights of molybdate ions in nitric acid solutions do not show a maximum if the solution contains a high amount of ammonium nitrate and, within certain limits, are independent of the acid concentration. Molybdate ions can be determined in nitric acid solutions within the same range of concentration with an accuracy of ±2 per cent if the solution contains 400 grams or more of ammonium nitrate per liter. The fact that molybdate ions can be determined in acid solutions in the presence of a large excess of ammonium nitrate makes it possible to determine phosphate ions directly, without special adjustment of the pH or addition of free lactic acid.

Uhl described a second wave caused by the reduction of molybdate ions, occurring shortly before the tangent potential of the high and well-formed wave is reached. In nitric acid, as well as in neutral solutions, under the conditions employed in this work a second wave could not be observed clearly. As may be seen in Figure 1, the sudden rise in current shortly before the tangent potential of the high wave is reached might indicate the existence of a second wave. This sudden rise in current is not observed at all in solutions which contain a higher concentration of ammonium nitrate (Figure 2). Only one wave appears which can be used for quantitative determinations. The conditions under which a second wave would appear in measurable form, as well as the explanation of the nature of the reduction products of the molybdate ion at the dropping electrode, require further study.

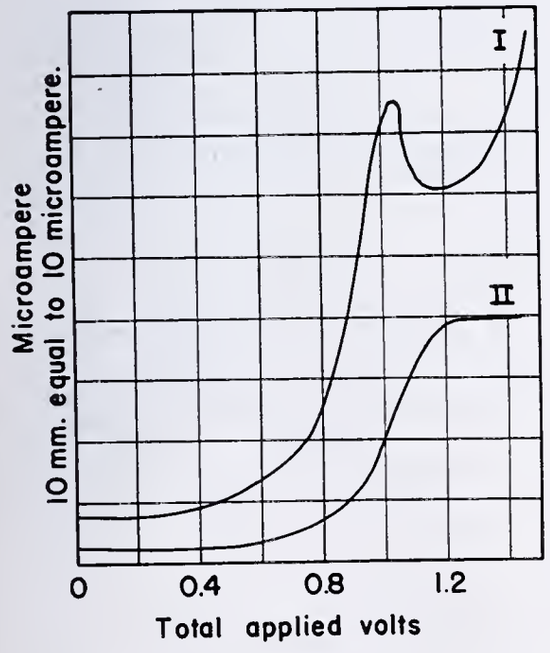

FIGURE 1. DETERMINATION OF MOLYBDATE

I. 0.001 gram of molybdenum per milliliter of 3 *N* nitric acid and 2 *N* ammonium nitrate solution
II. 0.001 gram of molybdenum per milliliter of 2 *N* ammonium nitrate solution containing 3 grams of methylcellulose per liter

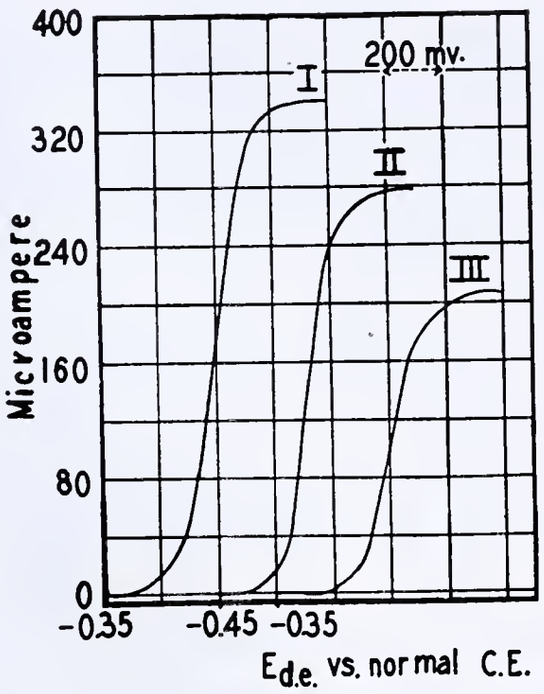

FIGURE 2. POLAROGRAPHIC WAVES OF MOLYBDATE SOLUTIONS

I. 1 ml. of standard molybdate solution in 50 ml. of regulating solution
II. Same solution after adding 1.0 × 10^{-4} gram of phosphorus (PO_4)
III. Same solution after adding 2.05 × 10^{-4} gram of phosphorus (PO_4)

Starting potential of each curve indicated on abscissa, and distance between each 10 mm. corresponds to 0.2 volt

Determination of Phosphate Ions in Pure Solutions

Different amounts of a standard phosphate solution (1 to 2.5 × 10^{-4} gram of phosphorus) are placed in 50-ml. volumetric flasks. Two milliliters of nitric acid (120 ml. of concentrated nitric acid diluted with 180 ml. of water) and 30 ml. of an ammonium nitrate solution (400 grams of ammonium nitrate in 1 liter) are added to each and heated to 35° C. To each flask is added 1 ml. of a standard solution of ammonium heptamolybdate (3.1 grams in 100 ml. of water). A yellow precipitate appears immediately. Approximately 15 minutes later the flasks are filled to the mark with ammonium nitrate solution and allowed to stand for several hours or overnight until the precipitation is complete. Ten milliliters of the supernatant solution are placed in the electrolytic cell and the polarogram is made with galvanometer sensitivities of $^1/_{1000}$ to $^1/_{2000}$, depending upon the concentration. The decrease in the wave heights given by the supernatant molybdate solution, plotted against the increase in the concentrations of the original phosphate solutions, given directly as phosphorus, provides a calibration curve upon which the phosphate-ion concentration of unknown solutions can be read. The accuracy of the determination is 1 to 2 per cent.

It is not necessary to weigh the ammonium heptamolybdate very accurately for preparation of the standard solution, if for a calibration and the corresponding series of determinations the same amount of the same standard solution is always employed. If another standard solution is prepared a new, complete calibration is essential.

Figure 2 shows three of the polarographic waves of solutions of different molybdate concentrations before and after the precipitation of phosphate and Figure 3 the calibration curve derived from the series of determinations. The heights of the waves corresponding to the various concentrations of phosphorus fall in a straight line which, when extrapolated to the ordinate, must intersect at a point identical with the wave height of 1 ml. of standard molybdate solution examined under the same conditions.

Table I. Illustrative Results of Phosphorus Determinations upon Foods, Urine, and Feces

Sample	Foods		Urine		Feces	
	Polarographic method	Chemical method	Polarographic method	Chemical method	Polarographic method	Chemical method
	Mg./0.1 g. dry weight		*Mg./1.0 ml. urine*		*Mg./10 mg. dried feces*	
I	0.341	0.336	0.733	0.721	0.200	0.205
II	0.325	0.329	0.690	0.698	0.204	0.201
III	0.289	0.284	0.623	0.620	0.245	0.232
IV	0.326	0.318	0.769	0.761	0.216	0.215
V	0.330	0.323	0.827	0.819	0.302	0.300
VI	0.336	0.336	0.800	0.804	0.285	0.283
VII	0.340	0.344	0.834	0.846	0.282	0.272

Determination of Phosphorus in Biological Materials

Phosphorus is one of the most important mineral elements in the living organism. In studies of mineral metabolism the chemical determination of this element in food, feces, and urine is time-consuming and requires relatively large amounts of material. For these reasons, a method whereby this element may be determined more rapidly and upon smaller amounts of material considerably facilitates routine analyses.

To determine phosphate ions, carefully collected samples of feces, urine, and foods must be dried and ashed. For this work food and feces were oven-dried at 60° to 75° C. and urine was dried from the frozen state under vacuum. Since minute amounts of phosphorus can be determined by the indirect polarographic method, only a small amount of dried material is required for analysis. It is difficult, however, if not impossible, to mix feces or food composites so that a homogeneous sample yielding only 0.1 gram of dry material can be ashed. For the determination on feces, 5 grams of mechanically mixed, dried material were ashed; for the food composites, 20 grams of the ground and dried material were ashed. For analysis of urine the dried substance of 250 ml. of urine was used.

Samples were ashed in a platinum crucible at 400° to 500° C. The ash was dissolved in concentrated hydrochloric acid (5 ml.) and filtered into a 100-ml. volumetric flask, the residue was reashed and added to the solution, and the flask was filled to the mark with distilled water. The amount of acid added should always be the same, so that the acidity of the regulating solution used later on for the determination of the remaining molybdate ions will be changed only slightly and always by approximately the same amount. Since these ash solutions contain a high concentration of phosphorus, only a small portion is needed for the polarographic determination. The 100 ml. of ash solution of feces (5 grams of dried material) contain 0.10 to 0.15 gram of phosphorus; 5 ml. of this solution are diluted to 25 ml. with distilled water and 1 ml. of this solution (0.1 to 0.3 mg. of phosphorus) is taken for analysis. Portions of the ash solutions of foods and urine are diluted to the same concentration. The remainder of the ash solutions may be used for the determination of other mineral constituents.

Phosphorus in the ash solutions is determined as in pure solutions, using 1 ml. of ash solution instead of the standard phosphate solution. The 1 ml. of ash solution should not contain more than 1 to 3 $\times 10^{-4}$ gram of phosphorus. The calibration curve obtained with pure solutions may be used for the determination of phosphorus in ash solutions, proving that the ash solutions do not contain interfering substances. Calibration using "internal standards" gives the same results. Recoveries are made within the same range of accuracy as the determinations themselves, 1 to 2 per cent.

The polarographic determination of molybdate ions in nitric acid solutions containing a large amount of ammonium nitrate is not influenced by the presence of alkali metals, alkaline earths, or such elements as iron, copper, aluminum, and manganese, if the latter are not present in excessive quantities. Feces, foods, and urine usually contain heavy elements in small amounts in comparison with the amount of phosphorus present. Since a large excess of molybdate must be added to precipitate the phosphate ions completely, the amount of molybdate remaining in the supernatant liquid after the precipitation is also large and must be determined using a relatively low galvanometer sensitivity ($^1/_{1000}$ to $^1/_{2000}$). With this sensitivity the galvanometer does not indicate a wave for any of the heavy elements which are present in low concentrations; hence, it is possible to determine molybdate ions directly in solutions containing all the elements present in the ash of feces, urine, and common foods. The conditions for the precipitation of phosphate ions with ammonium heptamolybdate, however, must be well controlled. Arsenic acid, which would interfere with the precipitation, is not present and the silica content normally is too low to be of any significance. In individual foods which may contain a high amount of silica, the silica must be removed. The very small amount of chloride ions in the ash solutions does not interfere.

The values obtained by indirect polarographic determination of phosphorus in food, urine, and feces (Table I) check very closely with the results of chemical determination upon the same samples by the method of Mackey and Butler (*3*).

Summary

The polarographic method for the direct determination of molybdate ions provides the basis for an indirect method for the determination of phosphorus in biological materials. Phosphorus is determined with an accuracy of 1 to 2 per cent as the difference between the concentrations of molybdate before and after precipitation of the phosphate ions as am-

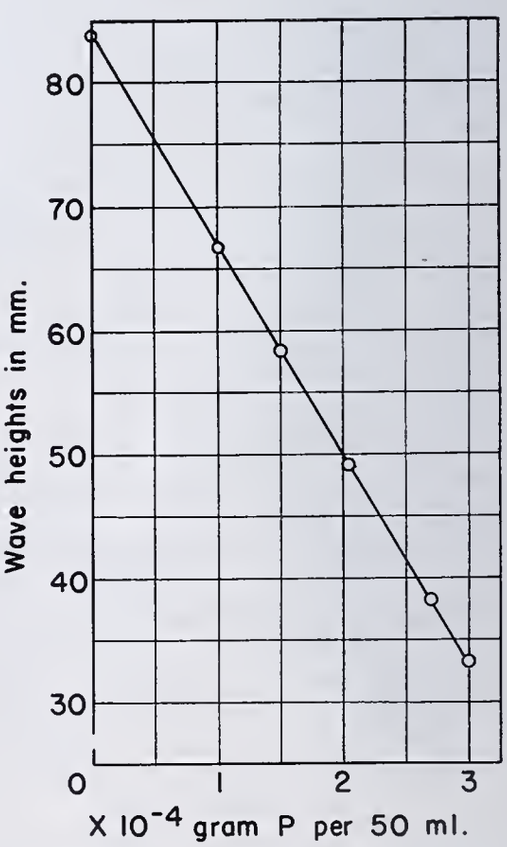

Figure 3. Calibration Curve for Determination of Phosphorus

Derived by plotting wave heights of curves shown in Figure 2 (and others in series) against amounts of phosphorus precipitated from solutions

monium phosphomolybdate. The method requires small amounts of material, effects a great saving of time in routine analyses, and eliminates some of the sources of errors involved in the chemical method.

The method may be applied to the determination of phosphorus in organic material as well as plant ash. Illustrative results of determinations upon foods, urine, and feces by both polarographic and chemical methods are presented.

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Spectrophotometric Determination of Phosphorus

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A NUMBER of investigators have described colorimetric methods for the determination of phosphorus based upon the reduction of molybdic acid. As far as can be determined, however, no complete spectral data on the characteristics of the blue color are available. In the course of investigations on cottonseed phospholipids and proteins, information of this nature has been obtained and an improved method for the determination of phosphorus has been devised.

In a study of the reaction mechanisms involved in the quantitative determination of phosphorus, Berenblum and Chain (*2*) demonstrated that the phosphate ion acted as a catalyst for the reduction of molybdic acid by stannous chloride; they concluded that optimal results for phosphorus could be obtained only within a very narrow range of acid concentration (1.1 *N*). An examination of their graph reveals that a fairly constant intensity of color is recorded also in the acid concentration region of 1.8 to 2.0 *N*, thereby suggesting a method wherein final concentration of acid does not require careful adjustment. Inasmuch as high acid concentrations inhibit the rate of the reduction of molybdic acid, it was found necessary to develop the color by heating, as recommended by Benedict and Theis (*1*) for the reduction by hydroquinone, and by Horecker, Ma, and Haas (*4*) for the reduction by 1,2,4-aminonaphtholsulfonic acid.

Experimental

A Coleman spectrophotometer (model 10S DM, 7.5 mμ slit) was used to measure the transmittance of the solutions. The spectral curves, from 400 to 950 mμ, for three concentrations of phosphorus in 2 *N* sulfuric acid are shown in Figure 1. It is apparent that a definite minimum transmittance occurs at 820 mμ. Dyer and Wrenshall (*3*) recorded a minimum at 660 mμ (photoelectric colorimeter with filters), although it was later reported that the stannous chloride which had been used was very impure (*7*). McCune and Weech (*6*) showed that up to 750 mμ a minimum was not reached (Hardy recording spectrophotometer), which is in line with the results presented in the figure. The nature of the spectral curve indicates that, if filters are used, they should transmit light in the red region of the spectrum, so that maximum sensitivity and minimum interference are obtained.

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Calibration data are set forth graphically in Figure 2. Beer's law applies for concentrations up to 1 microgram of phosphorus per ml. of the colored solution. The percentage transmittance was measured against blanks and the extrapolation to zero concentration of phosphorus gave a value of 98 per cent.

The results obtained when the final concentration of sulfuric acid was varied from 1.7 to 2.4 *N*, using a constant quantity of the other reagents, are given in Table I. Blanks in this acid range developed a faint yellow color which did not interfere with the determination. In acid concentrations less than 1.7 *N*, the blue color appeared in the blank and erratic results were obtained. It is evident that the error involved in determinations in which the acid concentration might vary from 1.7 to 2.1 *N* would be less than 2 per cent.

The amount of stannous chloride reagent may be doubled without any change in the transmittance of the standard at 820 mμ, although there is a slight increase in the yellow color of the blank.

A study of the stability of the color developed under the conditions finally adopted showed no variation in intensity over a period of 24 hours if the colored solutions were kept

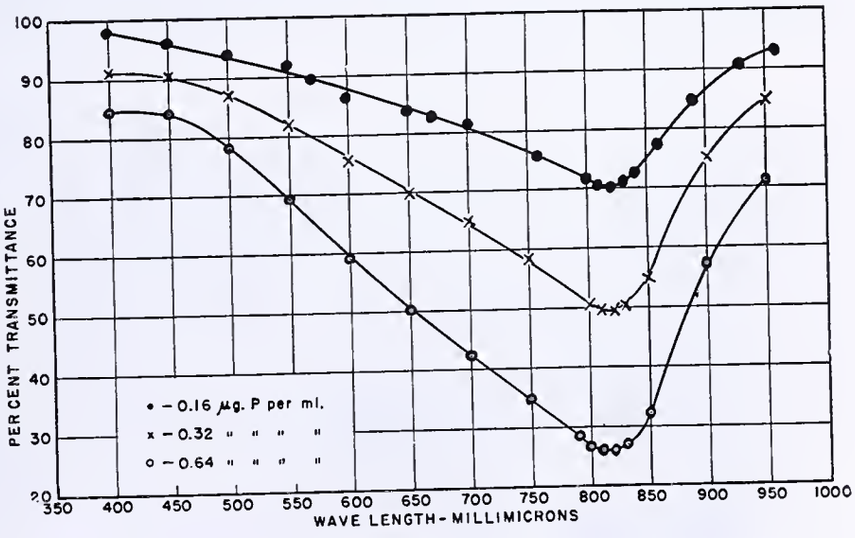

Figure 1. Transmittance–Wave Length Curves for Three Concentrations of Phosphorus

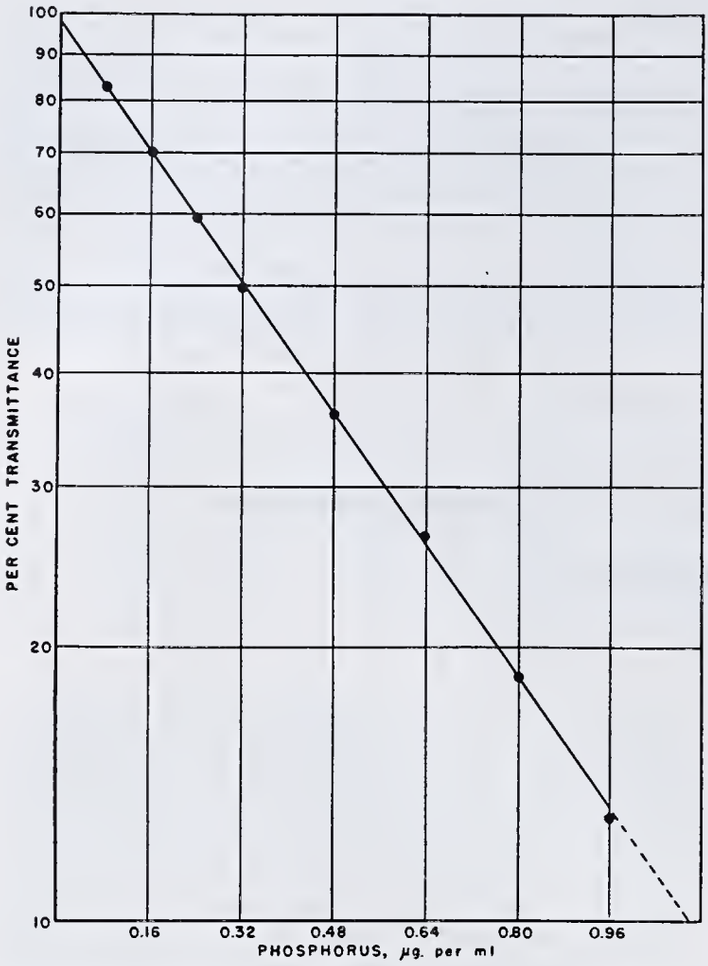

FIGURE 2. CONCENTRATION OF PHOSPHORUS *vs.* TRANSMITTANCE

in closed flasks. A maximum decrease in color of about 2 per cent occurred if the solution was allowed to stand in the cuvette of the spectrophotometer for 1 hour.

Reagents

Sodium molybdate (phosphate-free). Dissolve 75 grams in 1 liter of distilled water.

Stannous chloride. Dissolve 10 grams of the C. P. salt in 25 ml. of concentrated hydrochloric acid and store in a glass-stoppered brown bottle. Kuttner and Lichtenstein (*5*) suggest that the solution should not be used longer than 4 weeks. Dilute 1 ml. of the stock solution to 200 ml. with water for each set of determinations.

Standard phosphate solution. Dissolve 0.4389 gram of anhydrous potassium dihydrogen phosphate in 1 liter of distilled water (*5*). This solution (0.1 mg. of phosphorus per ml.) may be diluted to give the required concentrations.

Recommended Procedure

STANDARDIZATION. To known quantities of phosphate solution (2 to 24 micrograms of phosphorus) in a 25-ml. glass-stoppered volumetric flask add 5.0 ml. of 10 *N* sulfuric acid, 2.5 ml. of 7.5 per cent sodium molybdate solution, and sufficient water to bring the volume to approximately 22 ml. It is necessary to shake the mixture at this point in order to avoid the appearance of a premature blue color upon the addition of the reducing agent. Then add 2.5 ml. of stannous chloride and again mix the contents of the flask. Place the loosely stoppered flask in boiling water for 20 minutes to develop the color, cool to room temperature, and adjust the volume to 25 ml. Transfer approximately 5 ml. of this solution to the cuvette of the spectrophotometer and measure the percentage transmittance at 820 mμ, against a blank receiving identical treatment. The calibration curve needs to be determined only once.

UNKNOWN. To 15 to 20 mg. of phospholipid or 30 to 40 mg. of protein, in a micro-Kjeldahl flask, add 10 ml. of 10 *N* sulfuric acid. Heat the contents of the flask over an open flame until the water is removed and the sample is charred. After cooling, add a drop of 30 per cent hydrogen peroxide (C. P.) and again heat the mixture slowly, with shaking, in order to get maximum clarification. Repeat such treatment until the solution becomes clear, after which add water to the cooled digest and again heat the solution to boiling in order to convert the pyrophosphate to orthophosphate. Dilute the digest and measure an aliquot into a 25-ml. volumetric flask. Add sulfuric acid (10 *N*) in quantity sufficient to make the final concentration approximately 2 *N*. The remainder of the procedure has been described above. Compare the final color against a blank treated in exactly the same manner, but without added phosphorus. It is evident that, depending upon the size of sample and amount of phosphorus contained, the quantity of sulfuric acid used for digestion may be varied, because the adjustment to the desired final concentration is made later.

Advantages

The method described appears to have the following advantages: The reducing agent, stannous chloride, is cheaper and more readily available than the organic reducing agents that have been suggested. The amount of stannous chloride used can be varied without affecting the intensity of the developed color. It is not necessary to make an accurate adjustment of the final concentration of acid, which may vary from 1.7 to 2.1 *N*. The developed color is stable for at least 24 hours if the solution is kept in a closed flask. Greatest accuracy is attained by measuring the percentage transmittance at 820 mμ.

The difficulties observed by Willard and Center (*8*) in the determination of phosphorus in steels by the stannous chloride method might possibly be eliminated by the modifications here described.

TABLE I. INFLUENCE OF VARYING ACID CONCENTRATION ON COLOR INTENSITY

Concentration of Sulfuric Acid	Transmittance at 820 mμ[a]
N	%
1.7	49.8
1.8	49.6
1.9	49.5
2.0	49.4
2.1	49.2
2.2	48.8
2.4	47.5

[a] 0.32 microgram of phosphorus per ml.

Summary

The spectral curve for the blue color which is obtained from molybdic acid by reducing agents in the presence of phosphorus, and which is used for the quantitative determination of phosphorus, is described. Minimum transmittance occurs at 820 mμ. An improved micromethod, utilizing several procedures developed by previous investigators, is presented.

Acknowledgment

The author is indebted to H. S. Olcott for proposing the problem and for guidance in preparing the manuscript.

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Modifications in the Dumas Micromethod for Nitrogen

Automatic Apparatus Applicable for Combustion Micromethods

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THE application of automatic combustion technique to the Pregl micromethods has proved very satisfactory. Previous publications from this laboratory have described apparatus for the determination of ash in organic compounds (*6*) and of carbon and hydrogen (*8*).

The apparatus described here was designed after experience with several other automatic types. It is an adaptation of the Heraeus-type furnace (*4*) to an automatic combustion arrangement, which although similar in principle to that described by Hallett (*2*), is different in construction and operation. The Dumas micromethod described by Pregl (*7*) is essentially followed, but combustion rates, time of combustion, source of pure carbon dioxide, and method of calculation differ. While many of the points in the procedure and the apparatus are not original, the assembly and method of operation have proved to have many advantages. The apparatus was originally designed for the Dumas method, where the combustion tube must be removed from the stand to be filled for each determination, but, it can also be used for carbon-hydrogen, halogen, sulfur, etc.

Combustion Furnaces and Auxiliary Control Equipment

Figure 1 shows the automatic combustion furnace set up for the determination of nitrogen by the Dumas method. Figures 2 and 3 show close-up views of the stationary and movable furnaces.

Both furnaces can be moved in a lateral direction on sliding carriages fastened to the supporting bars. However, the stationary furnace is locked in position at the place where it meets the small furnace when the latter is at the end of its motion. The adjustable sleeves on the carriages are the bearing surfaces for this lateral motion. The furnaces proper are fastened to aprons which in turn slide on two bars, making possible a back-and-forth motion over the carriages.

The power for moving the small furnace is obtained from a two-speed governor-controlled phonograph motor. The drive from this motor is transferred by suitable gears to the horizontal screw which is located under the movable furnace. This screw is square cut; contact and release with the movable furnace carriage are obtained by use of a split nut arrangement, the opening and closing of which are controlled by a knob. When open, the furnace can be moved by hand in any direction desirable; when closed, it is moved by the screw.

The furnaces proper are encased in a housing made from standard 5.08-cm. (2-inch) square stock brass tubing. The Alfrax square tubing refractory lining is 2.54 cm. (1 inch) in inside diameter with 6.3-mm. (0.25-inch) walls (Carborundum Company, Niagara Falls, N. Y.). The front of the brass tubing is cut out, leaving a small edge to hold the refractory in place. The refractory tube is cut to give a beveled piece which can be fastened to the door of the furnace, so as to complete the lining when it is closed. The end plates of the furnace are made from Alfrax pieces and are held in position by brass plates (Figure 3, upper right). The brass end plates of the furnaces which come together (Figure 3, lower left) are inset into the refractory plates, so that the two refractory plates come together when the movable furnace reaches the fixed furnace. All brass parts are nickel-plated.

As previously described (*2*, *6*, *8*), the platinum resistance wire is wound onto four refractory rods which are held in position in the refractory end plates. In the large furnace 457.2 cm. (180 inches) of 0.406-mm. (0.016-inch) pure platinum wire are used, while the small furnace is wound with 457.2 cm. (180 inches) of 0.318-mm. (0.0125-inch) platinum wire. The wire is then coated with Alundum cement (Norton Co., Worcester, Mass., RA-562 fine) to protect the platinum from volatilization and give it a longer life.

Chromel-Alumel (Hoskins Mfg. Co., Detroit, Mich.) couples

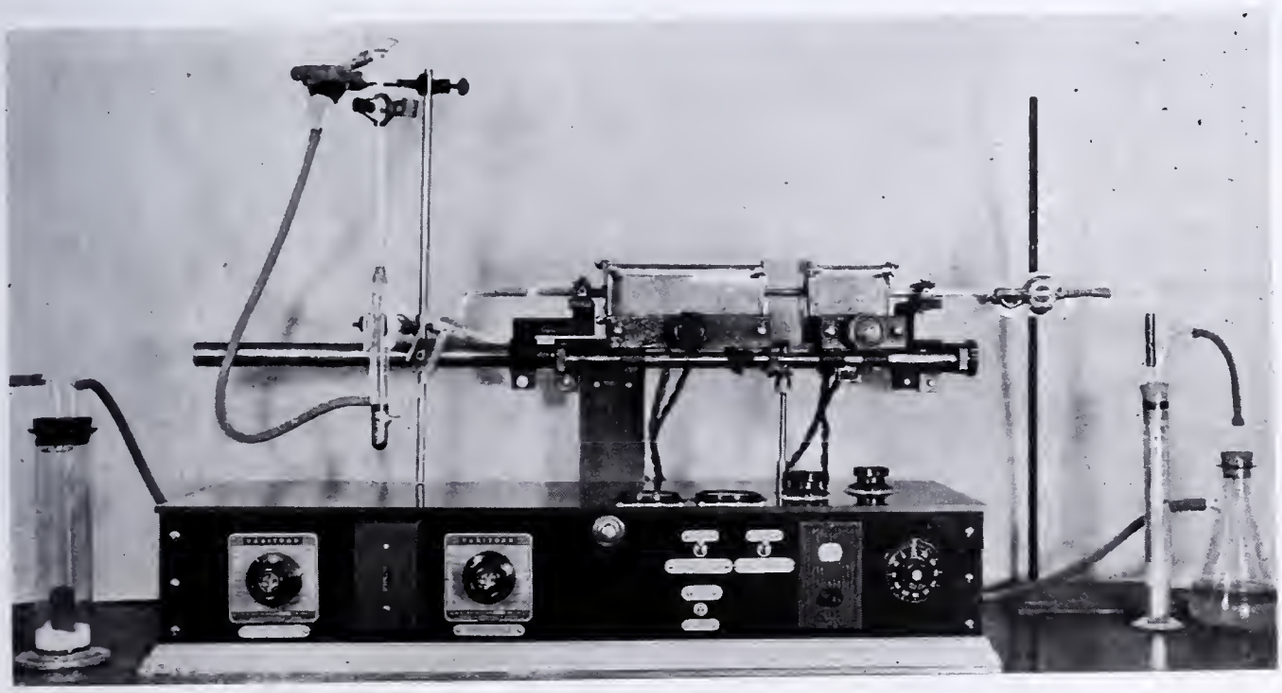

FIGURE 1. AUTOMATIC COMBUSTION FURNACE

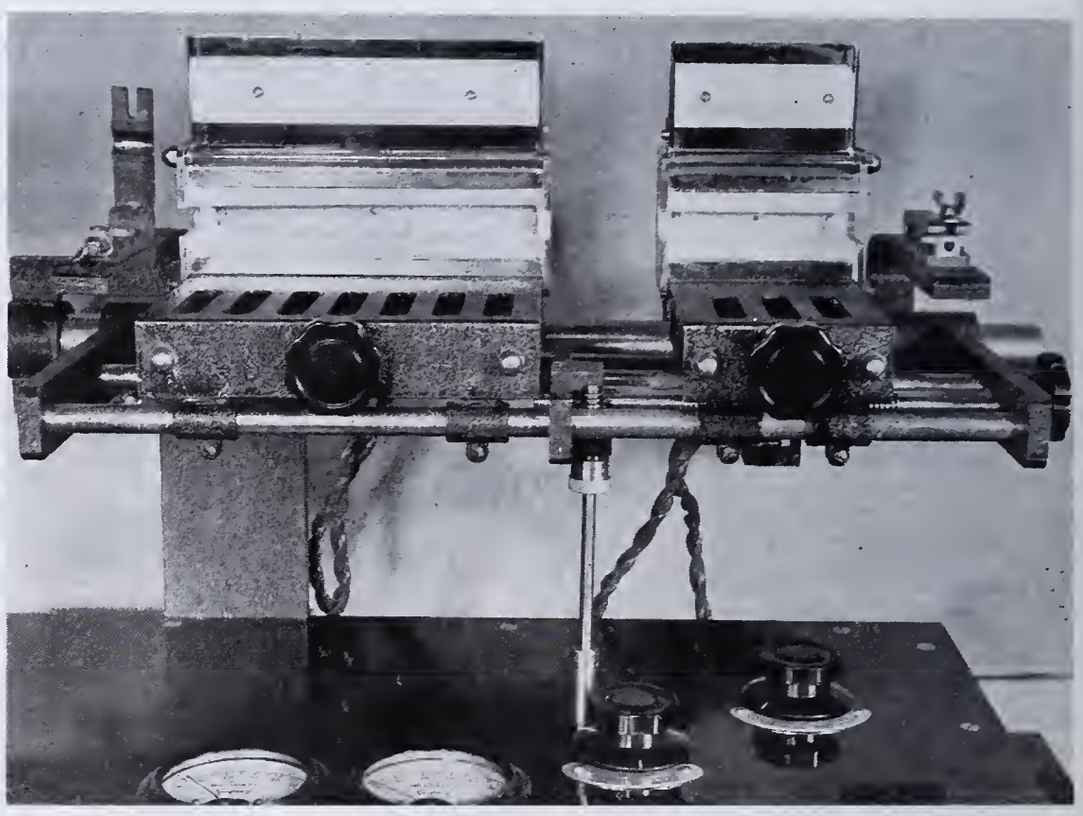

FIGURE 2. CLOSE-UP VIEW OF FURNACES

in each furnace lead to individual millivolt meters, calibrated by correlation with a standardized meter. The thermocouple from the standard meter is put into the combustion tube in the position at which the temperature is to be correlated.

The combustion furnaces with their assembly are mounted by means of two brackets on a supporting bar, which is in turn supported from a base in which auxiliary control equipment is located. Two Varitrans of 7.5-ampere capacity (United Transformer Corp., New York, N. Y.) serve as variable controls of the voltages for producing heat in the two furnaces. Two knobs which operate the governor and speed controls of the motor can be adjusted from the top of the base. An automatic switch (Walser Automatic Timer Co., New York, N. Y.) on the front panel controls the length of time the motor and the heat in the small furnace are in operation. A signal-type snap switch controls the power for the whole apparatus, while individual switches control the separate units which may have to be turned off independently.

Glass Apparatus

The dimensions of the glass apparatus follow those specified in the "Recommended Specifications for Microapparatus, Part II, Dumas Nitrogen" (*1*). Quartz combustion tubes have been found more satisfactory than glass and often last more than 6 months. Attempts to connect the apparatus by ground-glass joints have not been too successful because of the inflexibility of the setup.

Source of Pure Carbon Dioxide

The Kipp generator with various modifications to ensure delivery of pure carbon dioxide, generators producing pure carbon dioxide from carbonates by both acid treatment and heat, and apparatus for controlling the delivery of pure carbon dioxide from dry ice are used for the Dumas micro procedure. In this laboratory most of these have been tried and the latter has proved satisfactory for several years. Since the same carbon dioxide used to make dry ice is used to fill the tanks, it was decided to try to use tank carbon dioxide (conversation with J. A. Baty of the U. S. Rubber Company prompted this investigation). The Pure Carbonic Corporation (telephone conversation with Mr. Dean of the Newark office) suggested that 5 pounds of gas be bled off from a new tank while it was in a vertical position with valve up. This rapid exhaust should eliminate most of the air, which should be on top, and give as pure carbon dioxide as is obtained from dry ice. Experimentation in the use of tank carbon dioxide led to a procedure in which a constant blank of 0.010 cc. for a determination was obtained.

It was found that the pressure of the carbon dioxide entering the combustion tube affected the blank value obtained. If the pressure was low, high blank values were obtained. In using a gasometer (*5*) the pressure is rather high, about 38 cm. (15 inches) of mercury at the start of the determination. With the dry ice generators the pressure varies, depending upon the type of valve used. Using tank carbon dioxide, about 20 cm. (8 inches) of mercury contained in a 100-ml. cylinder with a T-tube served satisfactorily as the pressure regulator. The excess exhaust gas is washed with acid to eliminate mercury vapor and expelled through a hood to remove any health hazard. A container inserted in the exhaust carbon dioxide line stores ignited fine copper oxide, used in the temporary filling.

No explanation can be given for the high pressure necessary to obtain low blank values, but experimentation shows that a rather tight packing of the combustion tube and high pressure of carbon dioxide entering the tube give the best results. Using the high pressure, care must be taken to have all rubber connections in good condition and therefore the rubber stopper and the tubing connection to the azotometer must be changed frequently.

Procedure

The permanent filling of the combustion tube is made as follows: About 0.5 cm. of asbestos is packed down into the tip end of the tube, followed with about 13 cm. of coarse copper oxide and another 0.5-cm. plug of asbestos. A 6-cm. copper gauze, which has been previously reduced, is next inserted into the tube, then another 0.5-cm. plug of asbestos followed with about 6 cm. of coarse copper oxide. A final 0.5-cm. asbestos plug holds this permanent filling in position in the tube. This last plug should be at the end of the stationary furnace, and about 7 to 8 cm. of the copper oxide filling at the tip end should extend out of the other end of the stationary furnace.

In carrying out the analysis, 5 cm. of fine copper oxide are introduced into the combustion tube. The sample which has been weighed out into a porcelain boat is put into the tube and the boat is allowed to slide gently down the side of the tube. Inti-

mate mixing of the sample and the copper oxide is accomplished by a gentle up-and-down motion of the tube. A small additional amount of copper oxide is added to wash any small particles down into the tube, making the total filling at this point about 7 to 8 cm. of fine copper oxide. The one-hole rubber stopper is then inserted into the open end of the tube and is connected to the stopcock at which the carbon dioxide enters. It is important that this rubber stopper be kept in good condition and be changed often, as it may be a source of error if it becomes old and cracked. The azotometer is attached to the combustion tube by means of heavy-walled tubing, so that the azotometer and combustion tube meet glass to glass. The stopcocks are opened in such a manner that the gas flows through the tube, expelling the air which is in the tube to the atmosphere.

The stationary furnace is brought forward over the combustion tube, so that the permanent filling becomes heated. After about 2 minutes the stopcock at the right-hand side of the apparatus is closed and the two-way stopcock on the azotometer is turned, allowing the gas to pass into the azotometer. When microbubbles are obtained, the stopcock is closed and the small amount of gas which has collected in the top of the azotometer is removed by means of the leveling bulb; then the two-way stopcock is opened again to the azotometer. The movable furnace is then pulled into position, about 5 cm. from the stationary furnace, and brought over the tube proper by pulling the furnace forward. The automatic switch is set for about 20 minutes. During this 20 minutes the movable furnace moves over the sample, carrying out the combustion, and the motor is set so that the travel is about 5 cm. (2 inches) in 20 minutes. At the end of this time, the current is automatically shut off by the time switch. The operator now closes the stopcock on the azotometer and opens the stopcock from the source of carbon dioxide. The two-way stopcock is opened carefully to allow not more than one bubble per second to run into the azotometer. After a few minutes the rate can be increased, so that the entire washing out requires only about 10 to 12 minutes. During this time both furnaces are kept on the tube, but the movable furnace has been turned off and is gradually cooling down. When microbubbles are obtained, the stationary and movable furnaces are both pushed into the back position and the two-way stopcock on the azotometer is closed.

The volume of nitrogen is measured immediately after the leveling bulb has been brought to the level of the meniscus in the azotometer.

For the calculation of the per cent nitrogen, the procedure as outlined by Milner and Sherman (*3*) is used. The potassium hydroxide used in the azotometer is made up to a concentration of 41.6 per cent real, since the vapor pressure data for potassium hydroxide are available for this concentration. It has been found that 50 per cent real potassium hydroxide will not readily absorb pure carbon dioxide and very high blank values are obtained, but, in a concentration of about 40 per cent the absorption is satisfactory. It appears that the 50 per cent real potassium hydroxide is too viscous and that the carbon dioxide is not properly wet out by this viscous solution. For the calculation, the vapor pressure for the potassium hydroxide is subtracted from the barometric pressure to obtain the corrected pressure. The volume of the blank and 0.5 per cent of the observed volume are subtracted from the volume of gas collected to give the corrected volume of gas. This method of calculation is very much more satisfactory than any of the others suggested, especially with a variation in room temperature which over the entire year may be from 25° to 36° C. If the room temperatures vary only over narrow limits, a constant percentage correction is satisfactory.

Discussion

The procedure using automatic combustion for the Dumas method gives results which are more reproducible, and the

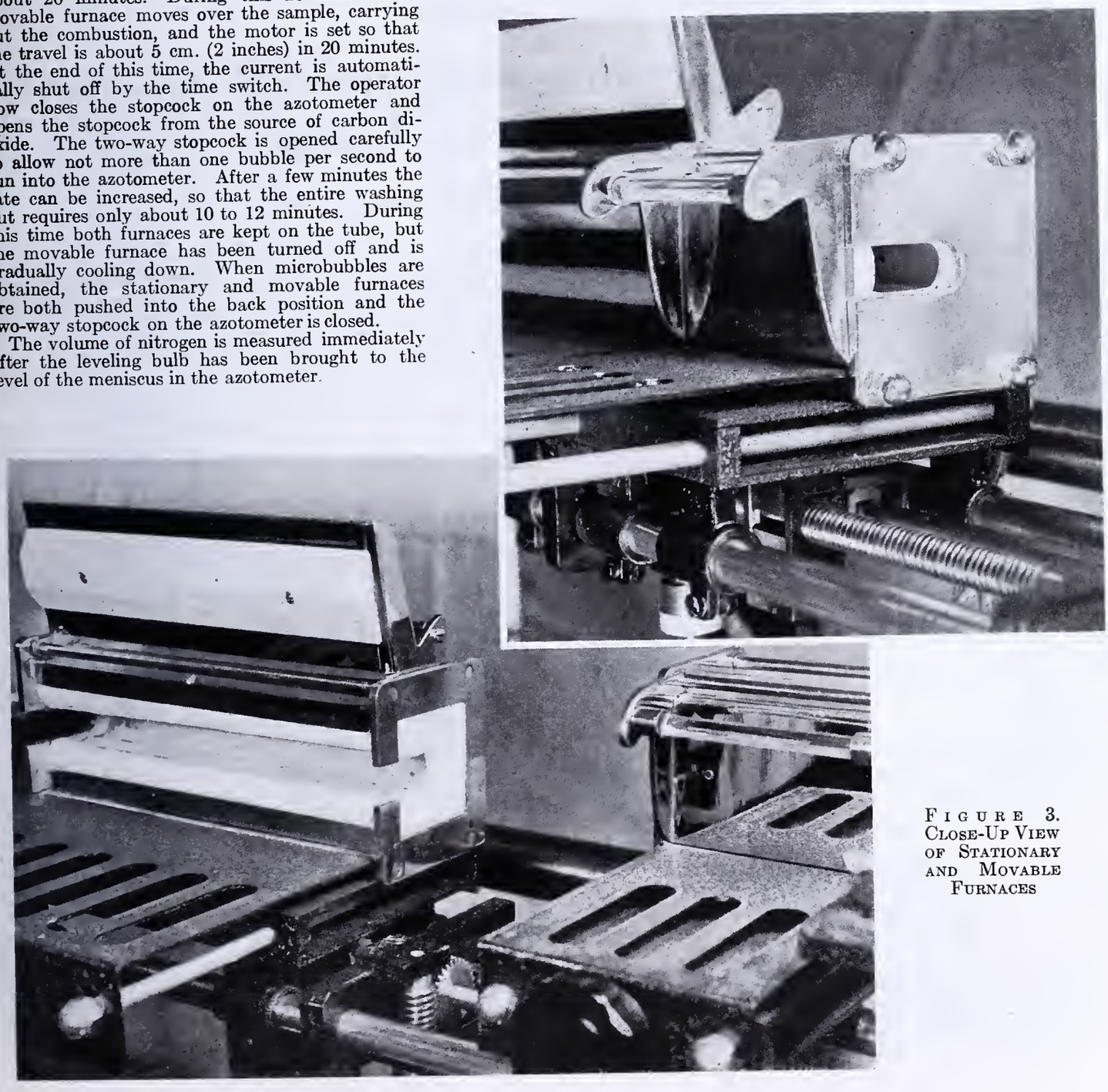

FIGURE 3. CLOSE-UP VIEW OF STATIONARY AND MOVABLE FURNACES

time necessary for a determination is less, than by the regular Pregl procedure. Another reason for automatic combustion is the reduction of man-hour time necessary for operation. Automatic combustion makes the burning rate uniform and therefore more reproducible conditions are obtained. The total elapsed time for a single analysis is about 40 minutes, of which about 20 minutes requires the operator's attention, including the time for weighing the sample.

The results obtained have the same accuracy as those reported for the Pregl microprocedure. The apparatus is checked once or twice a week on standard compounds. The average value for nitrogen obtained on 20 analyses of a standard sample of 2-amino-1-naphthalene sulfonic acid was 6.29 per cent with a limit of error (2σ) of ± 0.13 per cent. The theory for this compound was 6.28 per cent.

Acknowledgment

The authors wish to acknowledge the assistance of A. F. Mincz and W. L. Hatton in the construction and design of the automatic apparatus. Acknowledgment is also made to other members of the microchemical laboratory, especially to R. Koegel, for contributions made during the development of the procedure.

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Colorimetric Microdetermination of Arsenic after Evolution as Arsine

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SEVERAL methods for the colorimetric determination of arsenic after evolution as arsine have been described. Morris and Calvery (*3*) decompose the arsine by passing it through a heated fused silica tube, dissolve the deposited arsenic in nitric acid, and after the evaporation of the latter determine arsenic by the molybdenum blue method. Taubmann (*4*) absorbs the arsine in silver sulfate solution, precipitates the excess of silver with chloride, oxidizes the arsenic in the filtrate to the quinquevalent condition by boiling with bromine water, and finally applies the molybdenum blue method. The time required for a determination is about 3 hours.

The following method is based on the absorption of arsine in an acid solution of mercuric chloride containing permanganate. Arsine is thus oxidized in one step to arsenate, and arsenic can then be determined in the solution by adding an excess of ammonium molybdate–hydrazine sulfate and heating to obtain the molybdenum blue. Cassil and Wichmann (*2*) have used mercuric chloride solution for the absorption of arsine in a microvolumetric method for arsenic based on iodometric titration.

The following procedure is designed for amounts of arsenic ranging from 1 to 15 micrograms. Approximately one hour is required for a determination. The directions for the evolution of arsine are similar to those of the A. O. A. C. (*1*).

Apparatus

Cassil and Wichmann (*2*) have described an apparatus for the evolution and absorption of arsine in mercuric chloride solution, which doubtless can be adapted to the procedure below.

In the present work the apparatus consists of a 50-ml. Erlenmeyer flask closed by a one-hole rubber stopper from which leads a tube, *A* (Figure 1), containing at its lower end one or two plugs of glass wool impregnated with lead acetate. This tube is connected by means of a short section of rubber tubing to the delivery tube, *B*, which is drawn down to a capillary tip having an opening of about 0.5 mm. The absorption vessel, *C*, is drawn from a test tube, and should have a capacity of 8 to 10 ml., with the tapered portion of such dimensions that 1.35 ml. of absorbing solution will have a depth of 6 to 7 cm. A short piece of glass tubing, *D*, having an internal diameter approximately 1 mm. greater than the external diameter of tube *B*, is placed in the absorption vessel to break up the bubbles of gas and provide more absorption surface; it should be completely covered by solution.

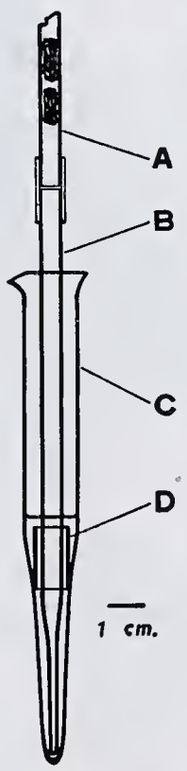

FIGURE 1. APPARATUS FOR ABSORPTION OF ARSINE

The apparatus used should be tested for its ability to absorb arsine completely by running a known amount of arsenic.

Reagents

Stannous chloride, 40 grams of stannous chloride dihydrate in 100 ml. of concentrated hydrochloric acid.

Potassium iodide, 15 grams in 100 ml. of water.

Zinc, 20- to 30-mesh, "arsenic-free".

Mercuric chloride, 1.5 grams in 100 ml. of water.

Potassium permanganate, 0.03 *N*, 0.10 gram in 100 ml. of water. Discard the solution when a precipitate of manganese dioxide forms.

Ammonium molybdate–hydrazine sulfate. Prepare fresh daily by mixing 10.0 ml. each of solutions A and B and diluting to 100 ml. with water. Solution A: Dissolve 1.0 gram of ammonium molybdate in 10 ml. of water and add 90 ml. of 6 *N* sulfuric acid. Solution B: Dissolve 0.15 gram of hydrazine sulfate in 100 ml. of water.

Standard arsenic solution. To prepare a 0.100 per cent arsenic solution, dissolve 0.1320 gram of arsenic trioxide in 2 or 3 ml. of 1 *N* sodium hydroxide, dilute with water, make acidic with hydrochloric acid, and dilute to 100 ml. From this stock solution, prepare by dilution a standard solution containing 0.010 mg. of arsenic per milliliter.

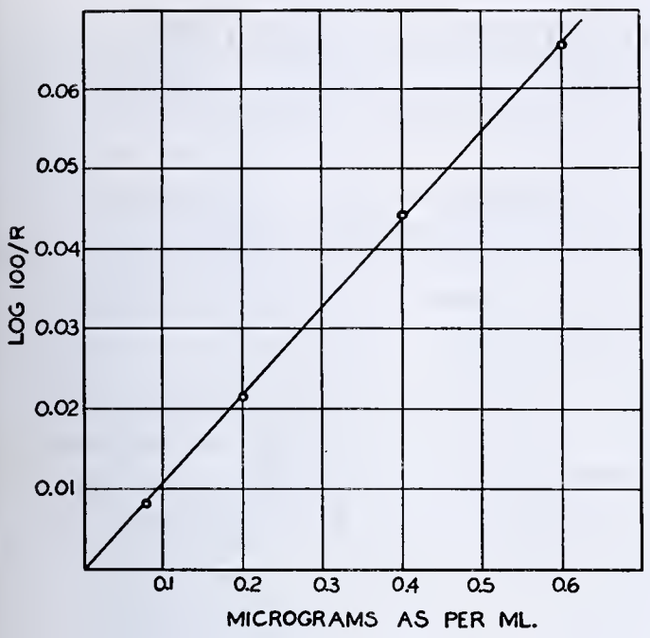

FIGURE 2. CALIBRATION CURVE (1-CM. CELL)

Procedure

The prepared sample must be free from substances that prevent the complete evolution of arsine.

Transfer the solution of the sample, conveniently having a volume of 25 ml. and containing not more than 15 micrograms of arsenic (20 micrograms of arsenious oxide), to the 50-ml. Erlenmeyer flask and add sufficient concentrated hydrochloric acid to make its total volume 5 ml., 2 ml. of potassium iodide solution, and 0.5 ml. of stannous chloride solution. Allow the mixture to stand at room temperature for 15 to 30 minutes to assure the complete reduction of arsenic from the quinquevalent to the trivalent condition. Alternatively heat to 80–90° C., keep at this temperature for 5 minutes, and then cool to room temperature.

Measure 1.0 ml. of mercuric chloride solution, 0.2 ml. of 6 *N* sulfuric acid, and 0.15 ml. of potassium permanganate solution into the absorption vessel. Mix with a thin glass rod. Connect the drawn-out delivery tube to the tube passing through the rubber stopper and lower the former into the absorption vessel, so that its tip nearly touches the surface of the solution. The absorbing solution should not be allowed to enter the delivery tube, for then a coating of mercury arsenide is likely to be formed later on the interior of the tube.

When all is in readiness quickly add 2.0 grams of zinc to the flask. Immediately insert the stopper and lower the delivery tube into the absorbing solution so that its tip barely touches the bottom of the vessel. Allow the gases to bubble through the solution for 25 or 30 minutes without heating the flask. At the end of this time, the solution should still contain some permanganate. With larger amounts of arsenic (10 micrograms) the solution will be more or less turbid because of the separation of hydrated manganese dioxide; this does no harm. Disconnect the delivery tube from the rest of the apparatus, leave it in the absorption vessel, add 5.0 ml. of ammonium molybdate–hydrazine sulfate reagent, and mix well. Heat for 15 minutes in a water bath at 95–100°, cool, transfer the solution to a 10- or 25-ml. volumetric flask, and make up to volume with water. Filter the solution through a small plug of fine glass wool in a small funnel and reject the first portion of the filtrate.

Obtain the transmittancy of the clear solution with a photoelectric colorimeter, using a red filter (preferably one showing maximum transmission at about 700 mμ). In the comparison cell place a reagent solution obtained by mixing 1 ml. of mercuric chloride solution, 0.2 ml. of 6 *N* sulfuric acid, and 0.10 ml. of potassium permanganate, and heating in a water bath for 5 minutes. Add 5.0 ml. of ammonium molybdate–hydrazine sulfate and heat for 15 minutes at 95°. Cool to room temperature, make up to the same volume as the unknown, and filter.

Since Beer's law holds for the color system (Figure 2), the reference curve can be constructed by finding the transmittancy of a single solution of known arsenic concentration. Mix 1.00 ml. of 0.001 per cent arsenic (as arsenious oxide) solution, 1.0 ml. of mercuric chloride, 0.2 ml. of 6 *N* sulfuric acid, and 0.10 ml. of potassium permanganate solution, and heat at 95° for 5 minutes. Then add 5.0 ml. of the molybdate-hydrazine reagent and proceed as described for the unknown.

Correct the result for a blank carried through the procedure.

Discussion

The results obtained in the application of the method, mostly to pure arsenic solutions, are given in Table I. Judging from the values obtained, 5- or 10-microgram quantities of arsenic can be determined with an accuracy of 5 per cent, and 1- or 2-microgram quantities with an accuracy of 10 per cent. The results tend to be low. Antimony in small amounts does not interfere appreciably, although it appears to lower the results somewhat (14 and 15, Table I). Phosphine, from phosphides, would be expected to give high results, but such error can be prevented by subjecting the sample to a preliminary oxidizing treatment.

The composition of the molybdate-hydrazine reagent has been altered from that recommended by others (*3*) by increasing the hydrazine sulfate concentration. The mixed reagent should be prepared fresh daily, because its reducing power decreases relatively rapidly on standing. The component solutions are stable for a long time, so that a reagent of constant reducing power is easily obtained by mixing these in the ratio specified, and the reference curve need not be reconstructed. However, the best practice is to construct the calibration curve the same day the determination is made. The blank solution, for the comparison cell, has a slight brownish color, but this is of no moment when a photoelectric colorimeter is used. A very slight difference in hue between unknown and standard solutions may be expected if a Duboscq-type colorimeter is used.

There is a slight precipitation of mercurous chloride when the absorbing solution is heated with the reagent. Although the precipitate is very small in amount, it must be filtered off when a photoelectric colorimeter is used. Filtration through a plug of fine glass wool is satisfactory. Repeated filtration of a reduced solution of arsenomolybdate through glass wool caused no change in the concentration of the colored compound.

An acid solution of potassium permanganate alone will not absorb arsine completely. Ceric sulfate and potassium bromate cannot be used in place of potassium permanganate in the absorbing solution. Potassium bromate (in hydrochloric acid solution) allowed a precipitate of mercury arsenide to form, and the same was true for ceric sulfate, but to a smaller extent. Even if ceric sulfate were effective as an oxidizer, its use would be inadvisable because of the common presence of phosphate in ceric salts.

TABLE I. DETERMINATION OF ARSENIC

(Volume of final solution 10 ml. in Nos. 1 and 2, 25 ml. in all others; cell thickness 1 cm.)

No.	As Taken γ	As Found γ	Error γ
1	1.0	0.9	−0.1
2	1.0	0.9	−0.1
3	2.0	1.8	−0.2
4	2.0	2.0	0.0
5	5.0	5.2	+0.2
6	5.0	4.9	−0.1
7	5.0	4.8	−0.2
8	7.5	7.3	−0.2
9	10.0	9.8	−0.2
10	10.0	9.7	−0.3
11	10.0	9.8	−0.2
12	10.0	10.3	+0.3
13	15.0	15.1	+0.1
14	10(+10γ Sb)	9.5	−0.5
15	10(+10γ Sb)	9.5	−0.5
16	10(+60 mg. Fe^{III})	9.8	−0.2

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A du Pont Type Semimicronitrometer

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The construction and operation of a semimicronitrometer are described. The nitrometer is of the compensating type, analogous to the du Pont nitrometer and the Lunge gasvolumeter, in which the quantity of gas is read at the volume it would occupy at standard conditions of temperature and pressure. Two models of the nitrometer have been developed, one hand-shaken like the macronitrometers and the other motor-shaken. Various inorganic and organic nitrates, including nitrocelluloses, have been analyzed. The apparatus can also be applied to the other determinations for which the usual Lunge or du Pont nitrometers are used.

The advantages of the semimicronitrometer as compared to the compensating macronitrometers are use of a much smaller amount of mercury, compactness, and easier operation. In addition, the motor-operated model lessens the danger of explosions and decreases the man-time required by allowing the operator to weigh out samples or run other nitrometers while the apparatus is being shaken. The precision is almost as good as that of the macroinstrument; the accuracy, slightly less.

THE nitrometer most widely used for the analysis of mixed acids and organic and inorganic nitrates is the du Pont nitrometer (*20*) which is a development of Lunge's gasvolumeter (*13, 14*). This instrument is used for one of the more important and frequent determinations made on explosives, the determination of nitrate and nitrite nitrogen. The du Pont nitrometer, which has been standardized as regards dimensions and operation (*1, 15*), is superior to the ordinary Lunge nitrometer in that a volume of gas in the measuring buret of the apparatus may be readily brought to the volume that it would occupy under standard conditions of temperature and pressure. This is accomplished by means of a compensating tube containing a quantity of gas whose volume at standard conditions is accurately known.

The disadvantage of the du Pont nitrometer is its large size, which necessitates the use of 11 to 18 kg. (25 to 40 pounds) of mercury for its operation and makes the manual shaking of the reaction tube rather cumbersome. The possibility that the reaction tube may be broken by an explosion while being shaken introduces an element of danger. In view of this, a nitrometer modeled on the du Pont (*1*) and Lunge (*14*) compensating nitrometers was developed which has one tenth the capacity of the du Pont nitrometer. To facilitate the operation of the apparatus, one model of the new nitrometer was mounted for mechanical shaking, thus eliminating the tedium of hand shaking and the danger to the operator of an explosion while shaking. An additional advantage of the mechanically shaken nitrometer is that several can be operated by the same operator, samples being weighed out while the apparatus is being shaken. Although no previous adaptation of the compensating type of nitrometer to smaller volumes or mechanical shaking could be found, Lunge semimicronitrometers have been described (*4, 12*) and a mechanically shaken Lunge nitrometer was recently mentioned (*24*).

Method and Application

The nitrogen in inorganic or organic nitrates or nitrites is readily evolved as nitric oxide by interaction of the substance with sulfuric acid and mercury. The volume of nitric oxide is measured and the percentage of nitrogen in the sample is calculated. This so-called "nitrometer reaction" is given by compounds containing the NO_2 or NO group attached to carbon through nitrogen (*5*) or attached to oxygen. This method is extensively used for the determination of nitric acid in oleum and mixed acids (*8*). The nitrometer method

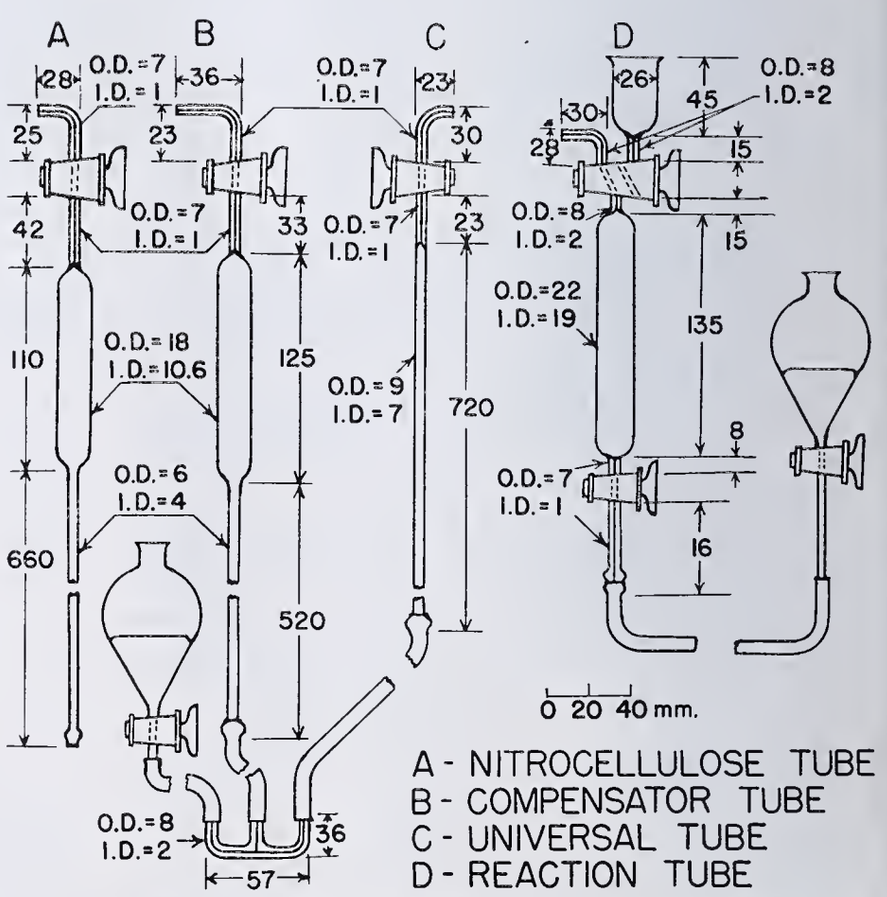

FIGURE 1. HAND-SHAKEN MODEL OF SEMIMICRONITROMETER

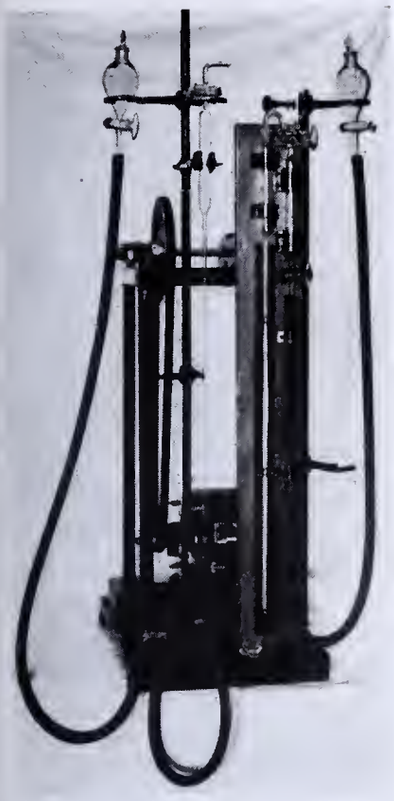

FIGURE 2. MOTOR-SHAKEN MODEL OF SEMIMICRONITROMETER

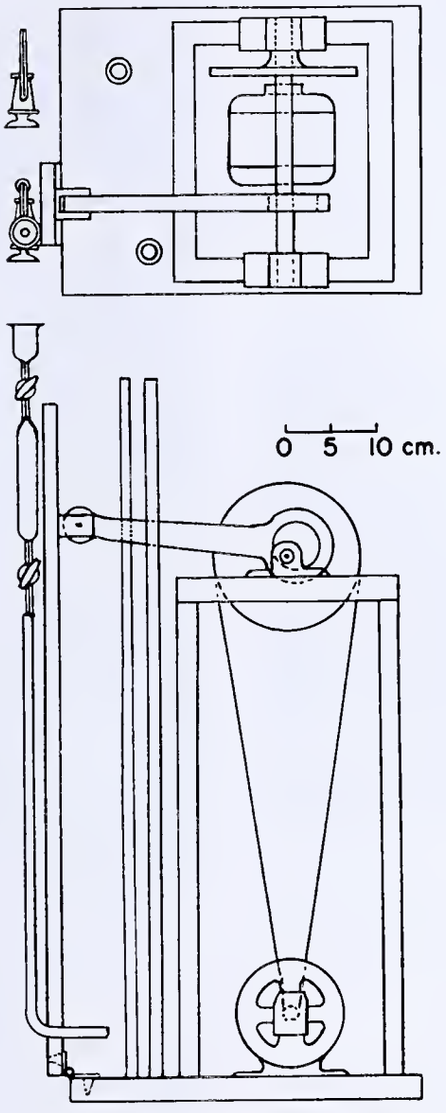

FIGURE 3. MOTOR-SHAKEN MODEL OF SEMIMICRONITROMETER, SIDE AND TOP VIEWS

and possible sources of error as applied to the determination of nitrogen, particularly in explosives, have been fully discussed in the literature and the interference of various substances which react with the nitric oxide has been pointed out (*2*, *3*, *6*, *8*, *10*, *11*, *16–19*, *21*, *22*, *23*). The chief sources of error are the solubility of nitric oxide in sulfuric acid, the formation of ammonia on prolonged contact of the nitrate with sulfuric acid before shaking with mercury, the formation of carbon dioxide from cellulose nitrate under the same conditions, and the formation of nitrous oxide and nitrogen on prolonged shaking with mercury. The equation given (*8*) for the case of potassium nitrate is

$$2KNO_3 + 4H_2SO_4 + 3Hg = K_2SO_4 + 3HgSO_4 + 4H_2O + 2NO$$

The compensating type of nitrometer can be used to analyze other materials which liberate a gas on suitable treatment. It has been applied to the determination of available oxygen in peroxides, hypochlorites, and salts of peroxy-acids, and to the valuation of oxalic acid, formaldehyde, metals, and carbonates (*8*, *14*, *26*).

In the case of the simple Lunge nitrometer, the volume of gas has to be reduced to standard conditions in order to calculate the weight of gas evolved. This process of calculation is avoided by compensating the measuring buret with a volume of gas which has been calibrated to standard conditions of temperature and pressure by bringing a gas in the measuring buret to the volume it would occupy at standard conditions. The manner of doing this is explained in the standardization procedure described later in this article.

Apparatus

Two models of the semimicronitrometer were developed. The hand-shaken model, shown in Figure 1, has one tenth the capacity of the standard du Pont nitrometer (*1*) and was designed to give the required accuracy in reading the gaseous volume. The apparatus is made entirely of Pyrex glass, except for the leveling bulbs which are 60-ml. separatory funnels. The lower stopcock on the reaction tube may be omitted. Red rubber tubing of 4.8-mm. internal diameter and 4.8-mm. wall thickness is used. The capacity of each buret is about 25 ml.; the capacity of the reaction tube is 30 ml. Graph paper scales are pasted securely on the two measuring burets; one unit on this scale equals 1 cm. The scale on the nitrocellulose tube starts at the bottom of the bulb, on the universal tube at the bottom of the capillary from the stopcock. The two measuring tubes were calibrated by weighing the mercury delivered and calculating the volume. Weighings were made every 5 cm. of tube length. The universal tube is used for samples of unknown nitrogen content; the nitrocellulose tube for samples of approximately known nitrogen content, so that a sufficiently large sample can be taken to fill the large bulb with nitric oxide. The lesser diameter of the nitrocellulose tube permits a more accurate reading of the volume.

The motor-shaken model, shown in Figures 2, 3, and 4, is simplified by having the buret and reaction tube

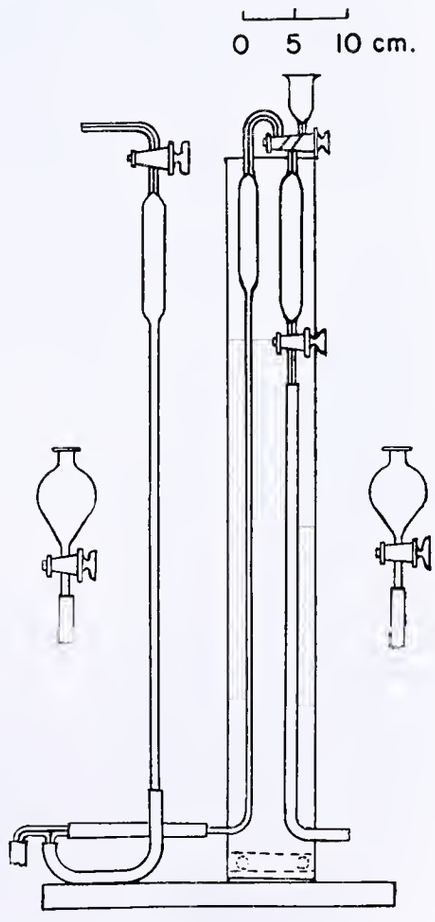

FIGURE 4. MOTOR-SHAKEN MODEL OF SEMIMICRONITROMETER, FRONT VIEW

connected together as closely as possible by a 2-mm. glass capillary tube, thus eliminating a stopcock and a rubber tubing connection. The universal and nitrocellulose tubes, each with its reaction tube attached, are mounted separately on 9 × 73 cm. boards. In operation, a board is hinged at the bottom to the base and is moved in a reciprocal motion near the top by an eccentric mechanism actuated by a 0.05-horsepower motor, being shaken about 300 times per minute. The reaction tube moves through a horizontal distance of about 3 cm. The boards containing the two burets can be readily interchanged by removing the two screws at the base and the one screw at the top. The buret and reaction tube and the rubber tubing connecting the reaction tube with its leveling bulb are fastened to the board with copper or lead straps.

The apparatus stand should be screwed to or clamped on a firm surface to avoid excess vibration when in use. The compensating tube, which can be readily moved up and down as necessary, is held in place with two buret clamps, one holding the enlarged bulb and one located at the bottom. Mounting the compensating tube on a rack and pinion arrangement would be advantageous. The three-branched connecting tube is held in a buret clamp with a moderately slack connection of 7.5 or 10 cm. (3 or 4 inches) of rubber tubing to the buret. About 45 cm. (18 inches) of rubber tubing should be allowed between the connecting tube and the compensating tube and 90 cm. (3 feet) of tubing between the connecting tube and its reservoir.

The two measuring tubes could be readily mounted on the same stand for shaking by the same motor. For large-scale installations, a number of nitrometers could be operated by the same motor in a compact arrangement. The use of burets with etched volume gradations would be advantageous, increasing the accuracy of reading volumes.

The air used in filling the compensating tube is desiccated by shaking with sulfuric acid in a separated reaction tube or in a similar tube having a simple two-way stopcock at its upper end and a capillary tube at its lower end. Mercury is used as confining liquid under the layer of sulfuric acid.

Four or five pounds of mercury, which is used as confining liquid, are ample for operating the semimicronitrometer. Concentrated sulfuric acid, C. P. grade of no or low nitrogen content, should be used. The mercuric sulfate formed can be recovered and reclaimed. The use of a cellulose acetate face mask is advisable as a precaution in case of explosion (*1*).

Procedure

The procedure for using the semimicronitrometer is based n large part on the instructions for operation of the du Pont nitrometer (*1*, *7*, *8*). The apparatus can be standardized with dry air or with nitric oxide from pure potassium nitrate. The latter procedure is the simpler and is recommended for routine use.

Standardization Using Dry Air. The compensating tube, buret, and all connections are completely filled with mercury.

The motor-shaken measuring burets are standardized as follows: The separate reaction tube filled with air, desiccated by shaking with sulfuric acid, is attached to the compensating tube. With the latter and the measuring tube in about the same position, desiccated air is drawn into the compensating tube to a point level with the 22-ml. mark on the measuring tube and the stopcock on the compensating tube is closed. Desiccated air from the attached reaction tube is then drawn into the measuring tube to the 22-ml. mark and the stopcock is closed. The gas in each tube is put under a slight positive pressure by having the mercury level in the leveling bulb slightly above that in the two tubes. The stopcock on each reaction tube is then opened to the air and their mercury reservoirs are adjusted so that the acid level in each is 2.5 cm. (1 inch) from the top. The stopcock on each reaction tube is given several complete turns, thus venting the air in the tube successively to the atmosphere and to the attached measuring tube.

By this process the mercury comes to the same level in the two tubes and in their leveling bulb, while the desiccated air in the tubes comes to atmospheric pressure. During this process no undesiccated air can enter the tubes because the gas in the tubes was at higher than atmospheric pressure before being vented to the atmosphere via the reaction tubes. After noting the atmospheric pressure and temperature, the volume of gas in the measuring tube is calculated to standard conditions. With the stopcocks on the tubes closed, the leveling bulb is raised until the gas in the measuring tube occupies the calculated volume, the compensating tube being moved so that its mercury level is the same as that in the measuring tube. A strip of paper or some other mark is placed on the compensating tube at the mercury level, completing the standardization.

To standardize the hand-shaken measuring burets, desiccated air is drawn into the compensating and measuring tubes as before and the stopcocks are closed. The mercury columns in the two tubes are balanced. After a U-tube containing sulfuric acid has been attached to the outlet of the measuring tube, the stopcock on the latter is opened and the sulfuric acid levels are balanced, putting the gas in the buret at atmospheric pressure. The volume of gas in the buret is calculated to standard conditions. With the stopcock on the buret closed, the leveling bulb is regulated to bring the mercury level in both burets to the same point, which marks the calculated volume in the buret. The compensating tube is marked at the new mercury level, completing the standardization.

Standardization Using Potassium Nitrate. The compensating tube, buret, reaction tube, and all connections are completely filled with mercury.

The separate reaction tube which has been filled with air, desiccated by shaking with sulfuric acid in the tube, is attached to the compensating tube. With the compensating tube and the measuring buret in about the same position, desiccated air is drawn into the compensating tube to a point about opposite the 22-ml. mark on the buret. The stopcock on the compensating tube is closed, and the attached reaction tube is removed. A 100-mg. sample of C. P. potassium nitrate, which has been recrystallized twice from water, washed with alcohol, ground to pass a 100-mesh screen, and dried at 135° to 150° C., is accurately weighed and placed in the cup of the reaction tube with 1 ml. of sulfuric acid. The sample is drawn into the reaction tube and the cup is rinsed with 5 1-ml. portions of sulfuric acid, care being taken to admit no air. The instrument is shaken for two 5-minute periods as described under Procedure for Analysis.

The gas generated is transferred to the buret. The reservoir and compensating tube are then adjusted so that the mercury levels in both the buret and the compensating tube are the same and stand at the 22.15-ml. mark on the buret, which is the volume of nitric oxide, calculated to standard conditions of temperature and pressure, corresponding to 13.86 per cent of nitrogen in 100 mg. of potassium nitrate. This is the theoretical per cent of nitrogen in potassium nitrate. A strip of paper is pasted on the compensating tube at the level of mercury, completing the standardization. When using the hand-shaken apparatus the reaction tube is disconnected for shaking. The introduction of air from the capillaries must be avoided, since the oxygen in the air interacts with nitric oxide, forming nitrogen dioxide which results in a change in volume and in the attack of the mercury in the buret and the formation of a coating of mercury salts on

Table I. Determination of Nitrogen by the Semimicronitrometer

Substance	Sample Weight	No. of Runs	Apparatus[a]	Volume of Nitric Oxide — Mean	Average deviation	Average deviation of mean	Nitrogen — Found	Nitrogen — Calculated
	Mg.			*Ml.*	*Ml.*	*Ml.*	%	%
Lead nitrate	165.0	3	U-M	22.13	0.00	0.00	8.39 ± 0.00	8.46
Potassium nitrate	100.0	21	N-H	22.13	0.16	0.03	13.84 ± 0.02	13.86
		15	N-M	22.04	0.07	0.02	13.79 ± 0.01	...
		12	U-M	22.05	0.07	0.02	13.79 ± 0.01	...
Sodium nitrate	85.0	5	U-M	22.36	0.07	0.03	16.46 ± 0.02	16.48
Cellulose nitrate A	125.0	11	N-H	20.65	0.36	0.11	10.33 ± 0.06	...
		5	U-M	20.59	0.03	0.01	10.31 ± 0.01	...
Cellulose nitrate B	125.0	4	N-H	22.59	0.03	0.02	11.31 ± 0.01	...
		5	U-M	22.58	0.05	0.02	11.30 ± 0.01	...
		5	N-M	22.60	0.03	0.01	11.31 ± 0.01	...
Cellulose nitrate C	100.0	7	U-M	21.20	0.03	0.01	13.26 ± 0.01	13.38
Cellulose nitrate D	100.0	3	U-M	20.08	0.02	0.01	12.56 ± 0.01	12.66
Nitroguanidine	100.0	3	U-M	21.44	0.04	0.03	13.41 ± 0.02	13.46
Pentaerythritol tetranitrate	75.0	3	U-M	20.99	0.02	0.01	17.51 ± 0.01	17.73
Tetryl	255.0	3	U-M	18.95	0.10	0.06	4.65 ± 0.01	4.88

[a] Apparatus used: N nitrocellulose tube, U universal tube, H hand-shaken apparatus, M motor-shaken apparatus.

the buret walls. Air can be excluded from the capillaries by filling them with mercury.

It is advisable to run a sample of potassium nitrate occasionally—e. g., at the beginning of each day's work—in order to check the calibration. The procedure using potassium nitrate given by the American Society for Testing Materials (*1*) calibrates the buret for 20° C. and 760-mm. pressure, while the procedure given here is for 0° C. and 760-mm. pressure.

For the permanent standardization of the apparatus, which can be done if the lower opening of the compensating tube is kept continuously sealed with mercury, the stopcock on the compensating tube is replaced by a capillary tube which can be sealed after the introduction of dry air.

Procedure for Analysis. The buret, reaction tube, and connections are completely filled with mercury.

The size of the sample used should be sufficiently great to give from 20 to 24 ml. of nitric oxide—e. g., 100 mg. for 14 per cent nitrogen, 125 mg. for 11 per cent nitrogen. The weighed sample is transferred to the cup of the reaction tube and is flushed into the tube with 1 ml. of sulfuric acid. In some cases the sample can be first dissolved in the acid. The cup is rinsed with five 1-ml. portions of sulfuric acid. Care must be taken that no air bubbles enter the tube when the acid is admitted. With the lower stopcock on the reaction tube open and the mercury reservoir a little below the mid-point of the reaction tube, the apparatus is shaken for 5 minutes. The motor is stopped, and the reservoir on the buret and compensating tube is raised above the buret. The stopcock connecting the reaction tube and buret is opened, and any acid which is held in the upper capillary of the reaction tube is swept back into the tube by the flow of mercury. The reservoir of the buret is immediately lowered and all the gas is transferred to the buret. The stopcock connecting the buret and the reaction bulb is closed. The reservoir on the reaction bulb is lowered to give a less than atmospheric pressure in the reaction tube. The apparatus is shaken for 5 minutes with the mercury in this position. The reduced pressure is especially helpful in removing dissolved gas from the sulfuric acid and the precipitate of mercuric sulfate.

The gas formed during the second shaking operation is then transferred to the buret. Care must be taken to bring the acid layer in the reaction bulb flush with the upper face of the stopcock. If a considerable amount of gas was generated in the second shaking, the shaking process should be continued until nitric oxide is no longer evolved. With the stopcock on the buret closed, the compensating tube and reservoir are so adjusted that the mercury level is the same in both tubes and comes to the standardization mark on the compensating tube. The volume found on the buret at this point is the volume from which the per cent of nitrogen is calculated. The analytical procedure when using the hand-shaken apparatus is the same, suitably modified.

In emptying the buret after a determination, the nitric oxide is transferred to the reaction tube, from which it can then be vented to the air. The gas may be prevented from entering the atmosphere of the room by holding a suction line over the cup of the reaction bulb as the gas emerges. The sulfuric acid and mercuric sulfate are removed from the cup by suction and can be recovered by inserting a trap in the suction line.

Calculations of Results.

$$\% \text{ N} = \frac{\text{vol. (ml.) of NO} \times \text{weight (mg.) of N in 1 ml. of NO at N. T. P.} \times 100}{\text{weight (mg.) of sample}}$$

The value given by the American Society for Testing Materials (*1*) for the volume of nitric oxide delivered at 20° C. and 760-mm. pressure by 1 gram of sample containing 14.01 per cent of nitrogen is 240.36 ml. The weight in milligrams of nitrogen in 1.00 ml. of nitric oxide at normal temperature and pressure would, therefore, be

$$\frac{0.1401 \times 1000}{240.36 \times \frac{273.1}{293.1}} = 0.6256$$

Practically the same value is obtained from Gray's (*9*) value for the density of nitric oxide, 1.00 ml. of nitric oxide at N. T. P. weighing 1.3406 mg. The weight of nitrogen in 1.00 ml. of nitric oxide at N. T. P. is

$$\frac{14.01}{30.01} \times 1.3406 = 0.6258 \text{ mg.}$$

Accordingly, the equation used to calculate the results is

$$\% \text{ N} = \frac{\text{vol. (ml.) of NO} \times 62.56}{\text{weight (mg.) of sample}}$$

Experimental Results

Table I shows the results obtained in analyzing various inorganic and organic compounds by the semimicronitrometer, using the "nitrometer reaction".

The inorganic substances used were potassium nitrate, Merck's reagent grade; sodium nitrate, J. T. Baker's c. p. grade; and lead nitrate, J. T. Baker's c. p. grade; these were recrystallized twice from water and dried at 150° C. The organic compounds used were the ordinarily obtainable commercial grades and were not further purified; they were dried at 100° C. In general, compounds like these tend to run slightly low in their nitrogen content. The sample of pentaerythritol tetranitrate was analyzed by a government testing laboratory, using a regular du Pont nitrometer. Their reported value of 17.55 per cent nitrogen is in good agreement with the value, 17.51 per cent, found with the nitrometer described. The theoretical value is 17.73 per cent. The tetryl required 30 minutes shaking for complete evolution of the nitric oxide. The values given for nitrocelluloses C and D were presumably obtained by the regular du Pont nitrometer. As indicated in the table, both the universal and nitrocellulose tubes were used with the hand-shaken and motor-shaken models. All determinations on potassium nitrate were made with the measuring buret calibrated with air. The results for the other materials are based on calibration of the buret with potassium nitrate.

The terms used in referring to the volumes of nitric oxide found have the following meaning: mean, the arithmetical average; average deviation, the sum of the deviations from the mean of the individual values, irrespective of sign, divided by the number of values; average deviation of the mean, the average deviation divided by the square root of the number of values. The value given for the per cent of nitrogen found is calculated from the mean value of the volume of nitric oxide, plus or minus the per cent equivalent to the average deviation of the mean.

The only experimental values omitted from Table I are those obtained in preliminary runs, testing the time of shaking necessary and other experimental factors, and those which were known to be in error. The precision of the results is seen to be excellent; in general the accuracy is not so good, the results tending to be on the low side. The latter source of error may be due to the solubility of nitric oxide in sulfuric acid. Normally, 10 ml. of concentrated sulfuric acid dissolve 0.20 to 0.35 ml. of nitric oxide (*16*, *25*) but by using the reduced pressure advocated in the Procedure for Analysis this figure is reduced. Further possible causes of low results are hygroscopicity of sample, loss in transfer of the sample to the cup of the reaction tube, and insufficient shaking.

Several nitrometers of the motor-shaken type have been built at Purdue for use elsewhere and have been entirely satisfactory.

Acknowledgment

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Electrophotometric Microdetermination of Phosphorus in Lipide Extracts

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A PROPOSED investigation of lipides in rat brain required a method for determining phosphorus with reasonable accuracy in minimal quantities of lipide extracts. This communication describes such a procedure, together with some observations of general interest in the application of electrophotometry to the determination of phosphorus.

McCune and Weech (*6*) noted that the blue color obtained in the method of Fiske and Subbarow (*3*) is not constant for a considerable time after development, as had been supposed, but continues to gain in intensity for at least 72 hours. They solved the problem of measuring the Fiske and Subbarow blue color with the electrophotometer by their discovery that the amount of absorption of light in a zone in the far violet region of the spectrum is constant for between 30 and 60 minutes after mixing the reagents. By using a combination of filters which transmitted a band extending from about 350 millimicrons in the ultraviolet to about 430 in the visible spectrum, satisfactory determinations were obtained.

In applying the observations of McCune and Weech to the electrophotometric determination of phosphorus in lipide extracts an unexpectedly high blank was encountered. Solutions containing the reagents in the concentrations recommended by Fiske and Subbarow absorbed more than half of the light transmitted by the filters of McCune and Weech. The absorption was not in the visible portion of the spectrum, since the solutions were entirely colorless to the eye (this statement applies to all blanks referred to in this paper), and must have taken place in the ultraviolet.

The high blank did not interfere with the procedure as employed by McCune and Weech (*5*) because in the instrument, of the test-tube type, which they used, the galvanometer was adjusted to a reading of 100 per cent transmission with the tube containing the blank in place before each measurement; this was impossible in the instrument (*7*) employed by the author, as the "air setting" was considerably higher than the "blank setting".

The high blank absorption not only had this practical disadvantage, but was undesirable on theoretical grounds. A test of each reagent separately in the concentrations used in developing the blue color revealed that none absorbed an appreciable proportion of the light except the aminonaphtholsulfonic acid–sulfite reagent, which showed a strong fluorescence and absorbed even more than the combination of all reagents. Therefore the concentration of aminonaphtholsulfonic acid was reduced, eventually to one fortieth of that of Fiske and Subbarow in the final solution, without appreciable diminution in the intensity of color yielded by phosphorus, even though in some experiments less aminonaphtholsulfonic acid than phosphorus by weight was present.

This, however, did not overcome the difficulty of the high blank. It was found that even though none of the reagents, including sulfite, gives an appreciable blank alone, the combination without aminonaphtholsulfonic acid absorbs almost as much light as with it, but does not fluoresce. Apparently the absorption shown by aminonaphtholsulfonic acid and sulfite alone largely disappears when the other reagents are added, but instead there is another type of absorption more or less independent of the aminonaphtholsulfonic acid. This absorption, representing the high blank of the combined reagents, could be lowered to a point where the air setting was considerably less than the blank setting (*7*), by reducing the total sulfite concentration to one tenth of that used by Fiske and Subbarow.

Highly erratic results were obtained when the dilute aminonaphtholsulfonic acid–sulfite reagent was applied to the determination of phosphorus. The extinction coefficient was much higher (100 per cent or more) with the smaller amounts of phosphorus (2 to 5 micrograms) than with the larger (10 to 20 microgram) quantities, and it was not reproducible from day to day. The difficulty was traced to the use of too concentrated reagents and too slow mixing (in cylinders or test tubes); it was avoided by carrying out the determination in Erlenmeyer flasks with rapid mixing as described herewith.

Reagents

Perchloric acid, 70 to 72 per cent; ammonium molybdate, 1.25 per cent; sulfite solution, containing 15 per cent of sodium bisulfite and 0.5 per cent of sodium sulfite.

Aminonaphtholsulfonic acid stock solution, made by dissolving 16 mg. of recrystallized aminonaphtholsulfonic acid (*3*) in 25 cc. of the sulfite solution with heating on the steam bath.

Dilute aminonaphtholsulfonic acid solution, made by diluting 1 cc. of the stock solution to 100 cc.

Porcelain chips, heated with cleaning mixture and washed until they give no blank.

Method

A scrupulously clean 50-cc. Erlenmeyer flask is clamped at an angle of about 45° over a steam bath, and a portion of a lipide solution containing from 2 to 25 micrograms of phosphorus is pipetted into the lowest "corner" of the flask. The solvent is removed; the position of the dry lipide is marked; 0.5 cc. of perchloric acid and 3 or 4 specially treated porcelain chips are added; the flask is placed on a digestion rack at a 45° angle with the lipide film down; a condenser (cold finger), previously cleaned by dipping in cleaning mixture and rinsing with distilled water, is inserted while still wet about halfway to the bottom of the flask (if the condenser is clean the small amount of water that adheres to it does not affect the result); and the sample is heated until colorless (usually 5 to 10 minutes). No white fumes escape past the cold finger except occasionally when bumping occurs. No loss of phosphorus has been observed even with the violent bumping which is frequently encountered when glass beads are used instead of porcelain chips. After cooling, the condenser is rinsed into the flask with 3 cc. of water from a pipet; the flask is removed from the rack and 2 cc. of the molybdate solution followed by 4 cc. of the dilute aminonaphtholsulfonic acid–sulfite solution are added from fast-flowing pipets with vigorous swirling of the flask during both additions.

The color is read in an electrophotometer equipped with the filters described by McCune and Weech (*6*). (Identical results were obtained with and without the light shade Aklo, No. 396, filter. The filter pack has a low transmission and it may be desirable to omit this filter if the light source is not sufficiently bright to give a full deflection of the galvanometer with the blank solution.) Blank solutions (usually 2 or 3) are prepared from the reagents in exactly the same way (without digestion), the average air setting of the instrument is determined for a blank setting of 100 per cent transmission, and the unknown sample is read against this setting 5 minutes or more after adding the aminonaphtholsulfonic acid solution. A cuvette 5 cm. long and 1 cm. in diameter is used. The volume of solution (9.5 cc.) is sufficient for two rinsings of the cuvette between readings. The method can be adapted to any electrophotometer equipped with the McCune and Weech filters, but the high sensitivity, down to 2 micrograms of phosphorus, depends on the development of color in a small volume and its reading in a long (5-cm.) cuvette.

The readings are translated directly into quantities of phosphorus from a previously determined standard curve, plotted on semilogarithmic coordinate paper.

Table I. Range of Variation in Determination of Phosphorus

(All data are actual readings of percentage transmission)

Sample	Phosphorus Present: 2γ[a]	5γ[a]	7.5γ	10γ[a]	12.5γ	15γ	20γ	25γ
1	77.2	54.2	41.1	31.1	24.1	18.8	11.8	7.1
2	77.9	54.0	40.9	31.2	24.0	18.7	11.8	7.2
3	77.5	54.1	41.1	31.1	24.1	18.6	11.6	7.1
4	77.1	53.9	40.9	31.1	24.0	18.7	11.7	7.1
5	77.6	53.9	..	31.2	24.0	..	..	7.1
6	77.7	54.0	..	31.4	24.0	..	..	...
7	77.6	54.0	..	31.1	..	..	..	...
8	77.5	54.0	..	31.1	..	..	..	...
9	77.4	54.1	..	31.2	..	..	..	...
10	77.3	54.1	..	31.1	..	..	..	...
11	77.8	53.9	..	31.4	..	..	..	...
12	77.3	53.9	..	31.2	..	..	..	...

[a] Coefficients of variation ($V = 100\sigma/\text{mean}$), calculated from the extinction coefficients, were 1.31, 0.35, and 0.30 per cent for the 2, 5, and 10γ samples, respectively.

Discussion

Fiske and Subbarow (*3*) emphasized the great advantage of rapid development of color in determining phosphorus; with their procedure the maximum was reached in 5 minutes, as far as could be determined with a colorimeter (*1*, *2*, *6*). The proposed modification retains this advantage. Most of the color (in the spectral zone measured) develops within 1 minute; there is usually a slight intensification up to 5 minutes, after which there is little or no change up to at least 1 hour.

Table II. Recovery of Phosphorus Added to Lipide Extracts

(0.2 cc. of extract added to each flask)

Extract 1 Sample	Phosphorus Added	Phosphorus Found	Phosphorus Recovered	Extract 2 Sample	Phosphorus Added	Phosphorus Found	Phosphorus Recovered
	Micrograms				*Micrograms*		
1	..	7.40	..	7	..	7.15	..
2	..	7.70	..	8	..	6.95	..
3	..	7.55	..	9	..	6.95	..
4	5	12.50	4.95	10	5	11.85	4.83
5	5	12.50	4.95	11	5	12.10	5.08
6	5	12.55	5.00	12	5	12.10	5.08
	Average percentage recovery 99.3				Average percentage recovery 99.9		

Sensitivity and Stability of Reagents. The concentration of none of the reagents is critical. Although there is little chance of losing perchloric acid with the condenser technique, it was found that the acid may be reduced by at least 20 per cent without materially affecting the result (*2*, *4*). The same values have been obtained with 1.5 as with 1.25 per cent ammonium molybdate, and varying the amount of aminonaphtholsulfonic acid in the stock solution from 12 to 20 mg. did not appreciably change the results.

As the ammonium molybdate solution becomes older the blank increases and the color intensity decreases somewhat. The reagent should be checked occasionally against a standard phosphorus solution and renewed if the recovery tends to be low.

The stock reagent gives some trouble due to the formation of crystals on standing, but if these are redissolved by heating the reagent appears to be as good as before. It is not recommended, however, that this reagent be used more than 2 weeks (*3*) and it may be necessary to renew it oftener.

It was anticipated that the dilute aminonaphtholsulfonic acid reagent would be unstable and throughout most of the work it was diluted just before use. However, excellent results have been obtained with this reagent kept in an ordinary flask in the laboratory for 5 days.

Precision. The extinction coefficient is not constant over the entire range of measurement, but the statement of McCune and Weech, "When plotted on semilogarithmic coordinates the regression line of galvanometer reading on concentration is so nearly linear throughout its entire extent that the regions between the 5 points that are determined in its preparation [8 points were determined in the present work] can be considered to be strictly linear without introducing significant error", applies to the findings with the proposed method. The actual data from which the calibration curve was plotted (Table I) show the range of variability of the method as applied to the determination of known quantities of phosphorus. (The calibration curve will probably vary somewhat with variations in light filters, etc.; it should be determined by each worker under his conditions.) Twenty-one 1-cc. portions of standard solutions were dried in 50-cc. flasks and carried through the digestion procedure. The samples covered the range from 2 to 20 micrograms of phosphorus; 11 contained 5 micrograms or less. The average recovery was 100.3 per cent and the standard deviation of the percentage error was 1.6 per cent. (This result shows that no pyrophosphate is formed during the digestion. In a series of determinations the solution was boiled after addition of water following digestion with no effect on the recovery of phosphorus.)

Recovery from Lipide Extracts. Samples containing 5 micrograms of phosphorus were dried in flasks, and 0.2-cc. portions of isopropyl ether extracts of brain were added and analyzed as described. The results (Table II) show a quantitative recovery.

Specificity. Fiske and Subbarow (Table II, *3*) tested a number of substances for their effect on the development of color with their method. Sodium chloride, potassium nitrate, or ammonium sulfate in the proportion of 10,000 times the quantity of phosphorus, sodium nitrite in the proportion of 100 times, and copper (added as copper sulfate) in the proportion of 10 times, do not interfere in the proposed modification. However, 50 micrograms of iron (added as ferric chloride) approximately double the intensity of color produced by 5 micrograms of phosphorus, and 1 mg. of silicon (added as sodium silicate) increases the color given by 5 micrograms of phosphorus by about one half. This result reduces the general applicability of the method, but does not seriously affect the use for which it was intended—i. e., the analysis of lipide extracts where appreciable quantities of iron or silicon would rarely, if ever, be present. The absence of significant solution of silicon during digestion with perchloric acid under the conditions described was shown by the quantitative recovery of phosphorus.

Effect of Temperature. Allen (*1*) found a considerable effect of variation in temperature on the development of color with the King (*4*, perchloric acid) modification of the Fiske and Subbarow method. With the proposed procedure identical readings were obtained with 5-microgram samples developed for 20 minutes at 20°, at room temperature (about 25°), and at 38° C.

Summary

A procedure for the electrophotometric determination of phosphorus (2 to 25 micrograms) in lipide extracts is described. To avoid a high blank, representing absorption in the ultraviolet, the concentration of aminonaphtholsulfonic acid was reduced to one fortieth, and of sulfite to one tenth of that employed in the procedure of Fiske and Subbarow (*3*). Full color development is obtained even though the quantity of aminonaphtholsulfonic acid may be less than that of phosphorus. Digestion is carried out with perchloric acid in a 50-cc. Erlenmeyer flask, using a condenser to facilitate rapid mixing which is essential in determining small quantities of phosphorus.

Acknowledgment

The author is indebted to A. Ashley Weech and to Donovan J. McCune for supplying unpublished details concerning their procedure.

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A Micromethod for Determination of Arsenic

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ACCURATE quantitative methods for the determination of 1 microgram and less of arsenic (*4*, *5*, *9*) are still problems for research, owing to interest in the arsenic content of normal tissue (*3*, *8*). Before it can be proved that arsenic is the causative agent in certain chronic conditions, a sensitive method for the analysis of normal tissue must be established. To this end much work has been done with modifications of the original Gutzeit method (*1*) as used by the Association of Official Agricultural Chemists. In 1936, the author (*2*) used this method during a study of the accumulation of arsenic in tissues of the albino rat. For comparative purposes, it was necessary to analyze normal tissue simultaneously; therefore, as a possible extension to smaller quantities, pieces of ordinary cotton thread, No. 60, were impregnated with mercuric bromide and used as arsenic detectors instead of the Hanford Pratt strips. More sensitive results were obtained.

It was thought, during the present investigation, that by using coarser thread in narrower capillary tubes, not only a more sensitive but also a more accurate and more definite stain could be produced, as How (*5*) has recently tried to prove with Morse and Kaley No. 8 knitting cotton. For this purpose two types of cotton threads were used in two types of capillaries: (1) No. 8 in a 1-mm. bore capillary, and (2) No. 24 in a 0.5-mm. bore capillary. The following investigation is a comparative study of these two types of impregnators and the Hanford Pratt strips, using in all analyses 1 microgram of arsenic.

Procedure and Experimental

The regular Gutzeit method of the Association of Official Agricultural Chemists was used, with the following modification:

In place of dental rolls impregnated with lead acetate in the scrubber tubes, glass heads (*7*) which had been soaked in saturated lead acetate were used. These scrubber tuhes were cleaned after every run, by taking out the beads, washing first with water, four times with concentrated hydrochloric acid, then four times with water. They were then soaked overnight in saturated lead acetate solution, poured out on filter paper, and put in the glass scrubber tuhes, which were half filled with the heads.

Instead of the regular detector tubes containing the Hanford Pratt strips of mercuric hromide paper (a series was run with these, however, for comparative purposes) capillary tubes of 1-mm. bore, each containing a piece of O. N. T. cotton thread No. 8 as a detector, were used in one series of determinations, and capillary tubes of 0.5-mm. bore, each containing a piece of Coat's cotton thread No. 24 as a detector, were used in a second series.

Impregnation of Thread. It was found necessary to suspend the thread in the mercuric bromide solution in such a way that each bit of thread would he completely impregnated, without contamination of any kind from the time of initial impregnation to the time of drying and placing in the capillary. To this end, a piece of glass rod of 5-mm. bore was drawn out and shaped as shown in Figure 1.

The device had two slight indentations at the top and one at the bottom, around which the thread was drawn loosely. The glass frame and thread were placed in a 50-ml. glass cylinder, and were held in place hy means of the handle hent in a right angle at one end, which allowed the frame to hang suspended in the center

of the mercuric bromide solution contained in the glass cylinder. The cylinder was filled to a height of about 1 cm. from its opening with 4 per cent mercuric bromide solution, and the thread was impregnated for 15 minutes, after which it was removed by means of the glass handle, without contamination from any outside lifting device, and placed upon a piece of glass rod, bent in the form of a square, to dry. When dry the thread was cut by scissors at both ends of the frame, thus giving the required length for the capillary tube. The individual threads were picked up by metal forceps and fed through the required capillary by means of a vacuum line. By this technique the thread was untouched from the time it was impregnated until it was fed through the capillary tube and prepared for reaction with the arsine.

ANALYSES. A standard solution of arsenious oxide was prepared according to Lachele (*6*). Dilutions of the original stock solution were made in such a way that the working standard contained 1 microgram in each 0.5 ml. One microgram was used in each of the following analyses (Table I).

A series of analyses was run using the Hanford Pratt strips, the stains being measured in millimeters, and tabulated.

A similar series was run, using the 1-mm. capillary and No. 8 thread.

A similar series was run, using the 0.5-mm. capillary and No. 24 thread.

Discussion

In practically 50 per cent of the analyses, using the Hanford Pratt strips, the stains resulting from 1 microgram of arsenic were not measurable. Where it was possible to measure them, the results were far from accurate, because of the indefinite termination of the stain and the wide divergence in length of the stain on both sides of the filter-paper strips.

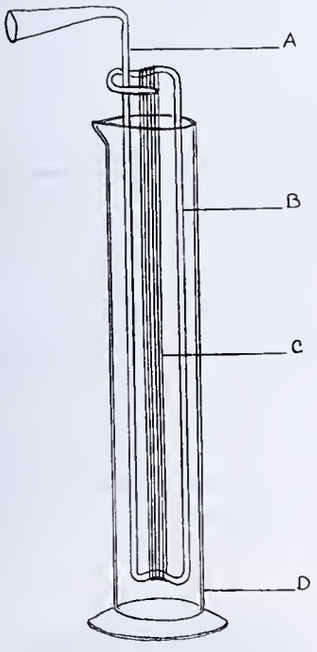

FIGURE 1. DEVICE FOR IMPREGNATING ARSENIC THREADS

A. Handle
B. Device for impregnating
C. Thread
D. Glass cylinder containing mercuric bromide

The average length of the stain on the threads was much greater than that on the Hanford Pratt strips, and for that reason could be measured more accurately. Including the thirty-three runs on each, the average lengths are as follows:

No. 24 thread in 0.5-mm. capillary, 6.1 mm.
No. 8 thread in 1-mm. capillary, 5.4 mm.
Hanford Pratt strips in 3-mm. capillary, 0.8 mm.

The variations in length of stain, using the two types of thread in different capillaries, are contained in Table II. The variation with thread 24 in the 0.5-mm. bore capillary is not so great as with No. 8 in the 1-mm. bore capillary, in the former 60 per cent of the results being between 6 and 6.5.

TABLE I. DETERMINATION FOR ONE MICROGRAM OF ARSENIC

(All analyses were done under same conditions of time and temperature. Temperature 18° to 20° C. Time of each run 1.5 hours)

No. of Run	Average Length of Stain: Thread 24, 0.5-mm. cap.	Thread 8, 1-mm. cap.	Hanford Pratt strips, 3-mm. cap.
	Mm.	*Mm.*	*Mm.*
1	7	6.7	1.5
2	6.7	7.0	[a]
3	6.0	7.0	1.5
4	6.0	6.4	1.5
5	6.0	5.0	1.5
6	5.8	7.0	[a]
7	7.5	5.2	1.0
8	6.5	7.7	1.0
9	5.8	6.5	1.0
10	5.5	5.5	0.5
11	6.5	5.0	1.5
12	6.8	4.0	1.0
13	6.0	4.0	0.5
14	6.3	4.0	[a]
15	5.0	5.0	[a]
16	6.0	5.0	[a]
17	6.0	5.5	1.5
18	6.3	5.0	1.0
19	6.0	6.0	2.0
20	6.5	6.0	1.0
21	6.0	5.0	1.5
22	6.0	4.0	1.5
23	6.0	5.0	1.5
24	6.3	4.8	2.0
25	6.0	4.5	[a]
26	6.0	4.5	[a]
27	5.0	5.0	[a]
28	6.0	4.8	[a]
29	6.5	4.5	0.5
30	6.0	4.5	[a]
31	6.3	6.2	[a]
32	6.0	6.5	[a]
33	6.0	6.5	[a]

[a] Not measurable.

TABLE II. VARIATIONS IN LENGTH OF STAIN

Length of Stain	Thread 24 in 0.5-Mm. Cap.	Thread 8 in 1-Mm. Cap.
Mm.	*Per cent of 33 runs*	
7.5 and over	3	3
7.0–7.4	3	9
6.5–6.9	18	12
6.0–6.4	61	12
5.5–5.9	9	6
5.0–5.4	6	28
4.5–4.9	0	18
4.0–4.4	0	12

Conclusion and Summary

For the determination of 1 microgram of arsenic, thread as an arsenic detector in glass capillary tubes gives a more sensitive, more constant, and more definite stain than the usual Hanford Pratt strips. When No. 24 thread was used in a 0.5-mm. bore capillary, and No. 8 thread in a 1-mm. bore capillary, the former thread gave more constant stains. It would seem, therefore, that for the range of 1 microgram of arsenic, the closer the arsenic detector fits its glass container the more constant the stain.

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Preparation of Pigment Mounts for Microscopy

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POWDERED materials of various chemical compositions and widely different particle size characteristics are used for many purposes in rubber compositions and in paints, enamels, lacquers, and plastics. In the rubber industry these materials are called "pigments" in a generic sense, regardless of whether or not they are used for coloring purposes; in paint formulations their true pigment use is more important. In any case these finely divided materials are evaluated frequently on the basis of their microscopic characteristics. It is essential, therefore, to have available a method of preparing mounts of materials ranging in size and character from channel black to asbestos fibers, from zinc oxide to ground whiting. In the microscopic evaluation of these materials the preparation of the mount is one of the most important steps.

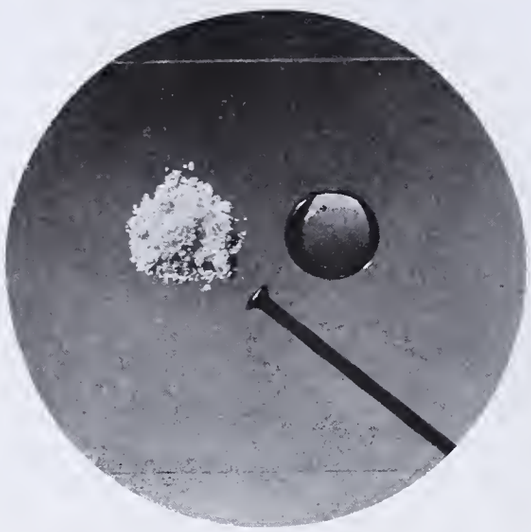

FIGURE 1. PIGMENT AND CEMENT (× 2)

In the course of development in this laboratory of the applications of microscopy to the rubber industry, many methods of dispersing and mounting dry pigments have been tried. The simplest method of performing the operation, stirring or rubbing the dry pigment into an oil or an aqueous medium, is frequently suitable for a coarse material such as ground whiting but unsatisfactory for use with materials that lie in the size range of zinc oxides, titanium dioxide, or carbon blacks. The shearing forces obtainable by rubbing or stirring are normally inadequate to ensure deflocculation of small particles, and even under the most favorable conditions, the use of a liquid dispersing medium frequently results in pronounced segregation of various sized fractions. Hence it is possible that the large particle-sized fractions will be overlooked, and, therefore, will not figure in the evaluation of the material.

Green (*1*) was one of the first to recognize and describe the requirements for completely satisfactory mounts.

The preparation of the mount is the really important factor involved in particle measurements. In order to obtain satisfactory results, the individual particles must be detached and evenly distributed—that is, all states of aggregation must be eliminated if we are interested in ultimate particle size. The particles must not be broken or ground during the process of dispersion; all the sizes of particles that exist in a sample must be present in their correct proportion; there must be a sufficient number of particles measured to give a true average; and the particles must lie in one plane and be free from motion.

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The technique employed by Green consisted in rubbing out the particles into an exceedingly thin layer with turpentine, and mounting them in glycerol or some other suitable medium. Later this method was modified by adopting a "multiple wedge dispersion" which provided a mount in which the pigment layer varied from 0 to 25 particles in depth. Concerning the method of Green, Haslam and Hall (*2*) stated that with a great deal of practice and care a slide can be prepared that will give fairly satisfactory results.

It was found in the Goodrich laboratory that the technique developed by Green possessed two rather serious pitfalls—a certain amount of segregation invariably persisted and some grinding of the large particles often occurred. The larger particles were swept to the sides where they could be overlooked. Even at best the method is seldom capable of providing a mount in which any limited area is completely representative of the pigment. Haslam and Hall (*2*) also recognized this disadvantage and overcame the difficulty by gathering all the pigment dispersed in turpentine in the center of the slide.

The pigment is first rubbed out in double-distilled turpentine until the lumps have been disintegrated and then, when the turpentine is practically evaporated, the sample is all pushed to the center of the slide and the remainder of the turpentine is driven off with heat. It is extremely important that all the pigment be gathered together in the center of the slide, for in the rubbing-out operation the coarse particles are carried to the edges of the slide and would be lost in the final mount. The pigment is then covered with dammar which is melted under the cover glass. Haslam and Hall state that this mount is far simpler to prepare successfully, better dispersion of the pigment is assured, a more permanent mount is secured and, owing to the rigidity of the dammar, it lends itself to easier manipulation to the high-power objectives on the microscope.

The method developed in the Goodrich laboratory has overcome most of the difficulties of these previous methods. It is rapid and reproducible, and effectively provides representative mounts of pigments of a wide range of sizes and compositions. The principle of the method is simple. The dry pigment is dispersed in high concentration in a suitable, optically clear cement, thereby utilizing the high shearing forces which may be created in a stiff, viscous mixture. This dispersion of high concentration is then diluted to form the final mount. This technique provides greater forces for rup-

FIGURE 2. COMPLETION OF FIRST DISPERSING STEP (× 2)

turing and dispersing flocculates or agglomerates of particles than are available from merely stirring in oil, while it avoids the actual grinding of large particles which can occur when a hard instrument is vigorously used in conjunction with a thin oil such as turpentine. The cement employed transmits forces in a more or less fluid fashion but prevents actual contact of the masticating instrument with the particles.

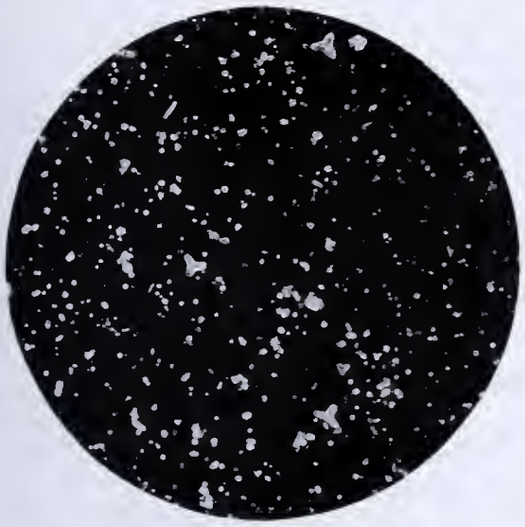

FIGURE 3. ZINC OXIDE (× 1000)

In the development of this method cements of various compositions were tried. Rubber cements were found in general to have a tendency to form a ball as the solvent evaporated, and thus present too much resistance to the working tool. In contrast, a cement having a gum base—e. g., dammar—possessed too little of the rubberlike characteristic which aids in good dispersion and dried to a hard mass difficult to cut and dilute. A satisfactory combination of the desired qualities was discovered in Duco household cement, which now is used for the preparation of mounts of pigments of a wide variety of sizes and compositions.

On a microslide is placed a drop of this cement, and beside it a suitable quantity of the dry pigment. The approximate amounts used are indicated in Figure 1, with the head of an ordinary pin for a size comparison. The pigment is kneaded into the cement with sweeping, rapid strokes of a small dissecting needle with spearhead, the cement hardening gradually as this operation proceeds. The strokes are 2 to 3 cm. long. The exact motion is difficult to describe, but an inexperienced technician learns quickly how best to manipulate the instrument and how to apply the proper pressure. After 8 to 10 strokes the mixture is rapidly scraped to a central point, from which it is spread out again immediately by additional rapid, sweeping strokes. This operation of gathering and respreading should be repeated 2 or 3 times.

When it is observed that the cement is approaching the state of unmanageable viscosity, the mass is gathered again and then spread quickly into a thin layer, as shown in Figure 2. The entire rubbing operation consumes 20 to 40 seconds. Figure 2 thus pictures the completion of the first dispersing step, and shows also the spearhead needle which is used. Proper manipulation to this point will have resulted in complete and uniform dispersion of the pigment particles in the cement. Very little segregation normally occurs, and in this concentrated suspension almost any small portion is representative of the whole.

The mix at this state is too concentrated for microscopic examination and must be diluted. For this purpose a very small piece (about 0.5 mm. square) of the hardened mix is cut off by the point of the spearhead, placed on a clean slide, and covered with a small drop (2 mm. in diameter) of fresh Duco cement. This combination is then rubbed out gently under a cover glass by imparting a squeezing and rotating motion with the index finger, which is protected with a thin, clean cloth to prevent soiling the cover glass.

In this operation the necessary dilution is normally accomplished with no segregation of any fraction; hence any area is usually representative of the whole. With rubber or paint pigments of small size—e. g., zinc oxide (Figure 3) or iron oxide (Figure 4)—the particles lie essentially in one plane. Obviously in a pigment possessing a wide variation in sizes, all the particles cannot lie in the same optical plane because the large particles may touch both the cover glass and the slide while the small particles are distributed in the space between, as may be observed in Figure 5, which shows a field of particles of ground barytes.

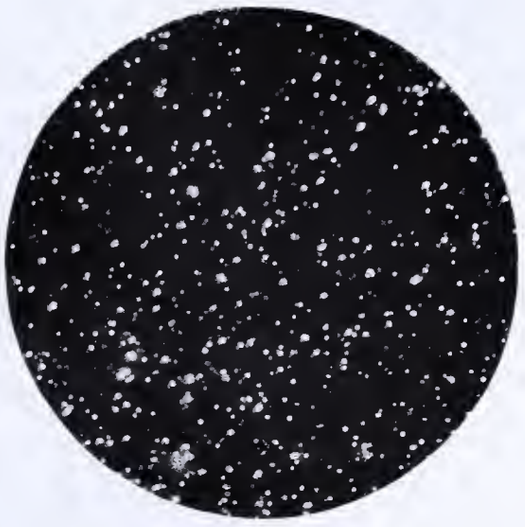

FIGURE 4. IRON OXIDE (× 100)

The refractive index of a mounting medium is important for two reasons: It should be sufficiently different from the refractive index of most of the pigments commonly examined so that the outlines of the particles may be observed clearly, and should be sufficiently high to permit utilizing the full resolving power of objectives of N. A. 1.40. Duco cement fulfills both these requirements. It is desirable, of course, that the particles in a mount be motionless. In this method the high viscosity of the matrix, even in fresh preparations, prevents sufficient movement of the individual particles to be annoying for observation; if, however, the preparation is to be photographed with an exposure of many seconds duration, it should be allowed to stand for a half hour or more before the particles are sufficiently motionless.

Disadvantages

One difficulty in the use of the present method is the slight movement of the dispersed particles immediately after preparation. This is not disadvantageous if visual observation

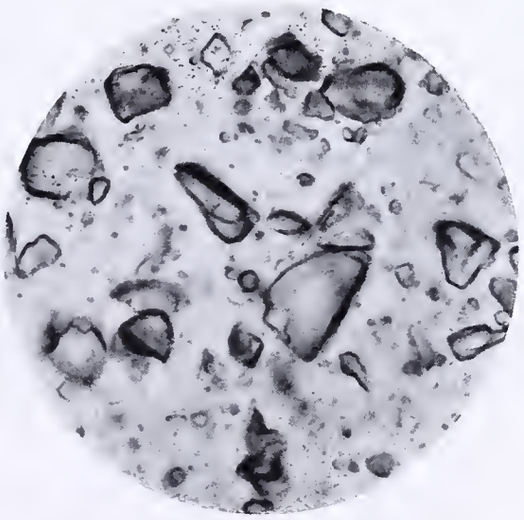

FIGURE 5. GROUND BARYTES (× 1000)

only is used, but if photography is employed, a setting period must be provided, long enough for the particles to come to rest. Moreover, with extremely fine particles, the movement, resulting partially from Brownian motion and partially from flow, may result in some degree of reflocculation.

Although no segregation of particles normally occurs, some segregation can result in the first step of the operation if the entire mass is not gathered into one spot several times during the kneading or rubbing. Whether or not an excess of the larger particles has been swept to the side can be determined by removing 0.5-mm. pieces from different localities (Figure 2), diluting, and examining them.

The mounts prepared according to this method are permanent if care is exercised to ensure a thin layer of cement in the final preparation. It is not always possible to arrive at this condition, however, particularly with coarse pigments. Where the largest particles are 40 to 50 microns in size, the cement layer also will be of that thickness and sometimes sufficient shrinkage will occur eventually to spoil the preparation. No other difficulties have been discovered in the use of this method in preparing some thousands of specimens for microscopic examination.

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PRESENTED before the Division of Microchemistry at the 97th Meeting of the AMERICAN CHEMICAL SOCIETY, Baltimore, Md.

Microtechnique of Organic Qualitative Analysis

Identification of Compounds Containing Nitrogen

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ACCORDING to the scheme of Mulliken and Huntress (*11*) for the analysis of organic compounds, the second order of compounds consists of substances which contain nitrogen in addition to carbon, hydrogen, and possibly oxygen. These authors divide this second order into two subgroups, the colored and the colorless compounds. Inasmuch as the former group is small, generic tests are unnecessary. The second group, however, is divided into three genera, based upon the results of titrations which place the substance in one of the following: (1) acidic species, (2) basic species, and (3) neutral species.

In developing a microtechnique for the analysis of such compounds, the authors of the present paper have endeavored to use a technique which would not require the use of special or unusual apparatus. For the concentrations given by Mulliken and Huntress (*11*) it is necessary to work with and measure with reasonable accuracy volumes of 0.012 to 0.06 ml. The possibility of using more dilute solutions was therefore studied. It is realized, of course, that using 1 ml. of a 0.1 *N* solution is not the same as using 0.1 ml. of a 0.01 *N* solution. However, since the division of these organic compounds into the three genera is more or less arbitrary, the theoretical considerations are not important. This becomes evident when one observes that many of the so-called acids do not have sufficiently high ionization constants to give clean-cut end points and that so-called pseudoacids are found in the acid genus, and amines such as diphenylamine are placed in the neutral genus. Since the division is an arbitrary one, it was decided that the best procedure would be actually to try out the titrations on a variety of compounds of all three genera to determine whether the use of more dilute solutions and a microsample would give the same results as the macroprocedure. Therefore, for example, anthranilic acid, nitrobenzene, 3-nitrophthalic anhydride, acetamide, aniline, diphenylamine, phthalimide, sulfanilic acid, and benzamide were used. The results obtained were in complete accord with the classification according to the tables of Mulliken.

Procedure

The titration setup is the same as that used in the microtitration of organic acids (*12*). Care must be taken to regulate the stream of air bubbles, so that very volatile substances will not be carried out by the air. Some difficulty was experienced with substances which were insoluble prior to their reaction with acid or base. The color of the indicator changed and then faded again as more of the substance went into solution and reacted with the excess of acid or base. In such cases the titration must be carried out slowly. The constantly changing cushion of purified air makes sure that the fading of the color is not due to absorption of carbon dioxide or ammonia from the atmosphere of the laboratory. The only precaution necessary is to avoid too great an excess of base which may destroy the indicator.

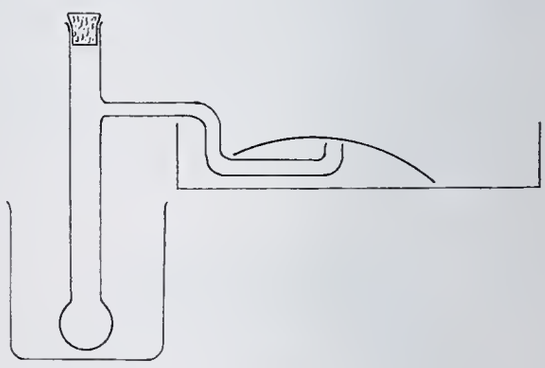

FIGURE 1

A. Two to 3 mg. of the substance (weighed on an analytical balance) are dissolved in 1 ml. of alcohol in a microbeaker and 0.05 ml. of 0.02 *N* sodium hydroxide is added drop by drop. If a color develops, the compound is in the acidic series, Genus 1. If no color develops, two drops of thymol blue indicator solution are added (from a capillary pipet). The addition of alkali is continued until a clear blue solution is obtained in which the color persists for 60 seconds.

B. If more than 0.1 ml. of 0.02 *N* sodium hydroxide is used in A, another 2 to 3-mg. sample is titrated, using water as the solvent in place of alcohol and phenolphthalein instead of thymol blue.

If more than 0.1 ml. of 0.02 *N* sodium hydroxide is used to bring about a permanent color change, the compound is placed in the acidic series, Genus 1.

If less than 0.1 ml. of 0.02 *N* sodium hydroxide is used, the compound is placed in the neutral series, Genus 3.

C. If less than 0.5 ml. of 0.02 *N* sodium hydroxide is used in A, another 2- to 3-mg. sample is titrated as in A but with 0.02 *N* hydrochloric acid in place of the sodium hydroxide until the solution remains pink for 1 minute.

If more than 0.1 ml. of 0.02 *N* hydrochloric acid is required, the compound is placed in the basic series, Genus 2.

If less than 0.1 ml. of 0.02 *N* hydrochloric acid is used, the compound is placed in the neutral series, Genus 3.

Tests for Genus 1

Millon's Test. A tiny particle (a few micrograms) of the substance is added to a small drop (2 mm. in diameter) of the reagent (*4*) on a microscope slide, and any color which develops is observed against a white background. The test drop may be warmed if necessary.

Mulder's Reaction. A tiny crystal (a few micrograms) of the substance is added to a small drop of concentrated nitric acid on a microscope slide. The color is observed against a white background, if necessary, warming the slide on a water bath. Then the slide is inverted over the mouth of a bottle of concentrated ammonia, and the color is again observed against a white background.

Murexide Reaction. One to 2 micrograms (or less) of the protein are added to a very small drop (2 to 3 mm. in diameter) of 6 *N* hydrochloric acid on a thin crucible cover and then a very small crystal (just visible) of potassium chlorate is added. The cover is heated on the water bath. If no color develops after evaporation, heating is continued cautiously over a free flame. Charring is to be avoided. A pink or brown color which turns purple-red on addition of a drop of ammonium hydroxide is positive.

Adamkiewicz-Hopkins-Cole Reaction. Three to 4 cu. mm. of the reagent are placed in a centrifuge cone and a few micrograms of the protein are added. Five to 6 cu. mm. of concentrated sulfuric acid are added by means of a capillary pipet to the bottom of the cone under the reagent. After standing for a few minutes, the two layers are mixed. If no color is visible after mixing, the liquid is drawn up into a coloriscopic capillary (*5*) and examined under the microscope or lens.

Tests for Genus 2

Specific Test for Ammonia. This test is carried out in a gas chamber consisting of a microscope slide, preferably one with a concave depression, as a base, a glass ring, and a 2.5-cm. (1 inch) watch glass as a cover.

A small drop of water is placed on the under side of the watch glass, a large drop of acetone or ether on the upper side. The evaporation of the latter keeps the watch glass cool. The drop of test solution is placed on the slide and the watch glass on the chamber. After warming the slide for a few minutes, the watch glass is taken off and the under side is touched to a clean slide, so that the drop of distillate is taken off on the slide. This drop is then tested for ammonia by means of the formation of the tetraiodide of hexamethylenetetramine.

Wacek and Löffler (*14*) use a gas chamber for the identification of volatile amines in the presence of ammonia. They use bases of varying strength to liberate ammonia and some of the amines: 5 per cent sodium carbonate solution for methyl amine, and 2 and 8 per cent sodium hydroxide for other amines.

Test for Salts of Ammonium Type. This test should be carried out before the generic tests, since ammonium salts are not listed in the tables. The same procedure and apparatus as above are used, except that 15 cu. mm. of water and 0.15 mg. of finely powdered magnesium oxide are added to 0.1 mg. of the substance. The complete gas chamber is set on a metal block, which is heated until the drop being distilled begins to decrease in size. The drop of distillate is tested as described in the test for ammonia. If ammonia is present, the acid must be identified in the residue or by preparation of the sodium salt.

Detection of Primary Amines with Nitrous Acid. The presence of primary amines is established by collecting the gas evolved when the sample is treated with nitrous acid. A small distilling flask is set up as shown in Figure 1. The flask is stoppered with a small cork, the side arm is placed in the Petri dish, and the latter is filled with alkaline permanganate solution. A 5-cm. (2-inch) watch glass is then inverted over the opening of the side arm in such a way that a small bubble (approximately 10 mm. in diameter) of air is entrapped under the glass and is brought over the mouth of the side arm. The presence of the bubble is necessary later to prevent sucking back of the permanganate solution due to the contraction of the gases in the distilling flask because of the initial absorption of the oxides of nitrogen. The circumference of this gas bubble is marked on the watch glass with a glass-marking pencil.

The cork is removed from the flask and the bulb and part of the neck of the flask are immersed in a beaker of ice water. The sample (0.5 mg.) and 0.1 ml. of ice-cold 6 *N* hydrochloric acid are placed in the bulb, and 0.02 ml. of ice-cold 3 *N* sodium nitrite solution is added. The flask is quickly stoppered and allowed to stand for 1 or 2 minutes. The water in the beaker is then slowly heated to boiling. The boiling must not be continued too long. The flask and contents are allowed to cool, keeping the outlet of the distilling flask open to the gas bubble at all times to prevent the sucking-back of the permanganate solution. The diameter of the final bubble of gas is measured and compared with the original and with that of a gas bubble obtained in a blank experiment run in the same way but without the sample.

Carbylamine Reaction for Primary Amines. This test has been described in detail by Emich (*6*).

Figure 2

Rimini Test for Primary Aliphatic Amines. One drop (1 mm. in a capillary) of the amine is placed in the depression of a spot plate. Twenty times as much water (20 mm. in the capillary) is added from an adjacent depression. Then 1 drop of pure acetone and 1 drop of 1 per cent sodium nitroprusside solution are added, both from a capillary pipet. A violet color is a positive test.

Simon Test for Secondary Aliphatic Amines. One drop (from a capillary) of the substance to be tested in a depression of a spot plate is treated with 12 times as much water and 1 drop of 1 per cent sodium nitroprusside solution. In an adjacent depression 1 to 2 drops of 50 per cent ethyl alcohol are placed. The end of a piece of copper wire is heated red hot and then immediately dipped into the alcohol. The heating and immersion are repeated. One drop of this solution is added to the test drop. A blue color fading to green and yellow is a positive test.

Acylation of Amines and Hydroxyl Compounds. *Direct Acylation.* To 3 to 6 mg. of the substance in a centrifuge cone is added twice as much acetic anhydride, and the mixture is heated in a metal block or on a water bath for 1 minute. After cooling, the product is dissolved in a hot solvent (water, dilute alcohol, or 95 per cent alcohol). The tube is cooled with ice and the sides are scratched until precipitation occurs. After centrifuging and decanting the supernatant liquid, the precipitate is rinsed sparingly with the solvent. A melting point is taken and, if necessary, the material is recrystallized.

Schotten-Baumann Reaction. Three to 6 mg. of substance are placed in a refluxing tube (Figure 2). A slight excess of 3 *N* sodium hydroxide is added (Mulliken and Huntress recommend potassium hydroxide for a better yield) followed by 4 to 7 cu. mm. of benzoyl chloride. The tube is sealed and allowed to stand for 0.5 hour with occasional centrifuging of the contents back and forth. The tube is then opened and if the odor of chloride is noticed, the tube is resealed and again allowed to stand for another half hour with centrifuging as before. If a precipitate appears when the reaction is complete, it is centrifuged out. If there is no precipitate, the reaction mixture is acidified with hydrochloric acid. The precipitate in either case is washed with water and extracted with cold ether, the extract being discarded.

The chief difficulty in this preparation lies in the purification of the product. The principal impurity is benzoic acid which should have been removed by the cold ether. However, since the derivative in some cases is also soluble in ether, this separation is not very efficient. The authors found that the benzoic acid can readily be removed by sublimation. A Johns melting point block, used in determining the melting points, made an excellent sublimator.

The residue from the cold ether treatment is placed on a cover slip on the block and heated to 90° to 100° C. A microscope slide is laid over the block. The shape of the block keeps the slide about 2 mm. above the cover slip and sample. When no more sublimate forms on a clean portion of the slide, another cover slip is placed on the sample and the temperature is raised to the melting point. An interesting observation was made in the case of the benzoyl derivative of diphenylamine. The melting point given in the literature is 178° C. In the authors' experiments the derivative obtained melted sharply at 107–9°, but on heating fur-

ther the melt solidified again between 130° and 140°, and melted again sharply at 176–7°. A suggested explanation of this behavior is that the benzoyl derivative of diphenylamine exists in two modifications with the melting points given and with a transition temperature somewhere between 130° and 140°.

Acylation in Pyridine. Procedure A. The reaction is carried out by dissolving 3 to 6 mg. of the substance in 30 to 60 cu. mm. of dry pyridine in a centrifuge cone. An equal quantity or slight excess of reagent is added quickly and with cooling under the tap if necessary. The mixture is diluted with approximately 0.6 ml. of water and stirred until precipitation occurs. If no precipitate appears after a reasonable time, the tube is set aside for 10 minutes, and then again stirred. The precipitate is centrifuged off, the supernatant liquid discarded, and the precipitate washed with 0.6 ml. of water, 0.6 ml. of 0.3 *N* sodium carbonate solution, and finally again with 0.6 ml. of water. The product is recrystallized from a suitable solvent to a constant melting point.

Procedure B. The procedure is the same as in A, except that after the reaction has taken place the mixture is dissolved as far as possible in 500 cu. mm. of ether and the pyridine is extracted by shaking three times with 500 cu. mm. of water. The product is washed twice with 50 cu. mm. of 0.3 *N* sodium carbonate and twice with 50 cu. mm. of water. The ether is evaporated and the residue allowed to stand in an evacuated vacuum desiccator to remove all volatile substances as completely as possible. The residue is then recrystallized from a suitable solvent to a constant melting point.

Picramides. Three to 6 mg. of the amine, 50 cu. mm. of ethanol, and 330 cu. mm. of a 2 per cent alcoholic solution of picryl chloride are mixed in a centrifuge cone. After standing for a few minutes, the mixture is heated to boiling. If necessary, water is added to produce a slight turbidity to induce crystallization. After a brief centrifuging to concentrate the solid at the lower part of the centrifuge cone (but not enough to pack it down), the larger part of the supernatant liquid is removed. The precipitate is stirred up with the remaining liquid and the slurry transferred to a microscope slide. Here the remaining liquid is drawn off with a piece of filter paper or a capillary pipet and the precipitate washed with alcohol. A melting point is then taken.

As the methyl amine derivative hydrolyzes very readily, the hydrochloride is used in place of the free amine. To 3 to 6 mg. of the hydrochloride, dissolved in 15 to 20 cu. mm. of alcohol, 100 cu. mm. of 2 per cent alcoholic picryl chloride are added. After mixing, 3 to 6 mg. of potassium hydroxide (powdered) are added, and mixed until the color becomes uniform. After centrifuging, the supernatant liquid is decanted to another centrifuge cone and treated with concentrated hydrochloric acid dropwise until the brown liquid turns yellow. The mixture is again centrifuged and the supernatant liquid removed and evaporated to dryness or to the formation of crystals. The solid is washed with alcohol, dried, and the melting point determined.

The methyl aniline derivative also gave difficulties by both these procedures, but by modifying Turpin's (*13*) procedure it is possible to prepare it. Five milligrams of picryl chloride are dissolved in boiling alcohol, then 3 mg. of methyl aniline and 3 mg. of anhydrous sodium acetate are added. The centrifuge cone is placed in the water bath or heating block and heated until half the alcohol has been evaporated. The cone is then placed in a vacuum desiccator until crystallization takes place.

ARYL SULFONYL DERIVATIVES OF PRIMARY AND SECONDARY AMINES. Behrens (*2*) suggested the use of the Hinsberg reagent for the separation of primary, secondary, and tertiary amines on a micro scale, but gave no experimental details. The following procedure as developed by the present authors gives good results.

To 3 mg. of the amine 5 to 9 cu. mm. of benzene sulfonyl chloride are added, and stirred until reaction takes place. The mixture is cooled under the tap or in ice water if necessary. Then 12 to 24 cu. mm. of 4 *N* potassium hydroxide solution are added and the mixture is heated slowly to slight boiling in a heating block to remove the excess reagent, stirring while heating. It is then centrifuged.

A. If the material dissolves completely in the potassium hydroxide, the substance is a primary amine. Any slight insoluble material is centrifuged off and the clear liquid is acidified with concentrated hydrochloric acid. The resulting precipitate is centrifuged out, the supernatant liquid discarded, and the derivative extracted from the residue with ether and recrystallized from ether or benzene. If no precipitate appears on the addition of the hydrochloric acid, the derivative must be extracted from the solution with benzene or ether.

B. If the derivative does not dissolve in the potassium hydroxide, the original substance is a secondary amine. It is centrifuged off and the supernatant liquid discarded. The derivative is extracted from the precipitate with ether and recrystallized from ether or benzene.

Alexander and McElvain (*1*) have suggested the use of 3-, nitrophthalic anhydride for the separation of primary, secondary, and tertiary amines but not on a micro scale.

SALTS OF AMINES. *Picrates.* The methods for the preparation of picrates vary according to the amine. Two examples will show the general technique.

Two to 3 mg. of quinoline are dissolved in alcohol and an equal volume of a saturated alcoholic solution of picric acid is added. The precipitate which forms immediately is centrifuged, the supernatant liquid removed, and the residue washed with five times the original volume of liquid, dried at 60°, and the melting point taken. In the case of substances such as urea, 3 to 6 mg. of the sample are dissolved in water and then an equal volume of a saturated aqueous solution of picric acid and a small drop of dilute hydrochloric acid are added. A precipitate will form in about 10 minutes. It is centrifuged out, the supernatant liquid is removed, and the residue is washed with very little water.

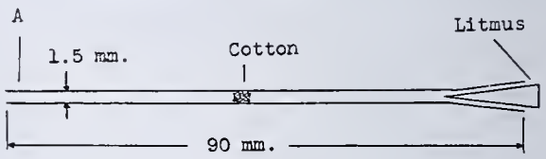

FIGURE 3

Salts of 3,5-Dinitrobenzoic Acid. Two milligrams of 3,5-dinitrobenzoic acid are dissolved in 25 to 50 cu. mm. of absolute alcohol. This solution is mixed with an equal volume of a solution containing 0.00001 mole of the amine in absolute alcohol. The solution is placed in a stream of dry air until the solution is evaporated. The solid is recrystallized from absolute alcohol or another anhydrous solvent such as benzene.

Salts of p-Toluene Sulfonic Acid. Three to 6 mg. of the monoamine or 2 to 4 mg. of a diamine are added to 20 cu. mm. of 2.5 *N* *p*-toluene sulfonic acid. The mixture is heated to gentle boiling on the heating block until the solution clears up. One to 2 mg. of decolorizing carbon are added and the solution is again boiled gently for a few minutes. While the solution is still hot, it is centrifuged and the hot liquid transferred to another centrifuge cone. The filtrate is cooled in ice water to crystallize out the salt. If no solid separates, the solution must be evaporated to some extent. The solid is recrystallized from hot water and dried in air at 110° C.

Tests for Genus 3

TEST FOR NITRO GROUP. The compound (0.1 to 0.2 mg. or less in 10 cu. mm. of 50 per cent hot ethyl alcohol) is treated with 5 to 6 drops (from a capillary) of 1 *N* calcium chloride solution and a pinch of zinc dust in a centrifuge cone. The mixture is heated in a water bath until a reaction takes place, kept hot until the reaction ceases, and then brought to a boil. After standing for 2 or 3 minutes, it is centrifuged. The supernatant liquid is siphoned off through a capillary into a second cone which contains 10 per cent silver nitrate solution. The appearance of a silver mirror or a gray or black precipitate indicates a nitro compound. A blank must be run on the reagents.

Hearon and Gustavson (*8*) describe a test for the nitro group carried out on a semimicro scale. Other color reactions for nitro compounds are given by Bost and Nicholson (*3*), Kulikov and Panova (*10*), and Kirchhof (*9*).

DIPHENYLAMINE REACTIONS FOR NITRATES, NITRITES, ALIPHATIC NITRO COMPOUNDS, AND NITROSOAMINES. The extreme sensitivity of this test even when carried out on the ordinary scale is sufficient to make a microprocedure unnecessary.

BIURET REACTION. This reaction has been described by Emich (*7*). It can be carried out on a white porcelain spot plate or in a centrifuge cone.

HYDROLYSIS OF SPECIES OF ORDER 2. *A. With Hydrochloric Acid.* The sample, 0.5 to 1 mg., of the substance is introduced into a capillary either by pushing the tube into the solid powder and then wiping off the outside or by dipping into the liquid. The same end is then dipped into 6 *N* hydrochloric acid (in a watch glass) until a 10-mm. column has risen in the tube. Both

ends of the capillary are sealed and it is heated 0.5 hour in the water bath. If a solid appears on cooling, it is centrifuged to one end of the capillary and this end is cut off just above the solid layer. The solid is recrystallized from water. Should no solid appear, the tube is cut open at both ends and the liquid is blown out on a slide, evaporated to dryness, and taken up in 5 cu. mm. of 10 per cent sodium hydroxide. The solution is taken up in a capillary pipet. The end of the pipet is sealed and the contents are centrifuged. If the liquid does not separate into two layers, it is extracted with ether or distilled in an Emich distillation tube.

B. With Sulfuric Acid. The sample is heated with 1 to 1 sulfuric acid as in A. After the heating, the solution is diluted with 5 to 10 cu. mm. of water. If a precipitate forms, the solution is cooled and filtered. In the case of anilides, the precipitate is aniline sulfate.

C. With Potassium Hydroxide. The same procedure as above is used but with 36 per cent alcoholic potassium hydroxide in place of the acids. The tube is centrifuged occasionally during the heating. After heating, the capillary is cut at both ends and the contents are blown out on a slide. The alcohol is allowed to evaporate. The capillary is washed out with 6 *N* hydrochloric acid onto the residue on the slide. The solution is diluted with water and the precipitate is washed and recrystallized.

Alkali Decomposition. A capillary of the size and shape shown in Figure 3 is used. The substance (0.1 mg. or less) is introduced into end *A* of the tube which has been previously fitted with a cotton plug and a piece of moist litmus as shown. The sample can be introduced easily by placing it on the end of a microscope slide, holding the end of the capillary horizontally against the end of the slide, and pushing the solid into the tube with a needle or glass rod. One milligram of powdered potassium hydroxide is introduced in the same way, 3.5 cu. mm. of alcohol are drawn in the same end, and the droplet of the resulting solution is allowed to slide down the tube for a distance of 2 to 3 mm. The end of the tube is sealed cautiously in the microflame. The litmus paper is observed during the sealing. If no change of color occurs, the sealed end of the tube is dipped into hot water, then cut off, and the contents of the tube are blown out onto a slide. The tube is rinsed out with dilute nitric acid onto the residue on the slide. If a clear solution does not result, the liquid is drawn up into a capillary, heated, and centrifuged, and the portion of the tube containing the precipitate is cut off and discarded. Silver nitrate solution is added to the clear solution.

Silver Nitrate Treatment of Original Substance. A few micrograms of the sample are introduced into a capillary. About 5 mm. of water or alcohol are added plus a few drops of 6 *N* nitric acid and a few drops of silver nitrate (in water or alcohol solution). If a precipitate forms, either in the cold or when warmed, the contents are blown out onto a slide and the drop is held over a bottle of ammonia.

Conclusion

All the melting points determined in this work were taken on a Johns melting point block. They were, in conformance with the experience of others, usually about 1° below the value given in the literature.

Spot tests for some nitrogen compounds and classes of compounds can be found in the literature but have not been considered in the present work.

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Improved Semimicrodetermination of Sulfur in Organic Materials

Peroxide-Carbon Fusion Followed by a Titration Using Tetrahydroxyquinone Indicator

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WHILE investigating the *p*-toluenesulfonic acid esters of certain carbohydrates, it became desirable to develop a simple rapid method for the determination of 0.5 to 5 mg. of combined sulfur. The titration of sulfate ion with tetrahydroxyquinone indicator has acquired considerable prominence because of its rapidity (*1*, *2*, *5–8*, *10*); but has been used in conjunction with oxidation procedures which were often lengthy, usually required special equipment, or were unsuited to all types of compounds. The authors have found that the oxidation of sulfur in organic compounds to sulfate by sodium peroxide–carbon fusion, originally developed for the analysis of coal (*3*), may be satisfactorily combined with the titration of sulfate ion with standard barium chloride solution, using tetrahydroxyquinone as an internal indicator.

The improved technique of Marvin and Schumb (*4*) was followed closely in the oxidation, and time was saved by using concentrated reagents in decomposing the fusion cake and in adjusting the pH of the resultant solution. The addition of a small amount of silver nitrate to the solution before titration was suggested by W. H. & L. D. Betz, Philadelphia, Penna., and has greatly increased the sensitivity of the indicator, making it unnecessary to subtract a titration blank. Common laboratory equipment was employed throughout and duplicate estimations were within the accepted accuracy of the analytical balance and the 10-ml. buret used in the titration. Four analyses were ordinarily begun and completed in less than an hour's time.

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Table I. Determination of Sulfur

Sample	Wt. of Sample	Wt. of Sulfur (Calcd.)	Sulfur Calcd.	Sulfur Found
	Mg.	*Mg.*	%	%
p-Toluenesulfonyl chloride	22.8	3.84	16.8	16.8
	13.8	2.32		16.8
Sulfanilamide	23.5	4.37	18.6	18.6
	26.9	5.00		18.6
2,5-Dichlorosulfanilamide	17.8	2.37	13.3	13.3
	16.0	2.12		13.5
Sulfonal	10.0	2.81	28.1	28.1
	10.1	2.84		28.2
Thiourea	12.9	5.41	42.2	42.2
	12.2	5.14		42.3
Sulfur	2.71	2.71	100.0	99.8
	2.54	2.54		99.9
$C_{30}H_{38}O_{11}S$ (*p*-toluenesulfonic acid ester of erythrose derivative)	9.1	0.61	6.73	6.8
	17.7	1.19		6.8
Wool (air-dried)	20.3	1.22	5.91[a]	6.00
	30.3	1.82	6.13	6.00
Rubber (laboratory tubing, contained no zinc)	46.5	0.981	2.115[b]	2.11
	49.2	1.05	2.11	2.11
Rubber (laboratory tubing, contained zinc)	35.7	0.705	1.976[b]	1.98[d]
	39.6	0.783	1.977	2.00
Mineral oil	45.3	0.49	1.09[c]	1.10
	48.4	0.53		1.13
Mineral oil	89.4	0.627	0.701[b]	0.71
	87.3	0.610	0.699	0.71

[a] Determined by Carius analysis.
[b] Determined by method of Waters (*9*).
[c] Determined by A. S. T. M. method D-129-39.
[d] Zinc removed by method of Sheen, Kahler, and Cline (*8*).

Materials and Reagents

A mixture of 15 parts by weight of granular sodium peroxide and 1 part of 30- to 60-mesh sugar carbon, kept in a well-stoppered bottle.

Hydrochloric acid, 12 *N*, reagent grade.

Ammonium hydroxide, 16 *N*, reagent grade.

Barium chloride solution, 0.01 *N*, standardized gravimetrically by precipitation as barium sulfate; 1 ml. = approximately 0.0003 gram of sulfur.

Silver nitrate solution, approximately 0.1 *N*.

Ethyl alcohol, 95 per cent, denatured with 5 per cent methyl alcohol.

Phenolphthalein indicator, 1 per cent solution.

Tetrahydroxyquinone indicator, as obtained from W. H. & L. D. Betz, Philadelphia, Penna.

Measuring dipper, capacity 0.15 gram of indicator.

Procedure

The sample, containing 0.5 to 5 mg. of sulfur, was weighed into a 30-ml. nickel crucible and covered with approximately 6 grams of the fusion mixture. The crucible was supported in a vessel so that its lower half was cooled by a current of cold water. A fuse, made by impregnating cotton string with potassium nitrate, was inserted part way into the charge. Combustion was carried out by igniting the fuse and promptly covering with a nickel lid. Too vigorous an oxidation could be avoided by increasing the sodium peroxide content of the fusion mixture. Upon cooling, the fusion cake was broken, removed by rapping the crucible sharply, and placed in a 250-ml. Erlenmeyer flask with 10 ml. of 12 *N* hydrochloric acid. The flask was covered with a watch glass until the vigorous reaction subsided. Hot aqueous washings of the crucible, its cover, and the watch glass were added, together with a few drops of phenolphthalein. The solution was boiled 5 minutes to destroy peroxides, adding, if necessary, more hydrochloric acid to maintain the acidity. The solution was cooled slightly and made just alkaline with 16 *N* ammonium hydroxide. Boiling was resumed to remove excess ammonia until the color of the indicator was permanently discharged. The volume of the solution was adjusted to approximately 25 ml.

After cooling to room temperature, 25 ml. of alcohol, 0.1 gram of tetrahydroxyquinone indicator ($^2/_3$ dipper), and 2 to 3 ml. of 0.1 *N* silver nitrate were added, and the mixture was shaken until the indicator dissolved. The cloudy yellow solution was titrated very slowly with the standardized barium chloride solution with vigorous shaking throughout. When all the sulfate had been precipitated, the presence of excess barium ions was denoted by a permanent pink coloration of the solution. The end point could be readily detected by observing the change in color of the particles of silver chloride under artificial illumination. The presence of precipitated salts, carbon, or large amounts of chloride ion did not affect the end point when determined in this manner.

The unpracticed eye may have severe difficulty in accurately detecting the end point of the titration. False indications of an end point are often given by the color change to pink in the bulk of the solution and on the surface of any sodium chloride precipitates. The true end point is given by the appearance of a pink coloration on the flocculent silver chloride coagulum, which settles more slowly than sodium chloride and is best viewed as a cloud of suspended particles by transmitted artificial light. It is often advantageous to add a few more drops of silver nitrate solution toward the end of the titration. The authors are indebted for these observations to Thomas S. Gardner, whose results entirely support their claims concerning the accuracy and reproducibility of the method.

The data in Table I show that the estimation of sulfur gave satisfactory results with a variety of pure compounds containing sulfur combined in different ways, and that the figures for wool, zinc-free rubber, and mineral oil agreed with those obtained by standard procedures. Zinc, as it occurred in rubber, interfered with the titration, but was conveniently removed after the peroxide-carbon fusion by the method of Sheen, Kahler, and Cline (*8*). The method has been applied to the analysis of a large number of *p*-toluenesulfonic acid esters of partly ethylated carbohydrates. In the following typical series, the per cent of sulfur is first given as directly determined and second as calculated from a single ethoxyl analysis: 2.50, 2.45; 2.84, 2.84; 2.90, 2.90; 3.01, 2.97; 3.10, 3.03; 3.21, 3.13; 3.22, 3.17; 3.25, 3.30; 3.30, 3.30; 3.57, 3.42; 3.70, 3.65; 3.74, 3.80; 3.90, 3.92; 4.04, 3.92; 4.25, 4.15; 4.79, 4.65; 5.22, 5.20; 5.32, 5.30; 5.41, 5.40. An average variation of 1.9 per cent between the two values was obtained.

Summary

Sulfur combined in a variety of nonvolatile organic compounds was oxidized to inorganic sulfate by a sodium peroxide–carbon fusion, and the sulfate ion was estimated by titration with standard barium chloride solution, using tetrahydroxyquinone as an internal indicator. No special apparatus was required, and 0.5- to 5-mg. quantities of combined sulfur were determined with speed and accuracy. The method should be especially useful for the routine analysis of materials like oil or rubber, with modification, if necessary, to remove interfering ions, and is apparently general for all types of sulfur-containing substances, including sulfonic acid derivatives.

Acknowledgment

The authors wish to acknowledge the assistance of W. H. & L. D. Betz in providing unpublished procedures for the determination of sulfur with tetrahydroxyquinone, and of C. B. Purves, A. A. Morton, G. G. Marvin, and A. G. Assaf.

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Research with the Polarograph

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LIKE many new analytical tools, the polarograph has been ballyhooed at times and belittled at others. The enthusiasm of early workers was misinterpreted as suggesting that any and all analyses could be carried out with this machine by any layman. As a result, there was skepticism and an unfavorable reaction when actual tests revealed that the polarograph had a definitely limited field of application and that a specially trained chemist was essential for its operation. Today it is generally agreed that this instrument is of unquestionable value, and this paper will serve to demonstrate some of its uses.

Polarography

EQUIPMENT. To simplify studies with the dropping mercury electrode, the polarograph was designed by Heyrovský and Shikata in 1925 (*22*) for plotting current-voltage curves automatically. As is shown in Figure 1, it consists of a potentiometric wheel, *P*, driven by a motor, *M*, and geared to a camera, *C*, in a fixed ratio for recording deflections of the galvanometer, *G*. (For simplicity, only this type of polarograph is discussed here. However, several other instruments for use with the dropping mercury electrode are produced by American manufacturers and sold under various trade names.) The current-voltage curves obtained with it (polarograms) are of value only if they indicate specifically the processes going on at one of the electrodes. It is therefore essential to have an electrolytic cell with one large nonpolarizable and one small easily polarizable electrode. A large calomel electrode and the dropping mercury electrode are undoubtedly the best combination meeting these requirements. (Other easily polarized electrodes can also be used under proper conditions, but they lack the advantage of an easily reproducible fresh surface and are seldom free from the effects of previous polarization.)

PRINCIPLES. Using such a cell it is possible to obtain with the polarograph what its name implies—that is, a graph showing the polarization of the small electrode with increasing applied voltage. The presence of any depolarizer at the electrode surface is indicated on such a graph by a flow of current. Under suitable, well-defined conditions, the depolarizer can reach the electrode only by simple diffusion, so that the current will be limited by the quantity of depolarizer which can diffuse to the electrode surface in unit time. This so-called "diffusion current" is proportional to the concentration of the depolarizer and serves for quantitative analysis (*25*). The potential at which the depolarization occurs is an indication of the nature of the depolarizer and serves for qualitative analysis (*20*). If more than one depolarizer acts over a given range of the applied voltage, a complex polarogram with several current increases at different potentials (waves) will be obtained, permitting the simultaneous qualitative and quantitative analysis of a number of substances. An example of such a curve in which optimum conditions prevail is shown in Figure 2.

Heyrovský and collaborators have made a systematic investigation of the factors which govern the polarographic wave and have been able to give a theoretical explanation of the observed facts, thus changing polarography from an empirical into an exact method. For details, the reader is referred to the original literature (*18, 20, 21, 25*) or to review papers (*27, 38, 39, 52*) and monographs (*19, 24, 28, 42*).

Theoretically, every substance can be analyzed polarographically if it is electroreducible or -oxidizable within the potential range of the electrode. The maximal range of the

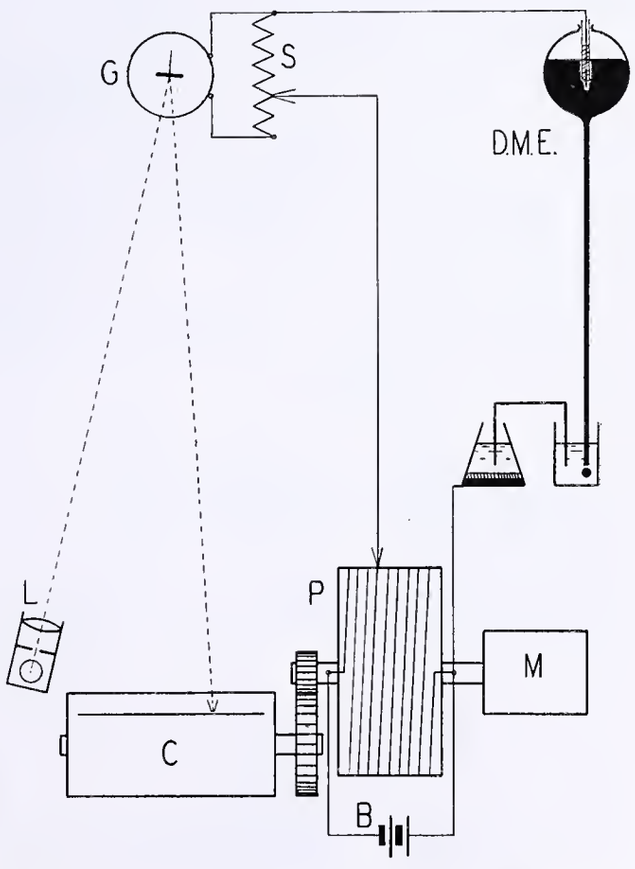


FIGURE 1. DIAGRAM OF POLAROGRAPH AND ELECTROLYTIC CELL

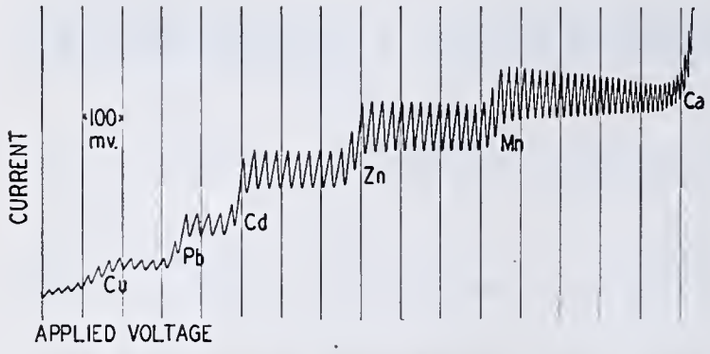


FIGURE 2. POLAROGRAM DEMONSTRATING SIMULTANEOUS ANALYSIS OF COPPER, LEAD, CADMIUM, ZINC, AND MANGANESE IN A SOLUTION OF CALCIUM CHLORIDE

dropping mercury electrode is from $E_{S.C.E.}$ + 0.6 to −2.6 volts, but in most solutions it is much smaller. If there is a great number of different electroactive substances in solution, the analysis becomes difficult, since some of them may have characteristic potentials (half-wave potentials, *20*) that overlap or come so close together that the resolution of individual waves becomes impossible. Such coincidences may sometimes be overcome by altering the pH of the medium in which the reaction is carried out, or by changing the reactants into complexes, from which they deposit at potentials far enough apart so that each wave can be measured separately. Figure 3 shows a chart of inorganic reduction

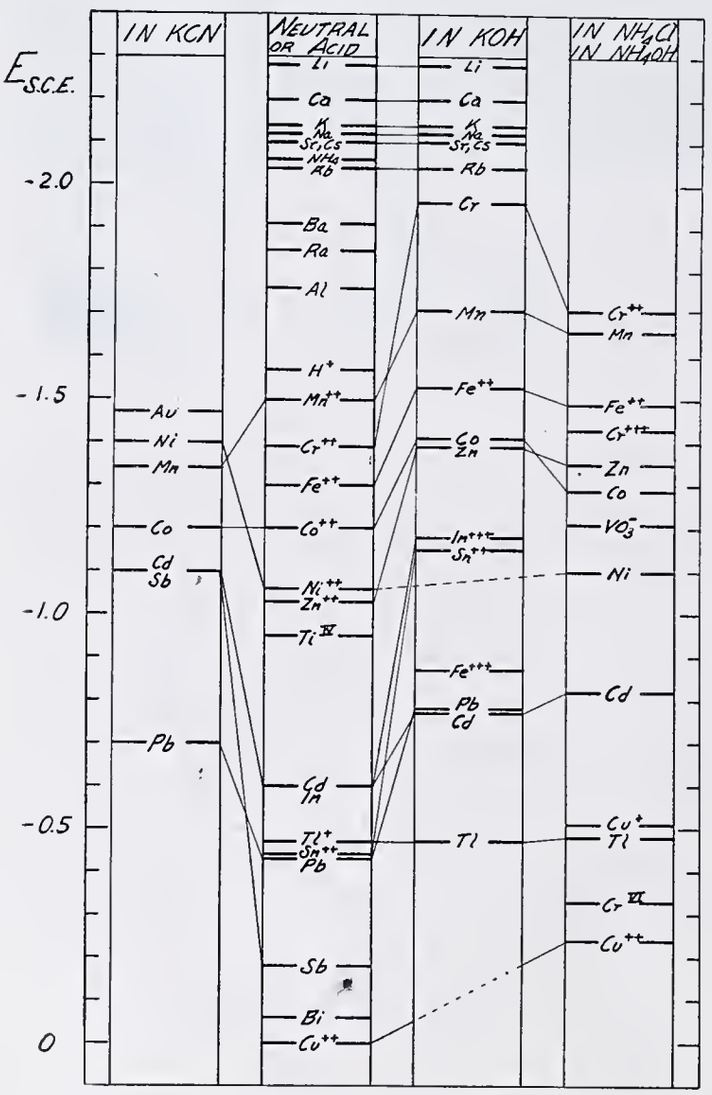


FIGURE 3. TABLE OF HALF-WAVE POTENTIALS OF METAL IONS (*42*)

potentials in different media (*42*). It may be seen that wave coincidences can be overcome in a good number of cases. If this is not possible, or if an ion with a more positive reduction potential is present in excess, recourse must be taken to ordinary chemical methods to bring about a preliminary separation. This is the case in most practical analyses which require a careful combination of electrochemical and general analytical procedures executed by a well-trained chemist. However, some analyses with the dropping mercury electrode, although possible, become so complicated that it is best to use a different method. These points are well demonstrated by the procedure for the analysis of brass (*24*) which is shown in Table I. Copper, zinc, nickel, lead, iron, and tin are all polarographically determinable. However, for simplicity, the procedure is divided into one gravimetric and three polarographic analyses in different media.

There are but few procedures published in as much detail as the one just mentioned, and even these should be used with reservation by either novice or expert. With rare exceptions, therefore, every contemplated polarographic analysis presents its own problem, for which the investigator must develop his own technique. This consists largely of finding supporting electrolytes or *Grundlösungen* which bring about the necessary separation of wave coincidences, so that every polarographic wave is significant and measurable. The reliability of each procedure must be tested by the construc-

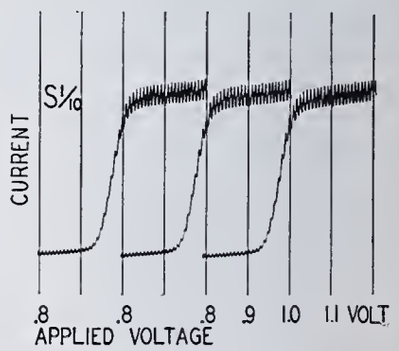


FIGURE 4. POLAROGRAPHIC ZINC WAVES REPEATED THREE TIMES

TABLE I. BRASS ANALYSIS

(Determination of Cu, Zn, Ni, Pb, Fe, and Sn according to Hohn, *24*)
Dissolve 0.5 gram of sample in concentrated HNO_3, evaporate to dryness; heat with 5 cc. of concentrated HNO_3, add water, and filter

SnO_2	Cu, Zn, Ni, Pb, Fe	
Determine gravimetrically	Add 15 cc. of concentrated NH_3 and 5 cc. of 2 *N* $(NH_4)_2CO_3$ and filter	
	Pb, Fe	Cu, Zn, Ni
	Dissolve in hot 2 *N* HNO_3, make up to fixed volume. Determine polarographically in slightly acid NH_4Cl solution	Make up to fixed volume. Determine polarographically (*a*) in NH_3 + NH_4Cl solution to get Cu and sum of Zn + Ni (*b*) in KCN + Na_2SO_3 solution to get Ni only

tion of calibration curves under identical conditions at which the analysis is to be carried out. A clear understanding of the fundamental principles is obviously essential. A person trained in physical chemistry should be able to acquire a good deal of experience in a relatively short time by carrying out the series of experiments described in the *Journal of Chemical Education* (*42*). For these no polarograph is needed and the necessary equipment should be available in most laboratories. Once a particular procedure has been fully developed, the analyses can be carried out by anyone with some training in chemical analysis—for instance, classes of medical students have been able to make polarometric oxygen and chloride determinations with but a few minutes of instruction. It is well, however, to have expert advice available, since experience has shown that, even in the simplest analyses, irregularities of unforeseen origin are bound to occur.

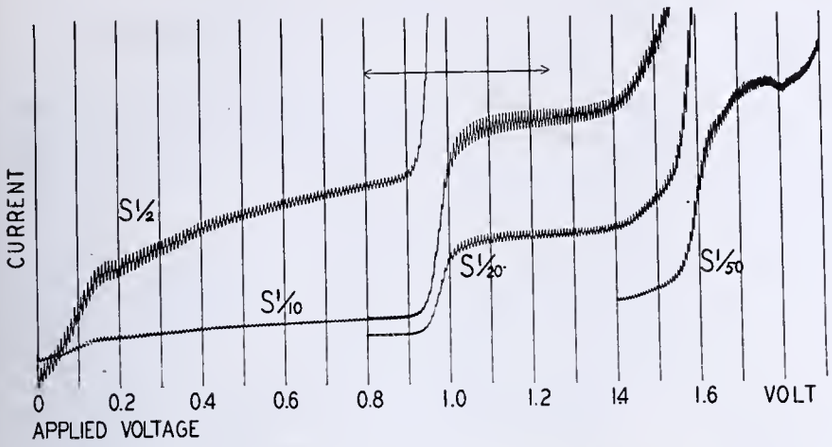


FIGURE 5. A STEP IN ANALYSIS OF A MAGNESIUM ALLOY

Looking for components of solution by recording curves at different sensitivities of galvanometer. Accidentally, middle (zinc) wave of curve *S* 1/10 was repeated over voltage increment indicated.

SENSITIVITY AND ACCURACY. The polarographic method is applicable over a concentration range of 10^{-6} to 10^{-2} molar; for best results, however, a 10^{-4} to 10^{-3} molar solution is desirable. In the latter case, an accuracy of ±1 per cent can be reached by repeating polarograms of the same solution several times (Figure 4) and averaging the individual measurements of the diffusion current. This can easily be done if the volume of the solution is 1 cc. or more, because the quantity of material electrolyzed is so small that the body of the solution remains unaltered and subsequent polarograms are identical. This is clearly demonstrated in the middle curve of Figure 5, where part of the curve was accidentally repeated, causing merely a thickening of the lines drawn. When smaller volumes of solution are analyzed, special precautions are necessary, since the quantity of material which is removed by electrolysis and also the end products formed become significant under these conditions. Furthermore, a residual current, which is inherent in the method, becomes appreciable in dilute solutions and necessitates a correction of the observed small currents. In general, therefore, the accuracy and sensitivity of the method are as follows: in practice, 1 cc. of a 10^{-5} *M* solution or 1 microgram of material (if its molecular weight is 100) can be analyzed with an accuracy of ±1 to 3 per cent. Under extreme conditions, 0.01 cc. of a 10^{-6} *M* solution or 0.001 microgram of material (if its molecular weight is 100) can be analyzed, but the error is about ±100 per cent.

SCOPE. The scope of the polarographic method includes a large variety of inorganic and organic ions and compounds. Analyses have been made of practically all metallic ions, of many anions which form complexes or precipitate with mercury, and of a number of complex anions such as bromate and nitrate ions. Even dissolved gases—e. g., oxygen, sulfur dioxide, and cyanogen—have been determined. In the organic field an ever-increasing number of compounds has been found suitable for polarographic analysis. Besides the group of known reversible oxidation-reduction systems, a great many compounds have been studied which are irreversibly reduced or oxidized at the dropping mercury electrode. In almost all these reactions, the pH of the solution has an influence on the reduction potential. For this reason and because reactions at the electrode can either produce or use up hydrogen ions (*39*, *41*), adequate buffering of the solution becomes essential in organic analyses.

REVERSIBLE REACTIONS. The ideal example of a reversible reaction at the dropping mercury electrode is given by quinhydrone and its components (*45*). In a well-buffered solution, the curve due to the reduction of quinone (Figure 6, *a*) will have the same characteristic half-wave potential (corrected for *IR*) as the curve due to the oxidation of hydroquinone (Figure 6, *c*). This potential is also obtained if quinhydrone is studied (Figure 6, *b*). Here one half of the curve represents an oxidation of hydroquinone at the mercury anode, while the other half represents a reduction of the quinone at the mercury electrode which is now cathode. Generalizing, one can say that an oxidation-reduction system is reversible in a thermodynamic sense whenever identical polarographic half-wave potentials are obtained in the reduction of the oxidant and oxidation of the reductant (*39*). These reversible reductions and oxidations are best for obtaining a clear understanding of the polarographic wave and will be discussed further herein.

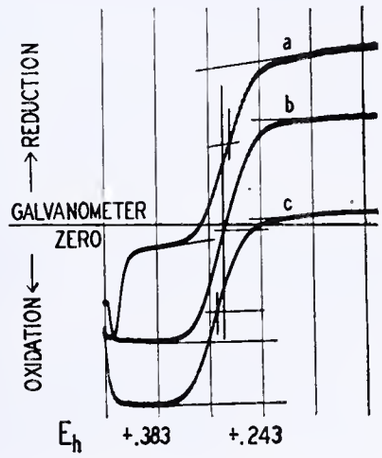
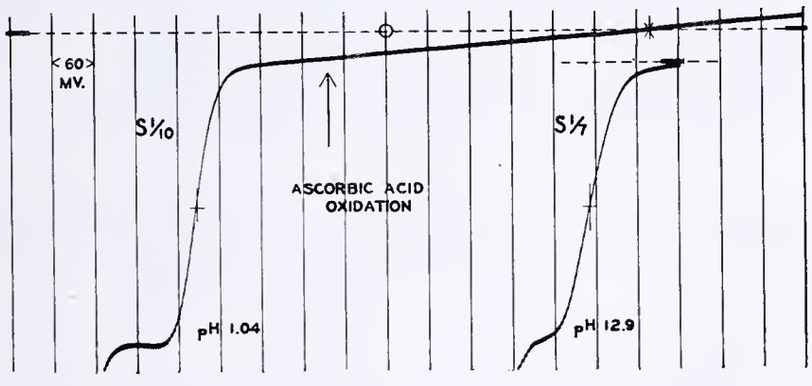


FIGURE 6. POLAROGRAM OF SOLUTION BUFFERED AT PH 6.67

a quinone, *b* quinhydrone, and *c* hydroquinone (*45*)



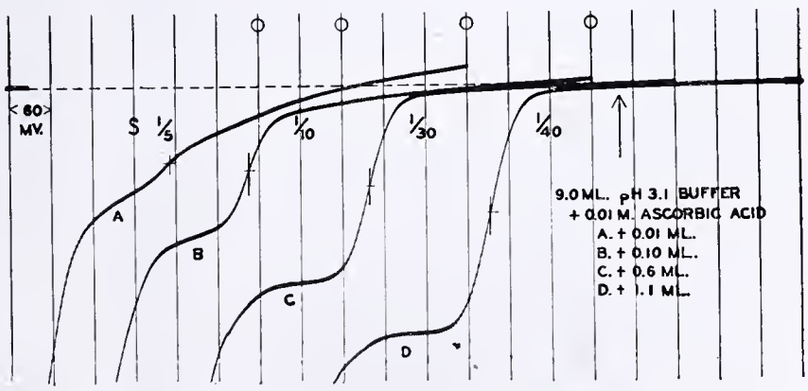


FIGURE 7. POLAROGRAMS SHOWING OXIDATION OF ASCORBIC ACID UNDER VARYING CONDITIONS (*46*)

For each curve, potential corresponding to that of calomel half-cell—i. e., when applied voltage is zero—is indicated by a circle

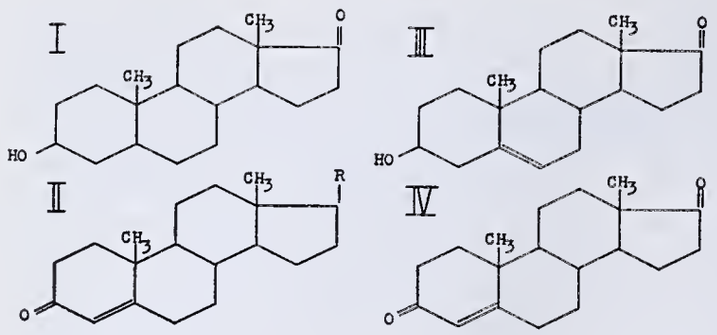


FIGURE 8

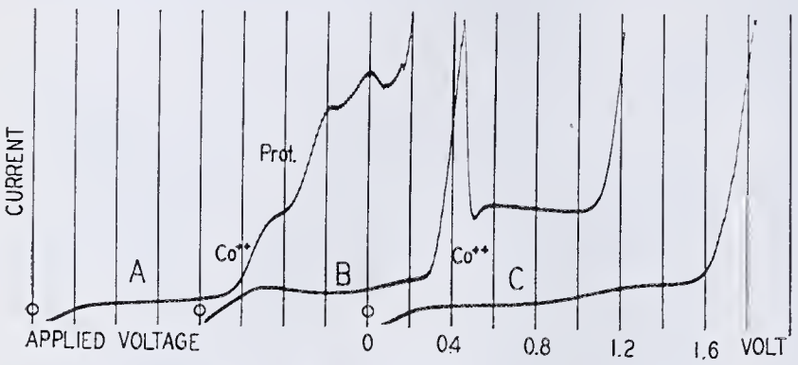


FIGURE 9. POLAROGRAMS DEMONSTRATING BRDIČKA'S REACTION (*9*)

A. 2 cc. of 8×10^{-3} *M* $CoCl_2$ + 1 cc. of 1 *N* NH_4Cl + 6 cc. of H_2O + 1 cc. of 1 *N* NH_4OH + 0.1 cc. of human blood plasma
B. Like *A* but without plasma
C. Like *A* but with H_2O instead of $CoCl_2$
Potential corresponding to calomel half-cell (applied voltage zero) indicated by circle

IRREVERSIBLE REACTIONS. By far the majority of organic analyses involve reactions which are not strictly reversible—that is, reactions in which the end products cannot be re-oxidized or reduced to the starting material at the same electrode potential. To this group belong the reductions of aldehydes, ketones, unsaturated acids, and nitro compounds. Although the potentialities of the method are great in this field, only few practical applications have so far been made.

An example of an irreversible oxidation (*46*) is given in Figure 7, which shows polarograms of the oxidation of ascorbic acid. This vitamin can be determined in fruits in an acid medium (*26*), but its polarographic determination in blood has not yet been accomplished. Of the other vitamins, members of the B group (*32*) and vitamin K (*16*), as well as substances related to vitamin E (*50*), have been found reducible in pure solution; procedures for their practical analysis should soon be developed.

Perhaps the best example of what can be done by a combination of organic chemistry and polarography is the work of Fieser and collaborators (*17*, *58*, *59*) who used the dropping mercury electrode for the determination of some sex hormones in urine. Eisenbrand and Picher (*15*) had found that the α-β unsaturated ketonic steroids (Figure 8, II) testosterone, progesterone, corticosterone, and desoxycorticosterone were polarographically determinable, while the 17-ketosteroids androsterone and isoandrosterone (Figure 8, I) as well as dehydroisoandrosterone (Figure 8, III) were not. The important contribution from Fieser's laboratory was the introduction of an active group into the electro-inactive molecule. This was done by treatment with Girard reagent *T* (trimethyl acethydrazide ammonium chloride). The otherwise inactive 17-ketosteroids thus not only were made reducible but they also showed a different reduction potential than the similarly treated Δ^4-ketosteroids (Figure 8, II). Therefore, by preparing the Girard derivatives, both types of compounds could be determined simultaneously (*59*). An additional accomplishment was the Oppenauer oxidation of dehydroisoandrosterone (Figure 8, III) with aluminum-*t*-butoxide in acetone, which changed the position of the double bond in the rings and oxidized the hydroxyl group at position 3 to form Δ^4-androstenedione-3,17 (Figure 8, IV). The Girard derivative of this compound showed two waves, as might be expected. Similar treatment of cholesterol and stigmasterol has also provided a means to analyze these compounds polarographically (*17*).

CATALYTIC REACTIONS. In addition to these reversible and irreversible direct reductions at the dropping mercury electrode, there are some which seem to be catalytic in nature.

Brdička (*9*) found that sulfur-containing proteins produce a large double wave (Figure 9, *A*) on the polarogram if the reduction is carried out in a buffered cobalt or nickel solution. No such double wave is obtained in the absence of these metals (Figure 9, *C*). For this and other reasons which cannot be enumerated here, Brdička concluded that the hydrogen evolution from the sulfhydryl groups in the proteins was catalyzed by the metals (*10*). Cystine and cysteine react similarly but produce only a single wave on the polarogram (*11*). When a number of blood proteins from different individuals were studied, the interesting observation was made that serum from cancer patients produced a protein double wave that was definitely smaller than that resulting from the serum of normal individuals (*12*). This seemed to offer possibilities for the diagnosis of cancer and stimulated research on the subject (*7*, *8*, *13*, *55*). Unfortunately, other diseases—e. g., pneumonia (*14*) and arthritis (*36*)—were found to have a similar effect, so that the test is not specific for cancer. Much promising work, however, is still in progress and even now valuable information is being obtained on the course of disease.

THEORETICAL APPLICATIONS. Aside from the foregoing practical applications, there are numerous theoretical problems in which the polarograph has been of service.

To get a clear picture of the happenings at the electrode-solution interface, let us again consider the polarogram of quinhydrone in a well-buffered solution (Figure 10, *A*). Since the electrode-reaction is reversible in this case, the polarographic wave will be practically identical with the familiar electrometric titration curve (Figure 10, *B*). In both, the midpoint of the curve gives us E_0, the characterizing potential of the oxidation-reduction system, yet the conditions responsible for these curves are fundamentally different. The polarographic wave indicates only the condition of the electrode-solution interface. The current of electrons flowing from or to the electrode corresponds to the titrating agent added to this interface while the body of the solution remains unchanged. In the electrometric titrations, the whole of the solution is altered and the electrode serves only

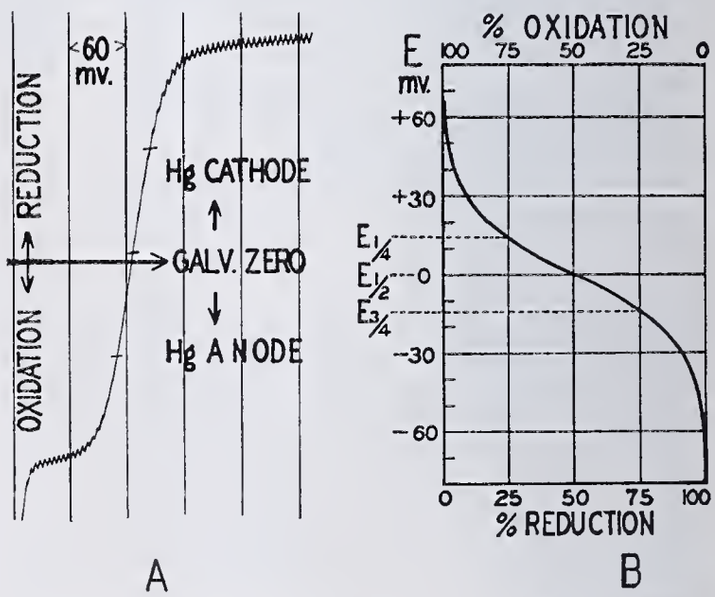


FIGURE 10. (*A*) POLAROGRAM OF QUINHYDRONE IN BUFFERED SOLUTION, REPRESENTING DIRECT TITRATION OF ELECTRODE-SOLUTION INTERFACE, WHILE BODY OF SOLUTION REMAINS PRACTICALLY UNCHANGED. (*B*) TYPICAL POTENTIOMETRIC OXIDATIVE OR REDUCTIVE TITRATION CURVE, REPRESENTING CHANGES THROUGHOUT BODY OF SOLUTION (*39*)

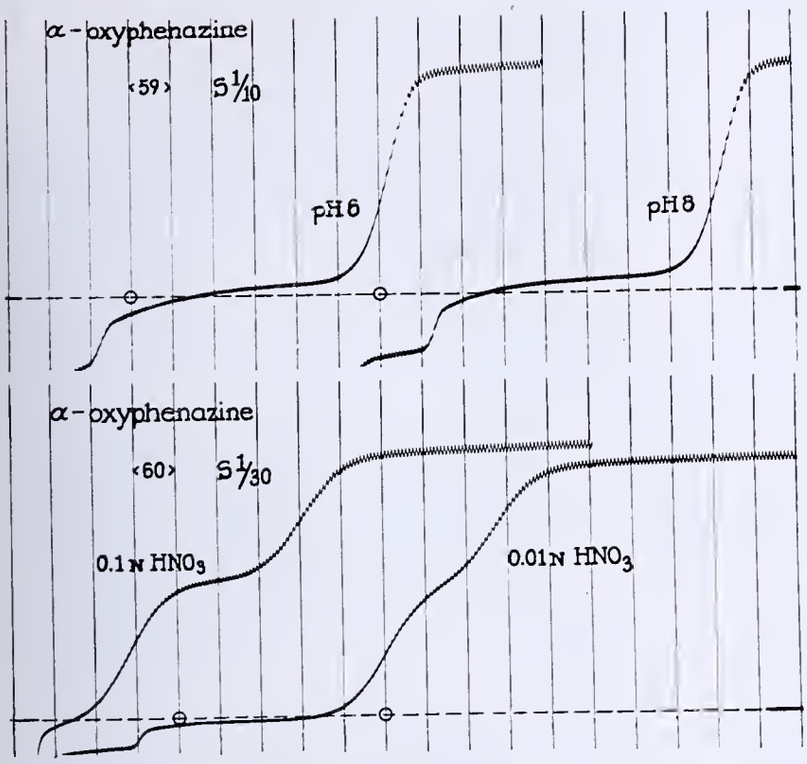


FIGURE 11. POLAROGRAMS OF α-OXYPHENAZINE

In McIlvaine buffers of pH 6 and 8, and in 0.1 and 0.01 *N* HNO_3 solutions (*37*). Potential corresponding to calomel half-cell (applied voltage zero) indicated by circle

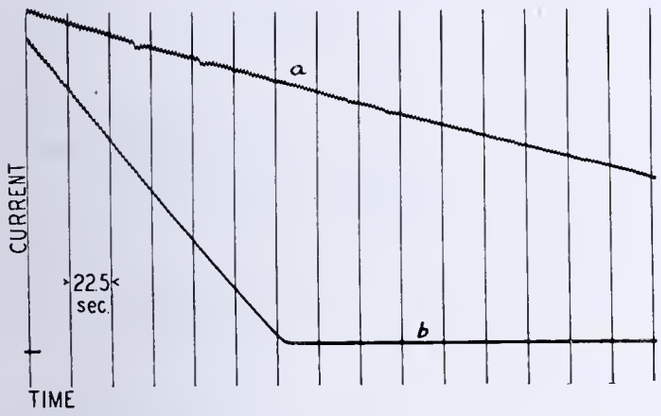


FIGURE 12. POLAROGRAPHIC RECORD OF CURRENT-TIME CURVES

Rates of oxygen consumption of yeast cells, (*a*) in absence of substrate, (*b*) in presence of glucose (*42*)

to demonstrate this change. The information gained in both cases is identical (*39*). Speed, ease of operation, and the absence of a need for a suitable titrating agent are advantages of the polarographic method, while higher accuracy and familiarity with the procedure speak for the electrometric titrations.

A good example of such studies is given in Figure 11, which shows the reduction of α-oxyphenazine (*37*), one of the semiquinone-forming compounds investigated by Michaelis (*35*). It is reduced in two steps in very acid solutions, while in more alkaline solutions the reduction proceeds in a single step. By means of the polarograph these studies have been extended (*37*) to measurements in the overvoltage range of potential and to irreversible systems. In the inorganic field similar investigations have been carried out with numerous complex metal ions (*31*).

There are many other theoretical polarographic problems in which the reductions are not reversible. To these belong studies on the effect of substitutions in an organic molecule upon its reduction potential (*1, 48, 49, 56*); rate studies such as the inversion of sucrose (*23*), or the tautomerization of an optically active azomethine (*6*); studies in keto-enol tautomerism and the polymerization of pyruvic acid (*43*); and studies of the effectiveness of buffers (*41*).

Polarometry

KINETIC STUDIES. All polarographic analyses require enough time to draw the curve and to evaluate the result. To save time, modifications of the technique have been developed wherein the currents obtained at a constant applied voltage are observed and recorded. This is possible whenever the reduction produces a wave with an extended plateau (*42*)—for instance, the oxygen concentration in a solution (*54*) has been determined by applying a fixed voltage of $E_{s.c.e.}$ −0.5 volt and noting the corresponding galvanometer deflections (*3, 5, 57*). Also, changes in oxygen concentration with time have been recorded by means of the polarograph. Figure 12 shows the rate of oxygen consumption of a yeast suspension (*a*) in the absence of substrate and (*b*) in the presence of glucose (*42*). These curves demonstrate the well-known fact that the rate of oxygen consumption of yeast is of zero order until very low oxygen pressures are reached.

TITRATIONS. The foregoing studies suggest reactions in which an analyzable component of the solution is altered by titration with a precipitating agent, while the current is observed at a constant applied voltage. Such titrations have been called polarometric titrations (*33*) to show their relationship to the polarographic method. [Kolthoff and Pan (*29*) have proposed the term "amperometric" to describe these titrations. As will be apparent from what is said below, their term fails to be specific, since it is also applicable to other unrelated methods in which current is measured.] Four main types are known at present and their respective titration curves are shown in Figure 13.

In *A*, the electroactive substance is removed from solution by precipitation with an inactive substance—e. g., lead titrated with sulfate ion (*30, 34, 51*). In *B*, to an inactive substance an electroactive precipitating agent is added—e. g., sulfate titrated with barium ion (*34*). In *C*, both the component of the solution and the precipitant are reducible at the given applied voltage—e. g., lead titrated with dichromate ion (*29, 47*). The end points

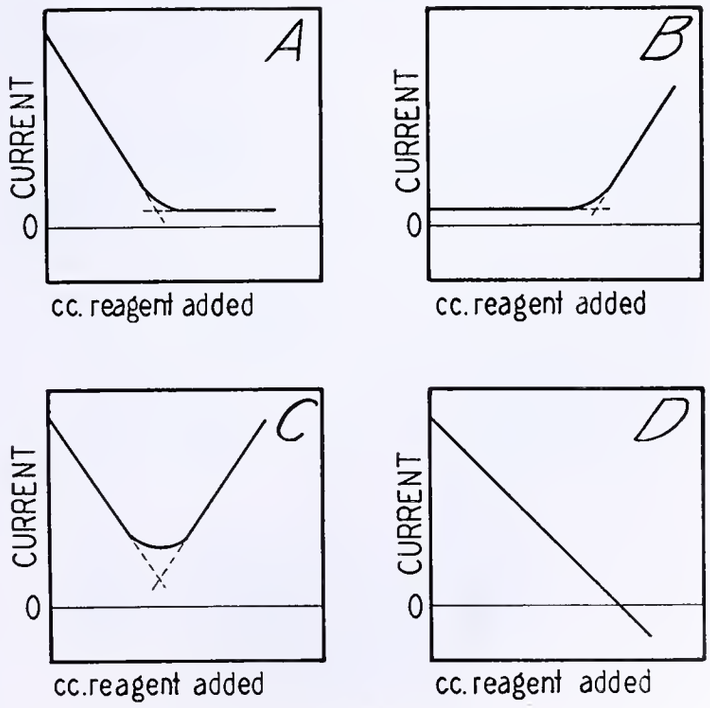


FIGURE 13. TYPES OF POLAROMETRIC TITRATION CURVES (*42*)

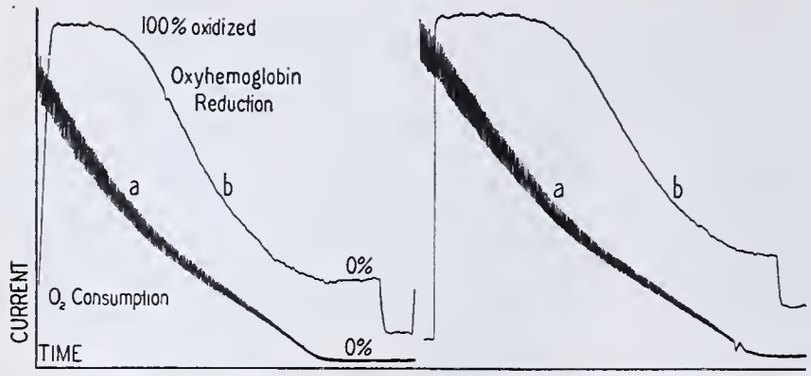


FIGURE 14. CHANGES DURING RESPIRATION OF YEAST IN RINGER'S SOLUTION (*3*)

In presence of glucose and defibrinated monkey blood.
a. Current-time curve obtained with dropping mercury electrode, showing consumption of oxygen
b. Current-time curve obtained with photocell and taken simultaneously with *a*, showing reduction of oxyhemoglobin accompanying change in oxygen consumption

of these titrations are usually obtained by extrapolation. Finally, in *D*, the titrating agent produces a reversal of current. This type of curve may be obtained either by an oxidative-reductive titration—e. g., titanium titrated with ferric ion (*53*)—or by precipitation of a polarographically determinable anion with a suitable cation—e. g., chloride titrated with silver ion (*42*). The end point in this case is reached when the current becomes zero (corrected for any residual current)—i. e., where the straight titration curve intersects the galvanometer zero line. These polarometric titrations are fast and accurate but have limitations similar to conductivity measurements.

Amperometry

Attention should also be called to some other, little known applications of the polarograph, in all of which it serves as a convenient device for the automatic recording of currents. These currents may be due to chemical reductions or oxidations as in polarography and polarometry, they may be created by the action of light on a photocell as in spectrophotometry or colorimetry, or they may be the sufficiently amplified very small currents flowing through vacuum tubes as used in potentiometry. Since current is measured in all of these studies, they can justly be grouped together as "amperometric". Examples of these techniques can be found in a paper by Baumberger (*3*).

Employing two galvanometers, Baumberger was able to record, simultaneously, changes in the potential of the solution and changes in its oxygen concentration during the respiration of a yeast suspension. Using a second dropping mercury electrode as an indicator electrode (*4*, *44*), he avoided the potential lag which had been always observed when platinum electrodes were used. In other studies he recorded the change in light transmission at a fixed wave length with a simultaneous change in the oxygen concentration of the solution during the respiration of yeast in the presence of red blood cells (Figure 14). From a single record, the oxygen dissociation curve of oxyhemoglobin could be constructed (*2*, *3*). Besides these current-time curves, Baumberger also recorded curves in which light transmission was plotted against wave length, by means of a suitable device which coordinated the movement of the prism of a spectrometer with the movement of the polarographic camera (*3*). As above, additional information could be plotted on these spectrophotometric records at the same time, by the use of extra galvanometers.

The polarograph has found still another application in studies of the passivity or periodicity of metals (*40*). Figure 15 is presented as an example. It shows a record of the current fluctuations with time when a constant voltage is applied to a small iron wire in a sulfuric acid solution. After an initial slow decrease in current, the wire becomes passive to be activated spontaneously at almost regular intervals.

Conclusion

It should be emphasized that this résumé of research with the polarograph necessarily had to be crowded and is therefore far from complete; the references given represent not even 10 per cent of the literature on this subject. It is hoped, however, that enough material has been incorporated in this paper to demonstrate the versatility of the polarograph and the general usefulness and limitations of polarography.

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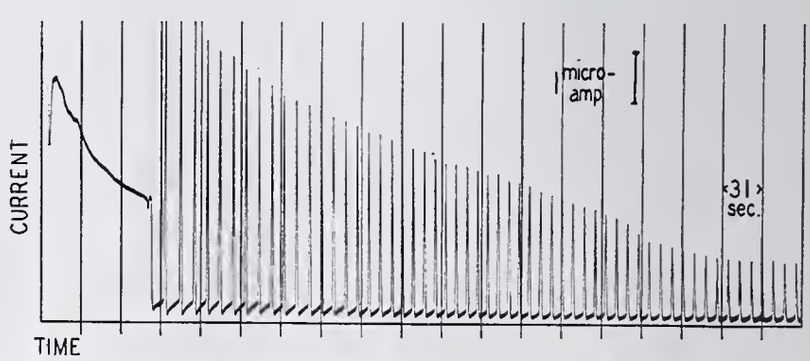

FIGURE 15. POLAROGRAPHIC CURRENT-TIME CURVE

Showing periodicity of a small iron wire in 1 *N* H_2SO_4 at constant applied voltage of E_h = +0.50 volt (*40*)

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Determination of the Aniline Point of Dark Petroleum Oils

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THE American Society for Testing Materials has defined aniline point (*1*) as the minimum equilibrium solution temperature for equal volumes of aniline and oil and has published a method for determining the aniline point by observing the temperature at which a 50/50 mixture of oil and aniline becomes turbid upon cooling. Since the use of the A. S. T. M. method is limited to oils of less than 8 A. S. T. M. color (*1*), the authors have developed a method for determining the aniline point of dark oils based on the visual observation of a thin film formed on the glass wall of the apparatus.

Previous investigators have observed the clouding of a thin film of aniline and oil and have made use of this principle to determine approximate aniline points. The method described by the Institution of Petroleum Technologists (*3*) suggests that the aniline point of dark oils can usually be determined with suitable illumination or by observation of the thin film of the mixture which is washed up on the sides of the tube during stirring. Donn (*2*) utilized the sharp break in the viscosity temperature curve to indicate the aniline point, but resorted to an approximate method for dark oils in which a drop of the hot solution was observed under a microscope. VanWijk and Boelhouwer (*5*) and Matteson, Zeitfuchs, and Eldredge (*4*) make use of the variation in transparency to infrared radiation.

Description of Apparatus

The apparatus shown in Figure 1 consists essentially of a double jacketed Pyrex test tube having a triple-junction copper-constantan thermocouple cemented to the inner wall of the tube. The aniline-oil mixture is stirred by swinging the apparatus back and forth over a small arc in such a manner that the contents run from one end of the tube to the other, alternately covering and exposing the thermocouple attached to the wall of the tube. This stirring action may be accomplished in a number of ways; the authors clamped the apparatus to a vertical arm which is pivoted at its lower end. The arm is moved back and forth about 25 times per minute by a slow-speed motor geared down to this speed. A small 7-watt light and shield are clamped on the arm to illuminate the film, and the apparatus is heated by a Bunsen burner which is also clamped on the oscillating arm. The temperature may be measured by any type of millivoltmeter or potentiometer which is calibrated to 0.1 millivolt in the range of 0 to 15 millivolts. An instrument calibrated to 0.01 millivolt should be used if greater than 0.5° F. accuracy is desired.

The inner tube (Figure 2) is 15 mm. in outside diameter and 125 mm. long (not including the standard-taper ground-glass joint which is sealed to the top of the tube to hold the glass stopper). The triple thermocouple junctions are brought into the tube through two small holes which are located about 90 mm. from the bottom of the tube. The junctions are separated to cover more of the tube surface and cemented in place with air-

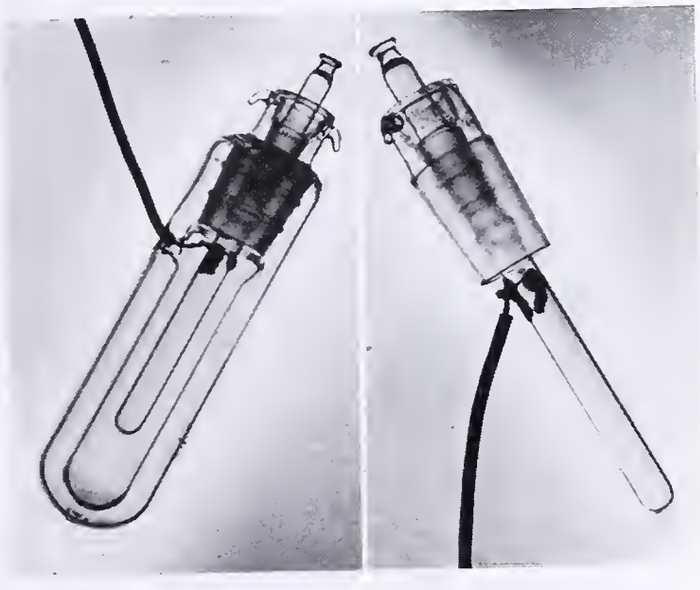

FIGURE 1 FIGURE 2

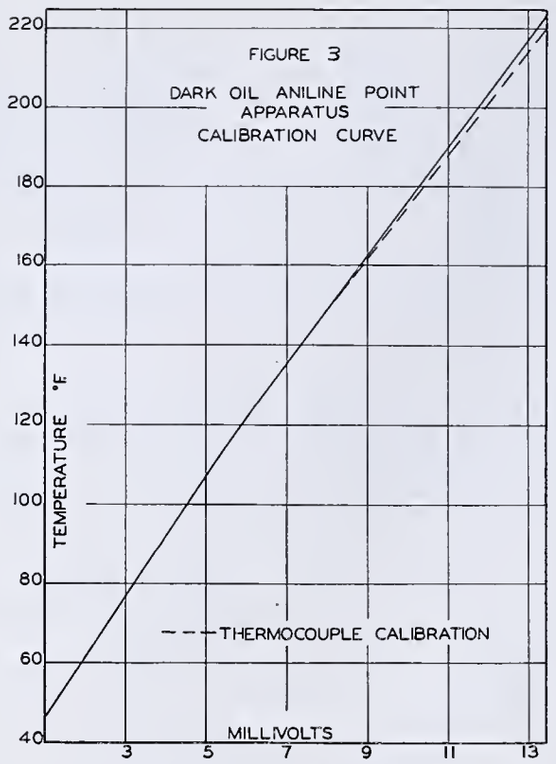

FIGURE 3

drying Insalute cement. The inner tube is supported in the jacket by means of a second ground-glass standard-taper joint.

The outer jacket is a double-walled tube 6 cm. in outside diameter and 19.5 cm. long, the inner surface of which is ground at the top to form half of the ground-glass joint which supports the inner tube. The jacket is vented near the top. A short section of glass tubing (not shown in the photograph) should be attached to the outer jacket just below the clamp to aid in blowing cold air through the jacket if this is necessary. A clamp is provided to hold the apparatus on the stirring mechanism.

Operation

Three cubic centimeters each of oil and aniline are pipetted into the inner test tube; the glass stopper is inserted and fastened by means of a rubber band. The apparatus is then clamped in a nearly horizontal position and the flame and stirring device are started. As the mixture is heated the inner tube should be vented at least every 50° F. The aniline point is taken as the temperature at which a cloudy film first appears on the glass wall surrounding the thermocouple junctions as the mixture is cooled from above its solution temperature. The rate of cooling should be controlled as specified in the A. S. T. M. procedure; however, when using a millivoltmeter, which may be read instantly, instead of a potentiometer the rate of cooling was found to have very little effect. The apparatus may be easily cleaned by pouring a suitable solvent into the tube and evacuating into a trap through a glass tube of small diameter. The oil sample and aniline should be prepared as described in the A. S. T. M. procedure.

Calibration

At high temperature, the temperature gradient through the apparatus becomes so large that a small correction must be added to the potentiometer reading, representing the temperature difference between the glass wall and the liquid film on the glass. This correction, which varies from about 0.5° F. at 150° F. to 2.5° F. at 215° F., was obtained by determining the aniline point of several light-colored oils in both the A. S. T. M. and the dark oil apparatus. A calibration curve for the apparatus is shown in Figure 3. The broken curve is the thermocouple calibration curve.

Table I gives a comparison of the results obtained by the A. S. T. M. method and the dark oil apparatus on a series of light-colored samples, and also shows the check results which are obtainable by different operators on samples of black oil.

The blends of No. 6 fuel oil in 180 viscosity neutral were made to bring the neutral to an 8 A. S. T. M. color.

Precision

The precision of this test is limited mainly by the precision with which the potentiometer can be read, since the potentiometer reading at which the film around the thermocouple becomes cloudy is highly reproducible. Duplicate determinations by different operators check within 0.5° F. The accuracy of the test depends largely on the thermocouple calibrations or the overall calibrations of the apparatus. The authors used constant-temperature (±0.025° F.) viscosity baths to ensure accurate calibrations; however, a periodic check should be made on the calibration. If the apparatus is carefully calibrated and corrected as shown in Figure 3 an accuracy of ±0.5° F. should be easily attainable.

TABLE I. COMPARATIVE RESULTS

Material	A. S. T. M. Aniline Point ° F.	Dark Oil Method ° F.
Kerosene	120	120.3
Kerosene	161	161.2
Kerosene distillate	168	168.0
Kerosene distillate	159	158.7
Cracked gasoline	97	97.0
No. 3 furnace oil	150	149.5
85 viscosity neutral	203	203.0
180 viscosity neutral	216	216.3
Blend fuel oil in neutral	209	209.2
Blend fuel oil in neutral	212	212.0
No. 6 fuel oil (black), operator 1	...	168.7
No. 6 fuel oil (black), operator 2	...	168.8
Still bottoms (black)	...	150.4
Black oil	...	146.0
700 E. P. gas oil (8 plus N. P. A. color)	...	118.0
20 gravity bottoms (Sweet Lake crude—black)		
Operator 1	...	221.0
Operator 2	...	221.2
Operator 3	...	221.3
28 gravity topped crude (Roscommon crude—black)		
Operator 1	...	178.6
Operator 2	...	178.7
Operator 3	...	178.2

Discussion

The simplicity in design and construction of the apparatus makes it available at very little cost to any laboratory which is equipped with the necessary millivoltmeter or potentiometer. In case of operation and time consumed the method is comparable with the proposed A. S. T. M. method for light-colored samples. The method is applicable to black stocks such as No. 6 fuel oil and most still bottoms; however, in the case of heavy tars the film may not be translucent even after 50 per cent dilution with aniline. If the solution temperature is below room temperature the aniline point may be determined by blowing cold dry air through the outer jacket, or by a method (*4*) involving the use of a standard diluent having an aniline point around 140° F. The aniline point of a 50/50 per cent by volume mixture of sample and diluent is determined, the aniline point of the sample then being obtained from a curve showing the relation between aniline points of oils and oil diluent blends. The aniline point of very black samples could presumably be determined in the same manner.

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Determination of Free Sulfur in Lubricating Oil

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THE increasing use of sulfurized fatty and mineral oils in lubricating oil has made important a method for determining free sulfur in such products.

In the sulfurization of fatty and mineral oils by direct reaction with elementary sulfur at elevated temperatures, control of the final free sulfur content is important because for some products, such as certain cutting and extreme pressure oils, a relatively high free sulfur content is desired, whereas for others, such as automotive oils, complete absence of free sulfur is commonly required. In those cases where presence of free sulfur has been considered desirable, the benefits have been variously attributed to cooling effect, increasing film strength, and antiwelding properties.

Various means have been used to determine sulfur in related products. Garner and Evans (*2*) describe a method for determining free sulfur in gasoline, involving refluxing the fuel with copper-bronze powder, solution of the metal and its sulfides in bromine water, oxidation to sulfate, and final recovery of the sulfur as barium sulfate. These authors also review the considerable previously published literature on what has been variously called free sulfur, active sulfur, corrosive sulfur, and loosely bound sulfur in gasoline and other light petroleum products. These generally involve the following steps:

Fixation of the sulfur as metallic sulfide by heating or agitating with a metal, usually sodium, mercury, copper, or an alloy.

Either determination of the increase in weight of the metal (calculated to sulfur), oxidation of the sulfide and final weighing of barium sulfate, or conversion of the sulfur to thiosulfate or hydrogen sulfide and its determination iodometrically.

An adaptation of the doctor test (alkaline plumbite solution) has been applied by Wirth and Strong (*5*), and Comay (*1*) has reduced the free sulfur of light petroleum distillates directly with nascent hydrogen.

Very little literature exists on the determination of free sulfur in heavier petroleum products such as lubricating oil. Sivertsev and Meerzon (*4*) shake the sample of oil with mercury, filter, treat the sulfide with hydrochloric acid and use hydrogen to sweep the hydrogen sulfide into cadmium chloride solution, filter the cadmium sulfide, and complete the determination iodometrically. Garner describes the difficulties of filtering the sulfides of mercury, particularly when free sulfur is high. Another disadvantage to the use of mercury is the lack of ready evidence of completion of sulfur removal. A purchaser's specification has been encountered which contains a method for active sulfur based on digesting the oil with copper powder at 149° C., filtering to remove copper and its sulfides, and determining total sulfur (bomb method) in the oil before and after this treatment. The present authors' experiments have shown that this treatment may cause a reduction of several per cent in the sulfur content of an oil though it actually contains no free sulfur.

Hardman and Barbehenn (*3*) determine free sulfur in rubber by extracting the sample with boiling acetone in the presence of copper, liberating hydrogen sulfide from the metallic sulfide by digesting with concentrated hydrochloric acid, and absorbing the hydrogen sulfide in ammoniacal cadmium chloride where it is determined iodometrically after acidification. This method is in many respects similar to some that had been applied to gasoline and discussed by Garner and Evans. Hardman and Barbehenn discuss previously published methods for determining free sulfur in rubber and most of these indicate a similarity to the problem as applied to gasoline.

Of the various methods to which reference is made, the principle of that employed by Hardman and Barbehenn for rubber seemed, and subsequently proved, most promising for free sulfur in oil. Their solvent had to be modified to ensure complete solution of oil samples and the apparatus and technique modified to ensure complete recovery of the large amount of free sulfur found in some products. Loss of sulfide had been reported by others (*2*) working with somewhat related methods on gasoline.

The following detailed method has proved satisfactory for determining free sulfur in pure petroleum lubricating oils, in such oils blended with fatty oils or sulfurized fatty or unsaponifiable oils, as well as in sulfurized fatty or sulfurized unsaponifiable oils themselves.

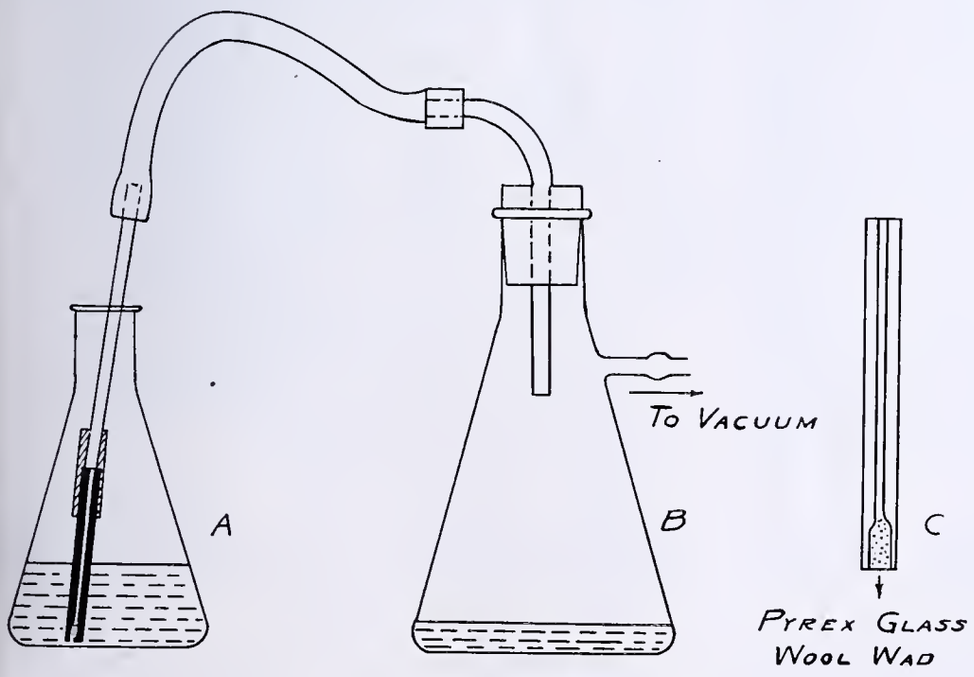

FIGURE 1. APPARATUS FOR REMOVING SOLVENT

Apparatus

Apparatus for removing solvent is shown in Figure 1. The filter stick, *C*, shown in place in *A*, is 70 to 80 mm. long and 2 mm. in bore, expanded to 6 mm. for a distance of 10 mm., the enlarged head being packed with fine glass wool.

Apparatus for liberating and determining hydrogen sulfide (Figure 2) includes a buret provided with rubber stopper at the bottom for tight fit in neck of flask *B*.

Reagents

Copper gauze, 40-mesh, cut into pieces 2.5 cm. (1 inch) square and folded double. These strips are reduced by heating in a flame to redness and promptly dropping into a test tube containing methyl alcohol. The strips, after rinsing with acetone, are stored under benzene until used.

Cadmium chloride stock solution, 36 grams of $CdCl_2.2^1/_2H_2O$ dissolved in 300 ml. of water, to which a mixture of 800

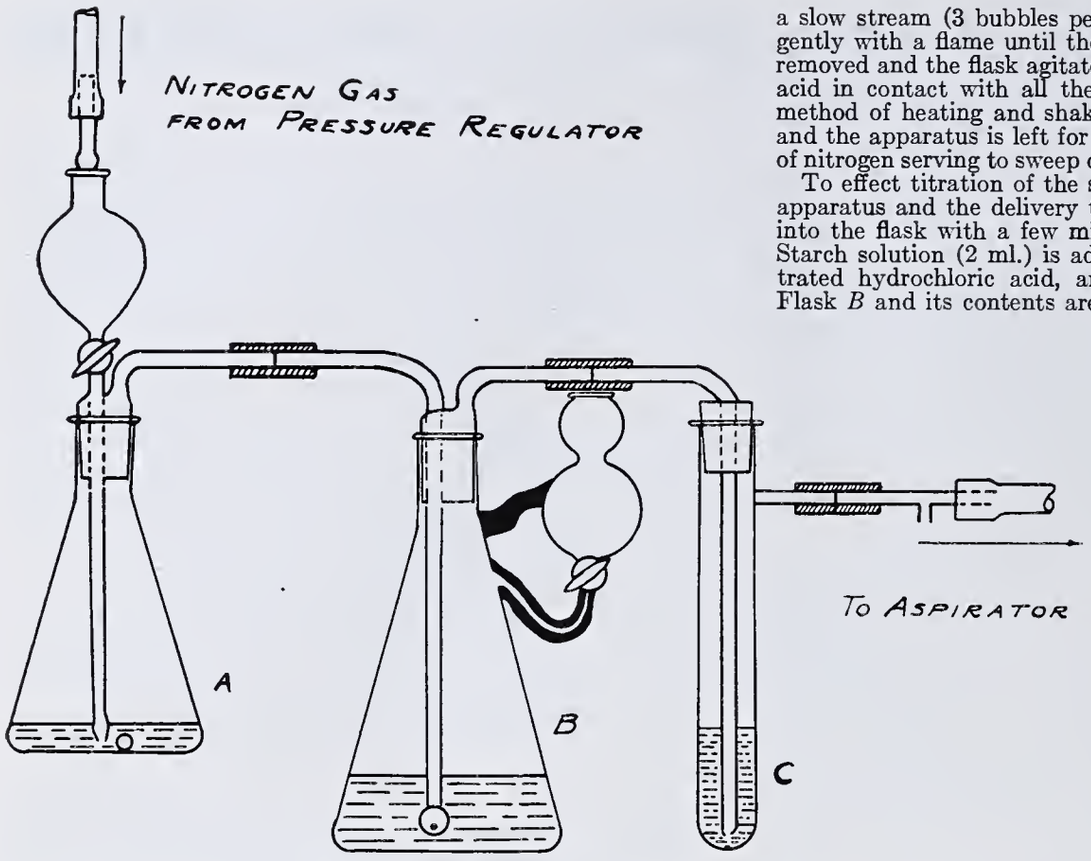

FIGURE 2. APPARATUS FOR LIBERATING AND DETERMINING HYDROGEN SULFIDE

A. 300-cc. Erlenmeyer flask equipped with 60-cc. separatory funnel through ground-glass stopper
B. Special flask (ground-glass joint) for absorption and titration. Attached 25-cc. separatory funnel is joined at its lower end to flask by 2-mm. (inside diameter) glass tubing
C. Test tube (trap)

ml. of water and 1200 ml. of concentrated ammonium hydroxide is added.

Standard iodine solution, 0.025 *N;* 1 per cent aqueous starch solution; nitrogen (low purity); acetone, C. P., free of uncombined sulfur; and benzene, C. P., free of uncombined sulfur.

Procedure

The sample is weighed into a 300-cc. flask (*A*, Figure 1), the amount taken depending upon the free sulfur content; 1 gram is usually satisfactory. Fifty milliliters of C. P. benzene are added, with agitation to dissolve the sample, and 100 ml. of C. P. acetone are added. (The use of acetone, employed by Hardman and Barbehenn, was retained because it appeared to exert a catalytic effect in the reaction between copper and sulfur.) A piece of prepared copper gauze is put in the flask and the solution is refluxed under a water-cooled condenser for 2 hours. The copper is then inspected (through the bottom of the flask) to detect discoloration. If there is no discoloration, the sample does not contain free sulfur. If there is darkening of the gauze, three additional clean pieces of gauze are added (without removing the first) and heating is repeated. If the newly added gauze is discolored, the addition of fresh gauze and heating are repeated until newly added copper is not discolored.

The liquid in the flask is removed through the filter stick as illustrated in Figure 1, and discarded. The residue in the flask is washed successively with benzene and acetone, the washings being removed through the same filter stick. The wad of glass wool is pushed from the filter stick into flask *A* by means of a stiff iron wire and the filter stick is added to the flask. The small amount of solvent adhering to the flask is removed by warming on a steam bath while introducing a gentle stream of nitrogen, and the flask is then connected to the evolution apparatus (Figure 2, *A*).

Thirty milliliters of cadmium chloride stock solution are diluted with distilled water to 160 ml. and 135 ml. of this solution are placed in flask *B* (Figure 2), the remainder in test tube *C*. After 30 ml. of concentrated hydrochloric acid are added to the separatory funnel over flask *A* and all connections are made secure, nitrogen gas under low pressure (approximately 75 mm. of mercury) is allowed to pass into the separatory funnel and displace the acid. The stopcock of this separatory funnel is adjusted so that the gas continues to pass through the apparatus in a slow stream (3 bubbles per second). Flask *A* is then heated gently with a flame until the acid begins to boil. The flame is removed and the flask agitated for about 0.5 minute to bring the acid in contact with all the copper sulfide in the flask. This method of heating and shaking is repeated three or four times and the apparatus is left for 10 minutes, the continuous passage of nitrogen serving to sweep out the hydrogen sulfide.

To effect titration of the sulfide, flask *B* is removed from the apparatus and the delivery tube, attached to its neck, is rinsed into the flask with a few milliliters of dilute hydrochloric acid. Starch solution (2 ml.) is added, followed by 30 ml. of concentrated hydrochloric acid, and the flask is quickly stoppered. Flask *B* and its contents are cooled by tap water, the stopcock of the attached separatory funnel being opened momentarily to equalize pressure, and 20 ml. of dilute cadmium chloride solution (stock solution diluted with one volume of water) are added to the separatory funnel. The stopper on flask *B* is removed and a buret, with a rubber stopper attached to its tip, is promptly inserted to make a tight connection at the neck of the flask. The stopcock of the separatory funnel is then opened and standard iodine solution is added from the buret while the flask is agitated. The gas displaced by the iodine solution passes through the separatory funnel where hydrogen sulfide is trapped.

When the solution in the flask turns blue, the addition of iodine is discontinued and the stopper attached to the buret is loosened to permit the solution from the separatory funnel to enter the flask; the additional liberation of hydrogen sulfide commonly discharges the blue color. The stopper of the buret is again tightly inserted into the flask and the solution in test tube *C* is introduced into the separatory funnel of flask *B*. The titration is continued to the blue end point and the separatory funnel is drained as before. Finally the test tube is rinsed with dilute hydrochloric acid and water, the rinsings are added to flask *B* through the separatory funnel, and iodine reagent is added dropwise to the usual blue end point.

A blank test is run on the reagents and its titer is subtracted from that of the sample.

TABLE I. ANALYSIS OF SYNTHETIC SAMPLES

Oil Present	Sulfur Added	Sulfur Found	Free Sulfur Content Calculated	Free Sulfur Content Found
Mg.	*Mg.*	*Mg.*	%	%
1000	2.25	2.40	0.22	0.24
1000	2.25	2.35	0.22	0.23
1000	5.63	5.74	0.56	0.57
1000	5.63	5.71	0.56	0.57
1000	11.27	11.34	1.11	1.12
1000	11.27	11.45	1.11	1.13

Calculation

1 ml. of 0.025 *N* iodine ≏ 0.4 mg. of S

$$\text{Free sulfur } \% = \frac{(A - B) \times 0.4 \times 100}{\text{wt. of sample in mg.}}$$

A = ml. of iodine required for sample
B = ml. of iodine required for blank

Where mere qualitative information or confirmation is desired, the darkened copper gauze is rinsed with benzene and acetone to free it of oil, covered with 1 to 1 hydrochloric acid, and heated in a test tube, and the vapors are tested for hydrogen sulfide with lead acetate paper in the usual manner.

Sample knowns were made by adding a benzene solution of elementary sulfur to 1 gram of oil known to be free of un-

Table II. Analyses of Commercial Products

Sample	Total Sulfur (Bomb Method) %	Free Sulfur (Proposed Method) %
Topped Mexican crude[a]	4.0	0.00
Venezuela crude[a]	2.0	0.00
Special naphthenic Diesel lubricating oil SAE 30[a]	2.18	0.00
Special naphthenic Diesel lubricating oil SAE 40[a]	1.90	0.00
Special naphthenic motor oil[a]	2.42	0.00
Special cutting oil A	3.08	0.97–1.01
Special cutting oil B	2.06	1.09–1.11
Sulfurized gear oil A	1.30	0.56–0.58
Sulfurized gear oil B	2.25	1.27–1.33
Sulfurized mineral oil	1.03	0.52–0.56
Sulfurized fatty oil	10.01	0.00

[a] These products are possessed of their high total sulfur content, naturally.

combined sulfur. The results of typical analyses are given in Table I.

A variety of commercial petroleum products of relatively high total sulfur content were analyzed and consistent results for free sulfur obtained. Typical of these are the results in Table II.

The method has been used for over two years and proved suitable for routine testing. As little as 0.001 per cent of free sulfur has been determined with it. Investigation of the utility of the method for oils containing added soaps, halogenated materials, etc., has not been completed. However, tests made with mineral oils containing either 5 per cent of lead naphthenate, 5 per cent of lead linoleate, unsaturated acids (5 per cent oleic acid), unsaturated hydrocarbons (30 per cent pine oil), or large amounts of oxidized oils from engine tests or laboratory oxidation tests (contain peroxides) showed no significant interference by these substances.

Acknowledgment

The authors acknowledge their appreciation of the assistance of F. C. Gast, L. Donn, and Karl Uhrig in the development and testing of this method.

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Presented before the Division of Petroleum Chemistry at the 102nd Meeting of the American Chemical Society, Atlantic City, N. J.

Mercury Cathode Method for Determining Small Amounts of Titanium and Similar Metals in Alloys

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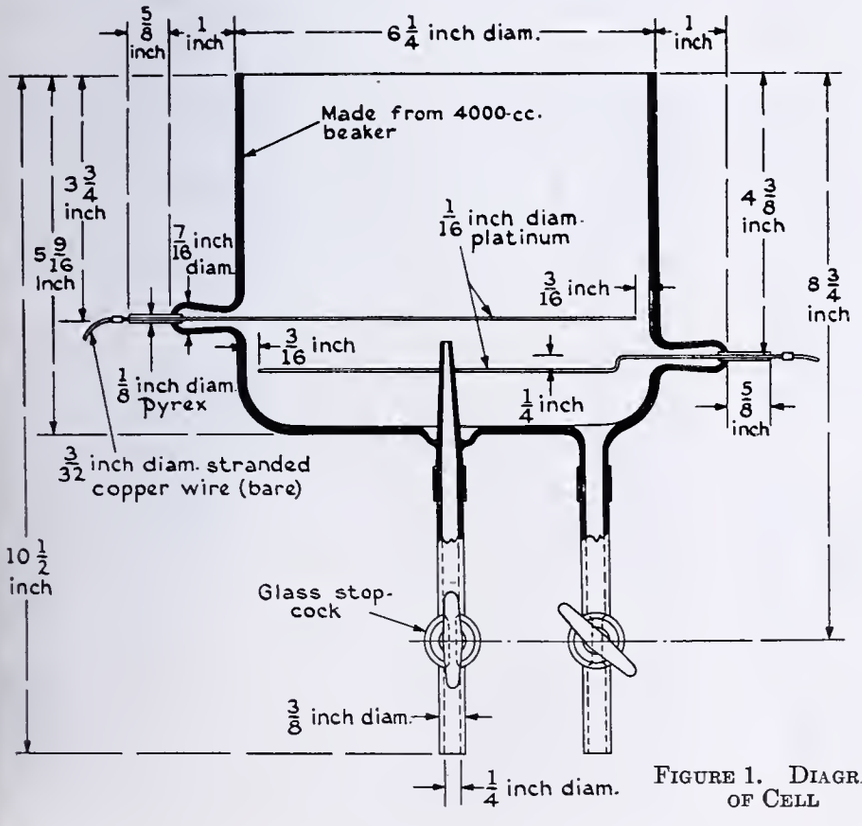

Figure 1. Diagram of Cell

THE mercury cathode method of analysis as described by Lundell, Hoffman, and Bright (*1*, *2*) has been used in this laboratory for many years. When using an average size cell and charge (0.5 gram) it takes overnight (14 hours) before all the amalgam-forming elements are deposited in the mercury.

To shorten time, when only small amounts or traces of titanium, aluminum, magnesium, or vanadium are present, a larger cell (Figure 1) has been used whereby a larger charge (5.0 gram) may be used and time shortened to 5 hours. For the larger cell, 2 to 3 amperes and 10 volts are used, as contrasted with the smaller cell using 1 ampere and 5 volts. In some cases where amounts of titanium, etc., are very small, two or three 5-gram charges are run through the cell, the electrolyzed solutions are combined, and analysis is carried out by usual methods.

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Determination of Lead in Sugars and Sugar Products

An Adaptation of the Diphenylthiocarbazone Reaction

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A VERY short and convenient dithizone method for the determination of lead in sugars and sugar products has been developed. This is accomplished without ashing the sugar product, without the use of a centrifuge for separating the aqueous and chloroform layers, and without the use of a shaking machine for dissolving and extracting samples. Only one concentration of dithizone is necessary for the whole procedure, including final colorimetric comparison and qualitative tests on water and reagents.

With the exception of lead nitrate, which should be recrystallized prior to use in preparation of standard lead solution, the reagents, including Eastman Kodak Company reagent grade dithizone, need no purification, as the blank is sufficiently low for ordinary testing. For analytical research, however, dithizone and other reagents may be purified according to methods of the Association of Official Agricultural Chemists (*1*, *2*).

The simple, inexpensive equipment required consists of a few burets, separatory funnels, Nessler tubes, Pyrex flasks and bottles, a vacuum pipet for taking aliquots of chloroform solutions, and a Pyrex still for making lead-free water.

A discussion of the theory of the diphenylthiocarbazone reaction and its application, as well as a detailed description of the procedure, is presented in this paper.

Many discussions of the dithizone reaction may be found in the literature. Particularly interesting are those relating to the examination of pharmaceutical chemicals (*6*), biological materials (*5*), soils (*7*), maple sirup (*8*), and foods (*1*); to experimental methods used in the study of the role of heavy metals in plant nutrition (*10*); and to a method used in delicate control of lead in cancer research (*9*).

Diphenylthiocarbazone Reaction

Diphenylthiocarbazone is phenylazothioformic acid phenylhydrazide, abbreviated in the literature to "dithizone". This highly colored organic compound produces a brilliant green color when dissolved in chloroform and combines with twelve or more metals, forming complexes which are violet, yellow, or red. These colors, when mixed with the green of uncombined dithizone, are changed to shades of green, violet, blue, and red, depending upon the concentration of the complexes formed and the concentration of the unused dithizone in the chloroform solution. The principal metals which form these complexes with dithizone are copper, silver, mercury, gold, zinc, cobalt, nickel, cadmium, stannous tin, lead, bismuth, and thallium.

Suppose a dilute solution (50 to 100 ml., containing 50 micrograms or less) of the chlorides or nitrates of any or all of these metals is placed in a separatory funnel and a dilute solution of dithizone in chloroform (10 ml. of a concentration of the order of 15 mg. per liter) is added. Dithizone is not specific, but under the proper conditions of hydrogen-ion concentration in the aqueous phase, these metals may be separated into groups. If the aqueous phase is made alkaline with ammonia (pH 8.0 to 11.0) and the separatory funnel is shaken vigorously, a group of the metals will pass from the aqueous phase to the chloroform phase and form colored complexes with the dithizone. After standing for a few minutes, the layers will separate and the colored dithizone layer can be drawn off into a suitable vessel. If another portion of the dithizone is added and the separatory funnel is shaken again, more metals are extracted, but this extract does not contain as high a proportion of colored complexes as the first. This process of extractive enrichment may be repeated until the dithizone layer remains pure green, an indication that all the metals which form complexes at that pH have been extracted.

If, in addition to making the aqueous phase alkaline (pH 8.0 to 11.0), we add potassium cyanide, complexes of all the metals of the alkaline group except lead, stannous tin, bismuth, and thallium will be formed with the potassium cyanide. These complexes will not react with dithizone. If the solution is then subjected to extractive enrichment with dithizone, the combined extracts will contain only lead, stannous tin, bismuth, and thallium.

The acid solution of these metals can be adjusted to the optimum pH at which bismuth and stannous tin react with dithizone and the dithizone extraction repeated for their removal. The remaining metals in the group are thallium and lead. Thallium is rare and its presence in ordinary work is unlikely (*3*). The solution containing lead can then be readjusted to pH 8.0 to 11.0, potassium cyanide added, and the lead isolated as a dithizone complex by extractive enrichment, as before.

The lead in the combined dithizone extracts is transferred to dilute acid of definite volume and concentration by shaking in a separatory funnel. The dithizone is drawn off and discarded. The acid-aqueous layer is adjusted to a definite hydrogen-ion concentration and is a mixture of ammonia, citric acid, and potassium cyanide of definite volume and concentration added for the purpose of forming fixation or competitive complexes, as before. Dithizone of a definite volume and concentration is then added, the mixture is shaken vigorously, and the dithizone is drawn off into a Nessler tube. A set of standards is prepared by using the technique described above, with known quantities of a standard lead solution. The standards are arranged in order in Nessler tubes and the lead content of the unknown is determined by matching its color with a standard.

Development of a Method Applicable to Sugar Products

A number of problems required solution before the dithizone reaction could be applied directly to sugar products. The method of the Association of Official Agricultural Chemists for lead in foods (*1*) is a long process, but results obtained by it must be assumed to approximate the truth very closely. It was the purpose of this investigation to develop a very short and convenient method which could be applied directly to the sugar product and at the same time produce correct results.

Solution of the Sample. Hydrochloric and citric acids at room temperature are used for dissolving the sample. Nitric acid cannot be used in a sugar solution because active substances are formed which combine with potassium cyanide and prevent the cyanide from forming complexes with zinc (*8*), which are necessary to prevent extraction of zinc by dithizone. A convenient concentration of hydrochloric acid for the solution of the sample is that used in the colorimetric comparison. This concentration (180 ml. of concentrated hydrochloric acid per liter) is used by Perlman (*8*) in the preparation of color standards. It has been found that all the lead in a 10-gram sample of a sugar product will be dissolved when agitated for 5 minutes in the cold with a mixture of 15 ml. of hydrochloric acid of this concentration, 10 ml. of 50 per cent citric acid, and 25 ml. of water.

Prevention of Precipitation of Ammonium Hydroxide Group. Before extraction with dithizone, the solution must be made alkaline with ammonia. Under ordinary circumstances, the iron and aluminum group, phosphates, and the alkaline earth group (calcium) would be precipitated; this precipitate would occlude lead and thus prevent its extraction with dithizone. Be-

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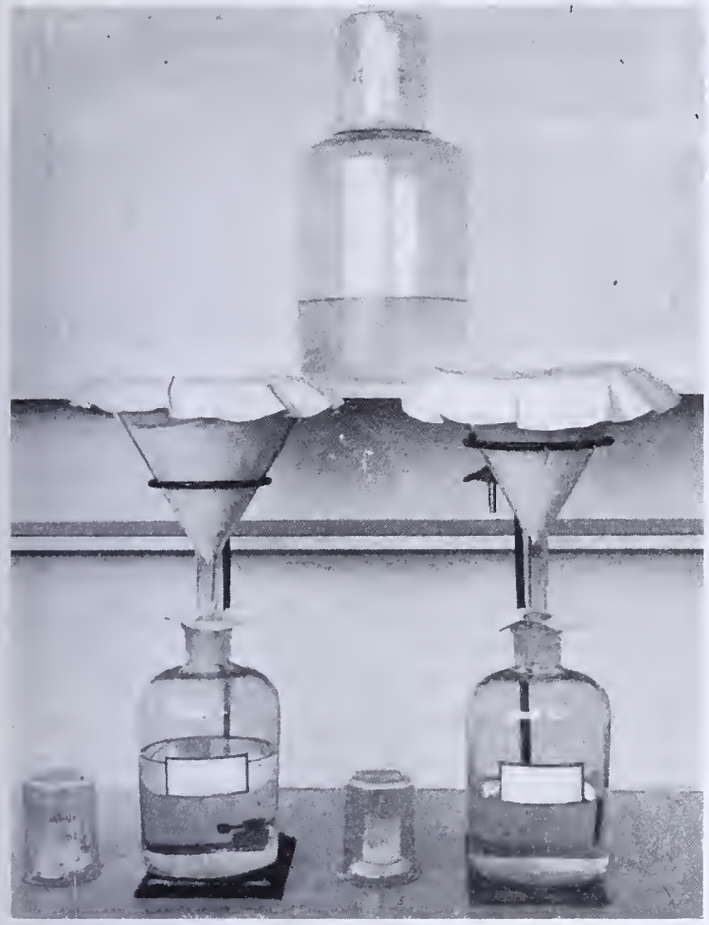


EQUIPMENT FOR PREPARATION OF LEAD-FREE WATER

cause dithizone reacts only with metals in solution, the solution must be perfectly clear after it is made alkaline with ammonia. The 10 ml. of citric acid added with the hydrochloric acid for the solution of the substance accomplishes this.

OPTIMUM pH. The optimum pH for dithizone extraction of lead from a sugar solution was found to be 9.5.

AMMONIUM HYDROXIDE REQUIRED FOR pH ADJUSTMENT. From 10 to 13 ml. of concentrated ammonium hydroxide are required for pH adjustment, depending upon the concentrations of the hydrochloric and citric acids and of the ammonium hydroxide. The presence of the 5- to 10- gram sample normally has little effect. The concentrations of the acids approximate the values given under "Solution of the Sample" and the ammonium hydroxide loses ammonia upon standing. The amount of ammonium hydroxide required for adjusting the pH to 9.5 must, therefore, be determined periodically.

POTASSIUM CYANIDE REQUIRED. For normal samples of sugar products, in the quantities used in this analysis, the presence of 1 gram of potassium cyanide is sufficient for the formation of competitive complexes. These complexes are formed with all the metals of the alkaline group except lead, bismuth, stannous tin, and thallium. Therefore, 5 ml. of a solution of potassium cyanide (20 grams per 100 ml.) are added before the dithizone extraction. The extract can contain only lead, bismuth, stannous tin, and thallium.

BISMUTH, STANNOUS TIN, AND THALLIUM. Tests made on sugar products by the method of the Association of Official Agricultural Chemists (*4*) show the absence of bismuth and stannous tin. Stannic tin is present in minute quantities in sugar products but it will not react with dithizone under the conditions given above. Thallium is rare and its presence in ordinary work is unlikely (*3*). Under the conditions outlined, the dithizone extract will contain lead only.

DITHIZONE CONCENTRATION AND VOLUME. When employing the usual technique for extracting a solute from a solution by means of an immiscible solvent, a partition of the solute between the two solvents nearly always exists and this makes it necessary to extract the solution many times with small quantities of the immiscible solvent to make the separation complete (*7*). This process is called "extractive enrichment". The chloroform layer settles out perfectly after each successive extraction when there is no sugar present in the aqueous layer. When sugar is present, and the solution has been agitated with a small volume of dithizone, the use of a centrifuge is necessary for breaking up the emulsion and separating the layers. It often requires 30 minutes' centrifuging to accomplish this. Extractive enrichment under these conditions would be very impractical, if not impossible. The problem becomes one of completely extracting the lead from a sugar solution under any conditions.

A technique has been devised in which this is accomplished with a single dithizone extraction and without the use of a centrifuge for separating the aqueous and chloroform layers. A large volume of dilute dithizone is used to extract a small quantity of lead from a comparatively small volume of solution; 15 micrograms or less of lead, in the presence of 10 grams of sugar in 75 ml. of solution, under the proper conditions of hydrogen-ion concentration, etc., will be completely extracted when shaken with 125 ml. of a chloroform solution of dithizone of a concentration of 7.5 mg. per liter.

SEPARATION OF AQUEOUS AND CHLOROFORM LAYERS. The extraction is made in a 500-ml. volumetric flask. The layers separate upon standing for 10 minutes. Centrifuging is not necessary.

TAKING AN ALIQUOT FROM THE CHLOROFORM-DITHIZONE LAYER. A 75-ml. aliquot is taken from the dithizone layer on the bottom of the flask by connecting the pipet to a source of vacuum. The vacuum pipet is described in detail below.

Preparation of Reagents

LEAD-FREE WATER. Redistill ordinary distilled water in an all-Pyrex glass still, or dissolve 4 grams of disodium phosphate in 12 liters of ordinary distilled water in a large Pyrex bottle, add 4 grams of calcium carbonate, and stir thoroughly. Let it settle overnight, and filter into 4-liter bottles with standard taper stoppers. The filter papers should be perfectly clean and about 45 cm. (15 inches) in diameter, and should cover the funnels. Test each bottle of prepared water for lead.

The author is indebted to P. R. Stout of the University of California for this method of preparing lead-free water.

HYDROCHLORIC ACID REAGENT. To 180 ml. of hydrochloric acid in a liter flask, add 300 to 400 ml. of lead-free water, cool to room temperature, and make up to 1 liter with lead-free water.

CITRIC ACID REAGENT. Heat 500 grams of citric acid in a minimum quantity of lead-free water in a beaker. After it has dissolved, cool and make up to 1 liter with lead-free water in a volumetric flask.

AMMONIUM HYDROXIDE REAGENT, concentrated ammonium hydroxide, 28 per cent.

POTASSIUM CYANIDE REAGENT. Dissolve 100 grams of potassium cyanide in lead-free water and make up to 500 ml. in a volumetric flask.

DITHIZONE REAGENT. Dissolve 7.5 mg. of diphenylthiocarbazone in 1 liter of chloroform.

AMMONIA-CYANIDE-CITRATE REAGENT. Dissolve 10 grams of citric acid in lead-free water, add 500 ml. of ammonium hydroxide, dissolve 20 grams of potassium cyanide in lead-free water, and add it to the ammoniacal solution in a volumetric flask. Make up to 1 liter with lead-free water.

LEAD NITRATE STOCK SOLUTION. Recrystallize from 5 to 10 grams of lead nitrate by evaporating an aqueous solution on the water bath. Filter and dry the crystals at 100° to constant weight. Preserve the product in a small, tightly capped reagent bottle. Weigh very accurately 3.197 grams of the purified salt, dissolve it in dilute nitric acid (0.1 to 1.0 per cent), and make up to 1 liter with lead-free water in an accurately calibrated volumetric flask; 1 ml. of this solution will contain 0.002 gram of lead.

STANDARD LEAD SOLUTION. Using accurately calibrated volumetric equipment, dilute 1.5 ml. of lead nitrate stock solution to 2000 ml. with hydrochloric acid reagent; 1 ml. of this solution will contain 1.5 micrograms of lead. This solution is used in the preparation of color standards.

Apparatus Required

The vacuum pipet consists simply of a convenient means of making and breaking connection between a vacuum line and a pipet, and of making and breaking connection between a

source of lead-free water and the pipet. The connections must be made of material which will cause no lead contamination in the pipet, as shown by a negative test for lead made on water which has passed through the connections and the pipet. By means of suitable clamps, two pipets, 75-ml. and 10-ml., are held rigidly in place at the proper distance above a sink to allow 500-ml. volumetric flasks and 125-ml. separatory funnels to be held under the tips.

Volumetric flasks, 500 ml., are required. The 500-ml. graduation marks are not used in the lead determinations. The neck of each flask must be of such length that the tip of a 75-ml. pipet will be 3 to 4 mm. from the bottom of the flask when taking an aliquot from the chloroform layer.

Separatory funnels, Nessler tubes, an all-Pyrex glass still, Pyrex bottles (one 12-liter and several of 4-liter capacity), and two or more 25 cm. (10-inch) glass funnels are also used. The Pyrex bottles and funnels are used in the preparation of lead-free water when an all-Pyrex glass still is not available.

Isolation and Determination of Lead

Weigh 5.5 to 10.0 grams of the sugar product in a 50-ml. Pyrex beaker, and measure 25 ml. of lead-free water into another 50-ml. beaker from a buret of large capacity. By transferring water portionwise from this beaker to the one containing the sample, wash the sugar or sirup into a 500-ml. Pyrex volumetric flask, and add 15 ml. of hydrochloric acid reagent and 10 ml. of citric acid reagent, accurately measured from burets. Rotate the flask for 5 minutes, and add the quantity of concentrated ammonium hydroxide (28 per cent) necessary for adjusting the pH to 9.5, determined periodically as described later.

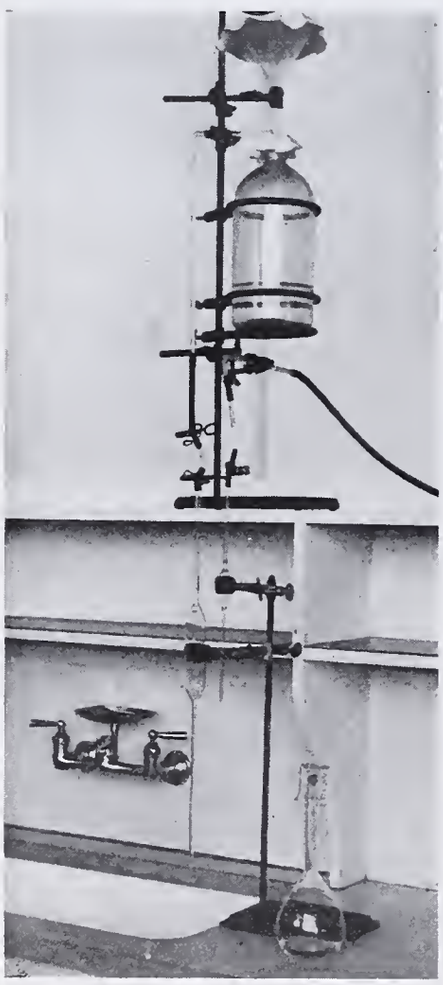
Place the flask in a cooling bath and allow it to remain for 5 minutes, agitating the contents occasionally while cooling. Remove it from the bath and add immediately 5 ml. of potassium cyanide solution and 125 ml. of dithizone. Without stoppering the flask, agitate the contents vigorously for 2 minutes, and let it stand for 10 minutes. Place the vacuum pipet in the flask so that the tip will be very close to the bottom, adjust the vacuum so that a minimum of disturbance will result, and draw out a 75-ml. aliquot of the chloroform-dithizone layer from the bottom of the flask. Allow the dithizone extract to run into a 125-ml. separatory funnel containing exactly 11 ml. of hydrochloric acid reagent. Connect the pipet to a source of lead-free water and rinse immediately with sufficient water to make it free from lead. Stopper the separatory funnel and shake vigorously for 1 minute. Let stand for 10 minutes. Draw off the chloroform-dithizone layer and discard; it may be run into a container for waste dithizone and the chloroform reclaimed later by distillation.

The lead is contained in the acid-aqueous layer remaining in the separatory funnel. With the vacuum pipet, draw out a 10-ml. aliquot of the acid solution and allow it to run into a 100-ml. Nessler tube. Connect the 10-ml. pipet to the source of lead-free water and rinse immediately with sufficient water to make it free from lead. To the solution in the Nessler tube add exactly 10 ml. of ammonia-cyanide-citrate reagent, mix well by rotating the tube, and allow it to stand for a few minutes to cool. Add exactly 10 ml. of dithizone, place a cork stopper in the tube, and shake for 1 minute. Remove the stopper and cover the tube with a 50-ml. beaker. Rinse the stopper with 200 ml., or more, of lead-free water.

Let the Nessler tube stand until the dithizone layer has settled. This layer will be colored green, blue, purple, red, or pink by the lead-dithizone complex and the excess dithizone. Compare the tube to a set of standards containing known quantities of lead, arranged in order from 0 to 15 micrograms. A convenient interval between them is 1.5 micrograms. This interval may be divided into three parts by estimation. If the exact quantity cannot be determined in this way, one or more extra standards may be prepared by measuring out the appropriate volumes of standard lead solution, which fall within this interval, into Nessler tubes and proceeding as directed under "Preparation of Standards". The lead content of extracts must be kept within the range of 0 to 15 micrograms by adjusting the quantity of the sample taken for extraction. If the color of an extract is found to match the standard which represents 15 micrograms of lead (the highest in this range), it is advisable to repeat the determination, using a smaller sample. The extract of the smaller sample will match a standard having a smaller lead content and all possibility of exceeding the range, without detection, will have been eliminated.

Make a blank determination on water and reagents, following the procedure given above, but substituting from 5 to 10 ml. of water for the sample of the sugar product.

Having determined the number of micrograms of lead contained in the extract, deduct the quantity found in the blank and calculate to parts per million in the sample. For example: The sample weighed into the volumetric flask was 9.52 grams. In the colorimetric procedure it was found that, after deducting the equivalent in milliliters of standard lead solution found in the



Vacuum Pipet

Burets, Reagents, and Extraction Flasks for Dithizone Colorimetric Procedure

blank determination, the sample contained the quantity of lead which is equivalent to 3.0 ml. of that solution:

$$3.0 \times 1.5 = 4.5 \text{ micrograms} = 0.0000045 \text{ gram of lead}$$

Two aliquots were taken during the analysis. Considering these ratios, the weight of the sample represented in the final colorimetric comparison was

$$\frac{75}{125} \times \frac{10}{11} \times 9.52 = 5.19 \text{ grams}$$

Then the sample contains

$$\frac{0.0000045 \times 1{,}000{,}000}{5.19} = 0.86 \text{ or } 0.9 \text{ part per million of lead}$$

Lead is eliminated from glassware by rinsing about six times with lead-free water, occasionally using dilute nitric acid before using water. Allow a small quantity of the water from the last rinsing to remain in each vessel. Pour the water from a dozen or more vessels into a large separatory funnel and make a qualitative test for lead. The hands of the operator may be immersed in 0.1 per cent nitric acid and rinsed with lead-free water as a precautionary measure. Rinse cork stoppers used in Nessler tubes with about 200 ml. of lead-free water immediately after use, and cover with beakers after rinsing. Before using new cork stoppers, shake them in a bottle with dithizone and dilute ammonia.

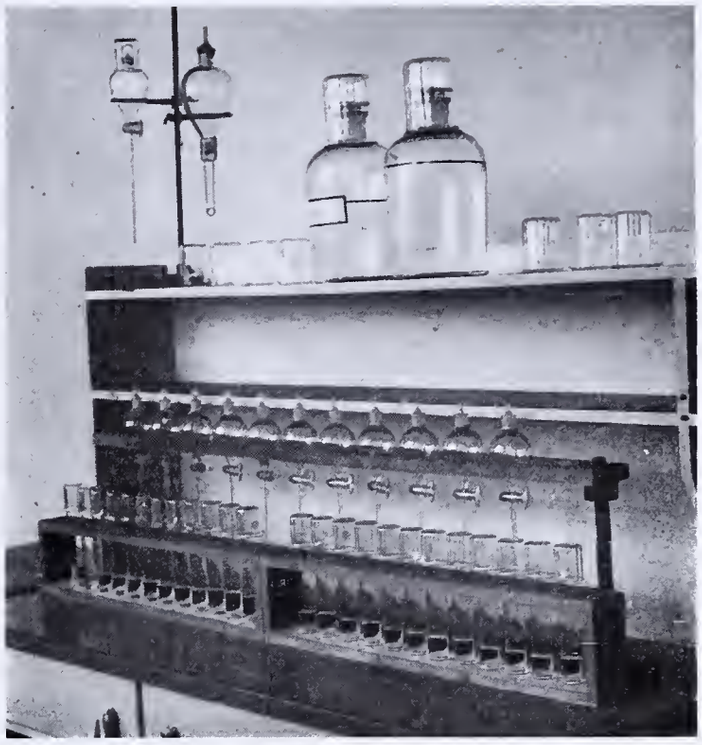
Dithizone extractions are made in three different types of containers—separatory funnels, Nessler tubes, and 500-ml. flasks. The usual technique of agitation for separatory funnels must be varied for extractions made in Nessler tubes and flasks. The appearance of the emulsion in a separatory funnel is noted while shaking dithizone with hydrochloric acid reagent, and Nessler tubes and 500-ml. flasks are agitated in a manner which will cause this same appearance of completeness in the emulsion. Nessler tubes will require a more gentle agitation and flasks will require a more vigorous shaking than separatory funnels to produce this result. Regardless of the type of vessel, the agitation should never be more vigorous than necessary to produce this appearance of completeness in the emulsion. The quantity of nonsugars should be kept to a minimum in the first extraction by using smaller samples for the analysis of products of very low purity. If the layers in a Nessler tube do not separate, the entire contents of the tube may be filtered into another tube through a pledget of cotton in a small funnel, and the clear filtrate compared with the standards. Permanence in the emulsion will rarely occur under the conditions outlined above. Filtration will not impair the accuracy of the test if the apparatus has been rinsed until free from lead.

Determination of Quantity of Ammonium Hydroxide Required for Adjustment of pH for Dithizone Extraction

Mix 30 to 35 ml. of lead-free water with exactly 15 ml. of hydrochloric acid reagent and exactly 10 ml. of citric acid reagent in a 100-ml. beaker. Add 9 ml. of concentrated ammonium hydroxide, cool in a water bath to about 20°, add 5 ml. of potassium cyanide solution, and determine the pH with a pH meter. Add ammonium hydroxide in quantities of about 1 ml. until the pH is 9.5. Note the number of milliliters required. Make this determination periodically, whenever new reagents (hydrochloric acid, citric acid, or potassium cyanide) are prepared, or a new bottle of ammonium hydroxide is used.

When determining lead in samples of sugar products whose composition is abnormal (old samples, damaged samples, sirups which have been subjected to some special treatment for experimental purposes, etc.) this procedure must be modified and a determination made for each abnormal sample.

Dissolve 5 to 10 grams of the sample in 25 ml. of lead-free water in a 100-ml. beaker, add exactly 15 ml. of hydrochloric acid reagent and exactly 10 ml. of citric acid reagent, and proceed as before.



Separatory Funnels and Nessler Tubes for Dithizone Colorimetric Procedure

Preparation of Standards

Arrange eleven Nessler tubes in a row and measure into them accurately, by means of calibrated burets, the following quantities of standard lead solution (1 ml. contains 0.0000015 gram of lead) and hydrochloric acid reagent:

Nessler tube number	1	2	3	4	5	6	7	8	9	10	11
Standard lead solution, ml.	0	1	2	3	4	5	6	7	8	9	10
Hydrochloric acid reagent, ml.	10	9	8	7	6	5	4	3	2	1	0

After mixing the contents by rotating the tubes, add exactly 10 ml. of ammonia-cyanide-citrate reagent to each tube. Mix well and allow them to stand until cool. Then add 10 ml. of dithizone, place cork stoppers in the tubes, and shake them for 1 minute. Remove the stoppers and cover with 50-ml. beakers. Wash each stopper with at least 200 ml. of lead-free water. Allow the Nessler tubes to stand until the dithizone layers have settled.

These layers will exhibit various shades from green in the first tube to pink in the last one, with intermediate blues, purples, and reds, depending upon the proportions of lead-dithizone complex and uncombined dithizone. The colors represent quantities of lead in the range of 0 to 15 micrograms and are arranged at intervals of 1.5 micrograms.

Qualitative Test for Lead

Water. Add 2 drops of citric acid to 30 to 50 ml. of water in a separatory funnel, make alkaline with ammonia, and then add 1 ml. of potassium cyanide. Mix well and add about 5 ml. of dithizone. Shake the separatory funnel vigorously and allow the layers to separate. A pink color in the chloroform layer shows the presence of lead. A green color or a mixed color containing green and red indicates that the excess dithizone has not been transferred from the chloroform phase to the aqueous phase. Add more water and ammonia or run the chloroform layer into another separatory funnel containing water and ammonia and potassium cyanide. In either instance, shake the separatory funnel vigorously and allow the layers to separate. A water-white chloroform layer is a negative test and a pink color in this layer is a positive test for lead.

Chloroform (new or reclaimed). To 50 ml. of chloroform in a separatory funnel add about 15 ml. of lead-free water, 2 drops of citric acid, and sufficient ammonia to make the aqueous phase alkaline. Add 1 ml. of potassium cyanide and about 10 ml. of dithizone, and shake vigorously. A pink color in the chloroform layer indicates the presence of lead. If a green color, due to ex-

Table I. Comparative Results

(A. Method of Association of Official Agricultural Chemists for lead in foods. B. Dithizone colorimetric procedure for lead in sugars and sugar products, described in this paper.)

Sample	Lead Found	
	A	B
	P. p. m.	*P. p. m.*
1	0.5	0.5
2	0.6	0.5
3	1.2	1.4
4	1.3	0.9
5	1.7	1.7
6	1.8	1.7
7	1.8	1.8
8	1.9	2.05
9	2.2	2.1
10	2.3	2.0
11	3.0	2.3
12	3.5	3.5
13	3.95	4.0
14	5.2	5.0

cess dithizone, is also present in the chloroform layer, remove it as in testing water.

Other Reagents. About 20 ml. of the reagent are treated in the same manner as a sample of water, when attempting to locate a source of contamination in quantitative or qualitative determinations. In the dithizone colorimetric procedure the combined contamination of all the reagents simply appears as a minute quantity of lead in the blank determination and, unless the blank becomes higher than usual, it is not necessary to make qualitative tests on individual reagents.

Results obtained with this method (Table I) agree with those obtained with the method of the Association of Official Agricultural Chemists for lead in foods (*1*). The accuracy of results obtained with the new procedure depends, to a very great extent, upon ability to match colors, upon ability and willingness to dispense exact quantities of solutions from burets, and upon the exclusive use of glassware which has been found by qualitative tests to be free from lead.

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Determination of Zinc in Rubber Compounds

A New Internal Indicator Method

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A SURVEY of the chemical literature reveals very little information regarding the determination of zinc in rubber compositions. Some of the earlier standard procedures involve the use of the gravimetric sulfide method, but the only direct references to the use of ferrocyanide as a volumetric precipitant with an external indicator applied to the analysis of rubber compounds are those of the A. S. T. M. (*1*) and Casazza (*3*). The A. S. T. M. method is used in the rubber industry with various modifications, and, in the absence of evidence to the contrary, is assumed to be the only volumetric method in general use for the determination of zinc.

The difficulty of manipulation and the low degree of accuracy of the external indicator procedure led to the consideration of a new method for this determination. The principal internal indicator methods for the determination of zinc have been considered by Kolthoff and Furman (*7*). One method employs ferrocyanide as precipitant and ferrous iron as an indicator and the other, based on the work of Cone and Cady (*4*) supplemented by that of Kolthoff (*6*), utilizes the ferricyanide-ferrocyanide oxidation-reduction system in equilibrium with precipitated zinc potassium ferrocyanide, using diphenylamine or diphenylbenzidine as indicator. With the latter method as a basis the experimental work for the development of a procedure suitable for the analysis of all types of rubber compounds was undertaken.

Experimental

With some modifications the diphenylbenzidine indicator method proved satisfactory in the presence of the ions usually found in an acid solution of the ash obtained from rubber compounds. It was also successful with rubber compounds of certain simple types, if the ash was treated in such a manner as to remove all oxidizing agents, reducing agents, and iron, if chlorides were present in only small quantities, and if there were no more than 10 grams of ammonium salts present in the solution to be titrated.

However, when this method was applied to the analysis of rubber stocks containing clay or other silicates, the zinc found was from 10 to 30 per cent low. This observation apparently has not been reported in the literature. Investigation of this phenomenon revealed that it occurs with any method of analysis which involves ashing as a preliminary step because of a reaction between zinc oxide and silicates which occurs even at the lowest possible ashing temperature. The reaction product is not decomposed by ordinary acid treatment and even prolonged acid digestion gives results 5 to 10 per cent low. The presence of lead oxide also causes low and inconsistent results, but the reasons for this effect have not been investigated yet.

Because of these phenomena it became necessary to resort either to fusion or to wet oxidation (digestion) in order to find a generally applicable method for the preliminary destruction of the sample. The latter method is the more practical. Concentrated nitric acid, bromine, and fuming nitric acid used successively will oxidize most of the organic material in a rubber compound. The best reagent for oxidizing the carbon black and organic matter remaining in the residue is perchloric acid, and when its addition is preceded by the above oxidizing agents this reagent is safe to use. Unfortunately, even small quantities of perchlorates had a

detrimental effect on the color change and position of the end point when diphenylamine or diphenylbenzidine was used as indicator. Since no way of overcoming this effect was found, it became necessary to discontinue the use of these indicators in connection with wet-oxidation methods of determination.

Because of these facts it was necessary to devise a new method suitable for determining the zinc after wet oxidation of the sample. Gravimetric methods were discarded as being impractical. Precipitation methods, such as precipitation with anthranilic acid or 8-hydroxyquinoline followed by volumetric determination of the precipitate, were discarded as being time-consuming and none too accurate in solutions containing as many ions and added salts as are inevitably present. The problem centered on finding a suitable indicator to be used with the ferricyanide-ferrocyanide system in the manner of diphenylbenzidine.

Only indicators from stock which were thought to possess oxidation potentials in the neighborhood of +0.75 volt were tried. The results obtained indicated that the potential of a suitable indicator must be no higher than +0.75 volt and probably not much lower. The compounds which proved to be unsatisfactory in color development or in reproducibility of end point were *p*-phenetidine, benzidine acetate, *N*-phenylanthranilic acid, *p*-aminodiphenylamine, 2,4-diaminodiphenylamine, diphenylcarbazide, and two *p*-phenylenediamine derivatives. All these indicators were tried under varying conditions of temperature and acidity, both by direct and back-titration. *p*-Anisidine showed promise as an indicator but proved to give very high results. *o*-Tolidine gave satisfactory results but the color change, yellow through yellow-green to blue-green, was not sharp enough to ensure accuracy without considerable experience.

Schulek and Rózsa (*10*) described a new multiform indicator, *p*-ethoxychrysoidine, which presumably had the properties desired for this purpose, although the literature does not describe this use for it. A sample of this dye was prepared as described below and it was found to be an excellent indicator under at least two sets of conditions applicable to the analysis of rubber compounds.

Tananaev and Georgobiani (*11*) reported the use of "blue ethyl acidic RR(B)", 2-(*p*-ethylaminophenylazo)-1,8-dihydroxynaphthalene-3,5-disulfonic acid sodium salt, as an indicator for lead titration with ferrocyanide and suggested its use for the determination of other metals. According to Rowe's Colour Index (*9*), Ethyl Acid Blue RR(B) is dye No. 59, which is the sodium salt of *p*-dimethylaminobenzeneazo-1,8-dihydroxynaphthalene-4-sulfonic acid. This was available from Eimer & Amend, New York, as Azo Acid Blue B, originally obtained from G. Grubler and Co., Leipzig. This dye was not received until the work described below was completed, but it was then tried as a substitute indicator. The end point was not quite so sharp as with *p*-ethoxychrysoidine and results were somewhat erratic, but further work on conditions of operation might prove it to be a satisfactory indicator.

As a result of the experimental work two methods of analysis were devised and tested. Method I is the more general and is, with slight modification, applicable to all rubber compounds, vulcanized and unvulcanized, and to latices. Method II is applicable only to rubber stocks and latices free of lead, silicates, or other fusible ash, and containing but small amounts of acid-insoluble fillers.

Method I. Wet-Oxidation Method

Reagents and Solutions. *Indicator.* *p*-Ethoxychrysoidine (*p*-ethoxyphenylazo-*m*-phenylenediamine), 0.2 per cent in sulfuric acid (sp. gr. 1.84, diluted 1 to 1 with water). The indicator is prepared by coupling diazotized *p*-phenetidine hydrochloride with *m*-phenylenediamine. An analogous preparation for chrysoidine is described by Houben-Weyl (*5*). Recrystallization of the dye is unnecessary. Store the solution in the dark.

Standard Potassium Ferrocyanide Solution. Approximately 0.04 *M* solution of reagent grade potassium ferrocyanide trihydrate. If available, highly purified crystals may be used as a primary standard but the solution is not stable (cf. standardization).

Standard Zinc Chloride Solution. Zinc solution (0.06 *M*) made from zinc of known purity dissolved in hydrochloric acid, or impure zinc compound dissolved in acid and standardized against potassium ferrocyanide (cf. standardization).

Potassium Ferricyanide. One per cent of the reagent grade crystals dissolved in water. This solution is stable only if kept in the dark.

Nitric Acid–Bromine Mixture, reagent grade nitric acid (sp. gr. 1.42) saturated with bromine. Free bromine must be present.

Perchloric Acid, reagent grade, 60 or 72 per cent acid.

Sulfuric Acid, reagent grade (sp. gr. 1.84) diluted 1 to 1.

Ammonium Hydroxide, reagent grade (sp. gr. 0.90).

Wash Solution, 25 grams of ammonium sulfate and 10 ml. of ammonium hydroxide per liter.

Fuming Nitric Acid. The red fuming type is preferred. Reagent grade.

Procedure A (for vulcanized rubber compounds only). Place the ground sample of rubber (0.5 to 2.0 grams) in a 400- to 500-ml. wide-mouthed flask. Add 15 ml. of nitric acid–bromine mixture, cover the flask, and let stand for 10 minutes or longer. Add 10 ml. of red fuming nitric acid, replace the cover, and place on a steam plate or bath until the rubber is disintegrated. Transfer to an electric hot plate or a burner, remove the cover, and evaporate to 10 to 15 ml. Cool partially, add 10 ml. of perchloric acid, and evaporate until all carbon and organic material are destroyed.

(*Caution.* To ensure safety to the operator, the operations involving the oxidation of organic matter with perchloric acid should be done behind a safety screen. The evaporation of perchloric acid to fumes should be done under a hood whose flue is free from condensed organic vapors, organic dust, and carbon or soot.)

This operation requires a temperature of about 200° C. Continue evaporation to about 5 ml. Dense white fumes should be evolved and the hot solution may be a pale yellow color but should become colorless on cooling unless much iron is present. If lead is present add 3 ml. of sulfuric acid and evaporate until dense fumes again appear.

Dilute to 50 ml., boil for a minute, cool, neutralize with ammonium hydroxide, add 5 ml. in excess, and boil for one minute. Filter hot and wash with four 25-ml. portions of hot wash solution. For greater accuracy when there is a large flocculent precipitate, wash only twice with 20-ml. portions of wash solution, wash the precipitate into the original flask, add 5 ml. of sulfuric acid, digest, precipitate as before, filter, and wash the precipitate with four 20-ml. portions of wash solution. Evaporate the combined filtrates to 100 to 125 ml., making sure in every case that nearly all of the ammonia is boiled off to prevent the formation of excess ammonium salts. Add 5 ml. of sulfuric acid and titrate in the manner described below. The temperature must be 90° C. at the start of the titration and should not fall below 70° C.

Add 4 to 6 drops of indicator and 4 to 7 drops of ferricyanide solution, the latter always in amounts equal to or greater than the former. Shake until the red color changes to yellow and titrate with standard ferrocyanide solution until a strong salmon-pink color persists. Back-titrate with standard zinc solution to a pale green color. Continue "back and forth" titration (as few times as possible) until a few drops of zinc solution cause the color to fade through a neutral shade to a very pale apple green. The last few drops must be added slowly, with shaking between drops, since the precipitation equilibrium is reached slowly. Because of the equilibrium conditions the zinc must always be added last. When only small quantities of zinc are present the indicator may not fade readily upon addition of ferrocyanide. This may be remedied by adding several milliliters of zinc solution first.

Standardization. If the zinc solution is a primary standard, standardize the ferrocyanide with it according to the method just described, using a hot solution containing the zinc, 5 ml. of sulfuric acid, and 5 grams of ammonium sulfate per 100 ml. of solution. If the zinc solution is not standard, standardize the ferrocyanide with 0.1 *N* potassium permanganate according to the method of Kolthoff and Furman (*8*). This method specifies that about 1 gram of ferrocyanide in 120 ml. of 1 *N* sulfuric acid be titrated with permanganate and that a fresh, approximately 1 per cent ferricyanide solution be used for color comparison at the end point. The ferrocyanide solution may also be standardized against permanganate or ceric sulfate using *o*-phenanthroline-ferrous complex (ferroin) as indicator. The zinc solution may then be standardized against the ferrocyanide solution.

Procedure B (for latices and unvulcanized rubber stocks). Because of the difficulty of destroying unvulcanized rubber readily with nitric acid–bromine mixture to such an extent that the use of perchloric acid is safe, the following modification of Procedure A is necessary.

Add 20 ml. of nitric acid–bromine mixture to the sample of latex or thinly sheeted unvulcanized rubber compound and warm until vigorous reaction starts, keeping the flask covered. If overnight digestion is convenient, add 20 ml. of red fuming nitric acid after the first reaction has subsided, cover the flask, and allow it to stand overnight on a steam plate or bath. After this treatment perchloric acid may be added and the evaporation safely continued as in Procedure A. If the determination must be completed without interruption, add 10 ml. of red fuming nitric acid after the first reaction has subsided, heat gently until the rubber is disintegrated, and evaporate nearly to dryness. Add 10 ml. of red fuming nitric acid and 10 ml. of perchloric acid and evaporate as in Procedure A. Whichever method of digestion is used, complete the determination according to Procedure A. The actual rubber content of samples should not exceed 2 grams. Do not evaporate or coagulate latices before the oxidation.

Method II. Ashing Method

This method may be used for unvulcanized and vulcanized compounds and latices which contain no clay or lead, and have a comparatively low acid-insoluble ash.

Reagents. Same as in Method I where specified with the addition of reagent grade hydrochloric acid (sp. gr. 1.2), saturated bromine water, and 1 per cent diphenylbenzidine in concentrated sulfuric acid.

Procedure A (*p*-ethoxychrysoidine as indicator). Ash a sample of from 0.5 to 2.0 grams of rubber in a crucible in an overnight ashing muffle furnace which is capable of slowly ashing the sample without combustion at a maximum temperature of 550° C. Ashing may be accomplished over a burner if the sample is not allowed to flame.

Treat the ash with 5 ml. of hydrochloric acid and 5 ml. of sulfuric acid, warm if necessary to free the precipitate from the crucible, and transfer to a beaker. Dilute to 50 ml., boil to dissolve all the ash possible, add 5 ml. of bromine water, cool, neutralize with ammonium hydroxide, and continue the preparation of the solution and the determination exactly as in Method I, Procedure A, except that the solution must be boiled for at least 10 minutes after acidification before titration.

Procedure B (diphenylbenzidine as indicator). Up to the point of adding the indicator, follow Method II, Procedure A, exactly. In place of *p*-ethoxychrysoidine add 4 drops of diphenylbenzidine and 4 to 5 drops of ferricyanide solution. If a deep blue color does not develop, add sulfuric acid in 2-ml. portions until it does appear on shaking. Titrate with ferrocyanide solution until the blue fades markedly to a gray-green, stop titrating, and shake with the addition of small portions of sulfuric acid until a deep purple color appears; then continue dropwise titration to a yellow-green color. Back-titration is permissible but the ferrocyanide must be added last.

Discussion of Methods

Indicators. The use of *p*-ethoxychrysoidine may require some experience in determining the best ratios of indicator to ferricyanide. When there was much variation in this ratio from that specified in the procedure the accuracy was affected to a small extent, because the equilibrium between the ions from the precipitate, the ferrocyanide ions from reduced ferricyanide, and the excess ferrocyanide ions necessary to reduce the indicator is sensitive to variations in the quantities of indicator and ferricyanide. Because of this equilibrium and because of the greater sharpness of the end-point color change, the titration must be finished with the zinc solution.

Since the diphenylbenzidine method checked well with electrometric titration and also with the *p*-ethoxychrysoidine method in standardization titrations, Method II, Procedure B, may be used as a standard to check experimental work with *p*-ethoxychrysoidine. However, the author found that *p*-ethoxychrysoidine end points were better than those obtained with diphenylbenzidine because the latter indicator fades during titration and it is frequently difficult to bring the color back sufficiently to obtain a sharp end point.

In standardization with diphenylbenzidine indicator, 5 grams of ammonium sulfate and 5 ml. of sulfuric acid per 100 ml. of solution must be present. More sulfuric acid usually must be added during titration as is noted in Method II, Procedure B.

Neither indicator works well with less than 10 mg. of zinc but standard zinc solution may be added to the reaction mixture as soon as there is evidence of too little zinc in the solution.

The use of ferricyanide incorporated in the standard ferrocyanide solution as advocated by Kolthoff and Furman (*7*) was not satisfactory with either indicator.

Interferences. Oxidizing and reducing agents which will affect the ferricyanide-ferrocyanide system or the indicator will interfere with both methods, but all such substances are removed by the preliminary treatments. Manganese and copper will interfere, but they are not usually found in rubber compounds except those containing certain organic copper rubber dyes. Any other metals which form precipitates with ferrocyanide in strong acid solution will interfere, with the exception of lead which is successfully removed by Method I but not by Method II. Results of trial determinations of mixtures containing lead indicate that there should be no more than a slight positive error when using Method I, even if some lead sulfate is present at the time of titration. About 5 grams of chloride ion can be present in Method I and about 3 grams in Method II without impairing the end-point colors. Ammonium sulfate must be present for quantitative precipitation, but amounts greater than 10 grams tend to diminish the intensity of the end-point colors.

Iron must be removed if present to an extent great enough to cause a very noticeable blue color when ferrocyanide is added. A very small trace of iron appears to sharpen the end-point colors but most rubber compounds contain too much iron to leave in the solution. A number of substances which form complex ions with ferric iron were employed in an attempt to eliminate the removal of iron. Tartrate, citrate, oxalate, fluoride, phosphate, and pyrophosphate were without effect, although the last was cited by Aruina (*2*) as being effective for this purpose.

Applications. In addition to being applicable to practically all rubber stocks and latices, the method will probably be equally effective with synthetic rubber mixtures which do not contain appreciable quantities of saturated hydrocarbons. Organic zinc compounds can be readily determined by either method. Inorganic zinc compounds can be determined by titration of an acid solution of the material using either indicator if interfering ions are removed. Application to alloys would be more difficult because of the necessity of removing interfering ions present and added during prior treatment. The removal of interfering ions by precipitation with hydrogen sulfide is not recommended because drastic treatment, such as evaporation with perchloric acid, would be necessary to remove the excess hydrogen sulfide, traces of which are very injurious to the determination. The determination of smaller quantities of zinc by means of a more dilute ferrocyanide solution, a larger sample, or both, is now being investigated.

Discussion of Results

The precision and accuracy of the titrations of standard zinc solution were tested by a series of comparisons of the *p*-ethoxychrysoidine method with the diphenylbenzidine method and with electrometric titration. The diphenylbenzidine end point coincided with that obtained with electrometric

TABLE I. RESULTS OF A STUDY OF THE METHODS

Sample No.	% Zinc Oxide Present	% Zinc Oxide Found, Average	Precision, Average % Deviation from Mean, ±	Accuracy, % Deviation of Mean from True Value
External Indicator Method, Uranyl Acetate on Spot Plate				
1[a]	4.20	4.59	1.3	+ 9.3
2[a]	4.17	3.52	6.0	−15.6
3[a]	4.65	3.40	3.0	−27.0
4[a]	21.10	19.50	2.5	− 7.6
Method II, Procedure B, Using Diphenylbenzidine				
1[a]	4.20	4.08	1.0	− 2.8
2[a]	4.17	3.12	4.0	−25.0
2[b]	4.17	3.30	9.0	−21.0
3[a]	4.65	3.82	2.3	−18.0
3[b]	4.65	3.90	2.5	−16.0
4[a]	21.10	20.75	0.96	− 1.7
4[b]	21.10	20.90	0.38	− 0.95
Method II, Procedure A, Using *p*-Ethoxychrysoidine				
1[a]	4.20	4.16	0.40	− 1.0
4[b]	21.10	21.03	0.10	− 0.3
6[b]	2.75	2.44	3.50	−12.7
Method I, Procedure A				
1[a]	4.20	4.17	0.54	− 0.7
2[a]	4.17	4.18	0.70	+ 0.2
2[b]	4.17	4.22	0.20	+ 1.2
3[a]	4.65	4.45	0.75	− 4.3
3[b]	4.65	4.52	0.70	− 2.9
4[a]	21.10	20.70	0.50	− 1.9
4[b]	21.10	21.10	0.10	0.0
5[b]	2.30	2.28	0.44	− 0.9
6[a]	2.75	2.74	0.91	− 0.3

[a] One precipitation of ammonia-insoluble material.
[b] Two precipitations of ammonia-insoluble material.

titration within ±0.2 per cent. The precision obtained in five successive titrations using diphenylbenzidine was ±0.2 per cent average deviation from the mean. The average of twenty successive *p*-ethoxychrysoidine titrations was the same as that of the diphenylbenzidine titrations and a precision of ±0.2 per cent was obtained with a maximum deviation of ±0.5 per cent. In this last series of titrations the ratio of indicator to ferricyanide was varied through all combinations of from 4 to 6 drops of indicator and from 4 to 7 drops of ferricyanide in which the ferricyanide was present in amounts equal to or greater than the indicator. When ratios outside these limits were used the values obtained were more erratic but still had a precision within ±0.5 per cent.

Table I shows the results of analysis of especially prepared rubber compounds by the uranyl acetate indicator method (*1*) by Methods II-B, II-A, and I-A. The experimental compounds were carefully weighed and mixed in 500- and 1000-gram batches. The quantities of zinc oxide added and the batch weights were carefully checked. The purity of the zinc oxide used was 100 ± 0.2 per cent, as determined by analysis according to a simplified form of Method I-A. The compounds were vulcanized in the form of sheets, 6 × 8 × 0.1 inch, and samples from various parts of the sheets were ground together for analysis. The experimental compounds contained the following principal fillers:

1. 21% carbon black
2. 21% whiting, 21% clay, and 10.5% titanium dioxide
3. 23% barytes and 23% clay
4. 21% whiting, 10.5% magnesium carbonate, and 2.5% ferric oxide
5. 31% barytes and 18.5% clay
6. 16.5% carbon black and 11% litharge

The results tabulated in Table I show very clearly the effect of ashing on the accuracy of zinc determinations in rubber compounds containing clay or lead. The data leave little choice between the accuracy and precision of Methods I and II, providing lead and clay are absent. The poor results obtained with the external indicator method are difficult to explain and may not be typical of the method, although difficulty has frequently been encountered in this laboratory with regard to obtaining the same type of end point in the determination as in the standardization. The amount of reagent used for a blank with the external indicator method is large and inconsistent.

In order to test Method I further, results obtained in routine analysis in the laboratory over a period of several months were examined for precision. Only those determinations in which two or more analyses were run were considered in compiling the averages. Eighteen samples, varying from 1.2 to 6.1 per cent in zinc oxide content, were analyzed with a precision (average percentage deviation from the mean) of ±1.14 per cent and a maximum single deviation from the mean of 2.60 per cent. Seven other samples containing from 14.0 to 45.0 per cent zinc oxide were analyzed with a precision of ±0.31 per cent and a maximum single deviation from the mean of 0.85 per cent.

A test of Method I, Procedure B, was made involving eight successive determinations of zinc oxide in a latex mix. The average value was 1.97 ± 0.9 per cent. This is satisfactory precision when it is considered that obtaining a homogeneous sample of a latex mix as heavily compounded as the one used in these tests is not always easy. It was found that, ordinarily, not more than two samples could be taken from a latex mix without thoroughly remixing the batch to maintain homogeneity.

To summarize the interpretation of the data obtained in testing these procedures: Any of the internal indicator methods described is capable of accuracy and precision sufficient for most analyses for research in rubber technology and is also well suited to the speed required in control analyses without appreciable loss of accuracy. Each method, however, must be used only under the conditions most favorable to its success.

A comparison of the merits of the methods by analysts in this laboratory resulted in a general preference for Method I over the external indicator method. No comparisons with Method II were made by the analysts, but the author's experience has been that the titration using *p*-ethoxychrysoidine as indicator with either method is more rapid than the titration using diphenylbenzidine. The back-titration necessary when *p*-ethoxychrysoidine is used is of advantage when small or unknown quantities of zinc are present, and there is no uncertainty concerning the approach of the end point as there frequently is with diphenylbenzidine. With diphenylbenzidine, the return of the purple color prior to final titration to an end point is frequently slow and the speed of color return appears to be a function of the proximity to the end point when titration is interrupted and to the quantity of sulfuric acid in the solution.

Summary

Two internal indicator volumetric methods for the determination of zinc in rubber compounds have been devised and investigated. The first method is a wet-oxidation method employing nitric acid, bromine, and perchloric acid as oxidants, and *p*-ethoxychrysoidine, a new oxidation-reduction indicator for this use, as indicator. It is of general application to all types of rubber compounds. The second method, less generally applicable to rubber compounds, utilizes either the new indicator or the well-known indicator, diphenylbenzidine. This method depends on ashing of the sample, and cannot be used for the analysis of zinc in rubber compounds containing lead or silicates, since it has been found that no method in which the sample is ashed will yield accurate results if these materials are present. Both methods use potassium ferrocyanide as the volumetric precipitant and potassium ferricyanide as an integral part of the indicator system. Both methods are applicable to other organic and

inorganic zinc-containing materials. The first method appears to have a slight advantage in ease of manipulation, total time of determination, and range of applicability. There is little choice between the methods or indicators as regards precision, accuracy, or the actual time used by the analyst for a determination, providing each method and indicator is used only under the proper conditions. Both methods are superior to the external indicator method using uranyl acetate, the method now in common use in rubber laboratories.

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Continuous Liquid Extractor for Large Volumes of Solution

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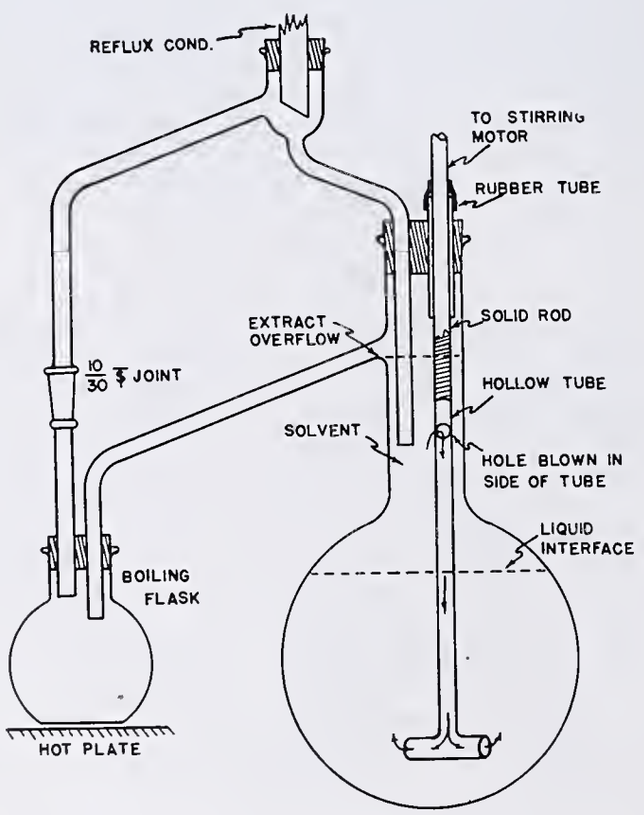
THE need for prolonged extraction of comparatively large quantities of solutions with an immiscible solvent of lesser specific gravity usually leads to the use of a continuous liquid-liquid extraction device similar to that described by Marshall (*1*), in which the solvent is made to bubble up through the solution by means of a long funnel tube leading from a reflux condenser. The solvent together with the extracted material is then returned through a suitable overflow to the boiling flask. Although having the advantage of simplicity in construction and operation, such an apparatus is necessarily inefficient because of limited contact of the solvent with the material to be extracted. Furthermore, it cannot be used conveniently with solutions, such as neutralized cook liquors from wood-pulping studies, that contain finely divided solids which occlude a part of the extractable material and settle out.



The apparatus described herein obviates the above difficulties by providing vigorous agitation and continuously recirculated dispersion of the solvent from the solvent layer through the lower layer being extracted. By so doing, the concentration of extractives in the solvent layer is maintained near the limit of distribution between the two phases. This concentrated extract is continuously replaced by fresh reflux from the boiling flask. The principle is that of a centrifugal pump, the rotor being the hollow T-stirrer (*2*) indicated in the diagram. The solvent is drawn from the solvent layer through a hole well up the stem of the stirrer, and flows down the stem of the T, from which, in a finely divided state, it is thrown into the solution by centrifugal force.

For complete extraction of a 2-liter batch of neutralized wood-cook liquor, the usual type of extractor required from 4 to 6 days, whereas the present apparatus required from 10 to 12 hours.

The diagram is self-explanatory. Pyrex glass was used throughout in the construction. The extraction flask is a distilling flask of from 2- to 5-liter capacity as required. The bearing for the stirrer is a short section of glass tubing of such size as will fit the stem snugly and is sealed at the outer end by means of a short section of well-lubricated rubber tubing.

The extraction flask is filled to within about 200 ml. of the side arm with the solution to be extracted, the remaining volume being occupied by the solvent. If the solution contains suspended solids which are preferentially wetted by the solvent, a small cotton plug placed in the entrance of the overflow tube serves well as a filter.

Should an emulsion form during operation, it may be suppressed by varying the speed of the stirrer: the more stable the emulsion, the slower the speed permissible to maintain the level of the emulsion slightly below the intake hole of the stirrer. The optimum speed for good circulation with a minimum of moderately stable emulsion was found to be about 500 r. p. m. when using a 3-cm. tip on a stirrer of 7-mm. bore.

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Rapid Turbidimetric Method for Determination of Sulfates

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SINCE benzene, phenols, and various other cyclic compounds alter the ratio of inorganic to ethereal (organic) sulfates in urine, it seemed desirable to obtain a rapid method for the determination of this ratio. Gravimetric methods have been used largely for this purpose but they are time-consuming. A nephelometric method for determination of inorganic sulfates in biological fluids has also been reported recently by Medes and Stavers (*2*).

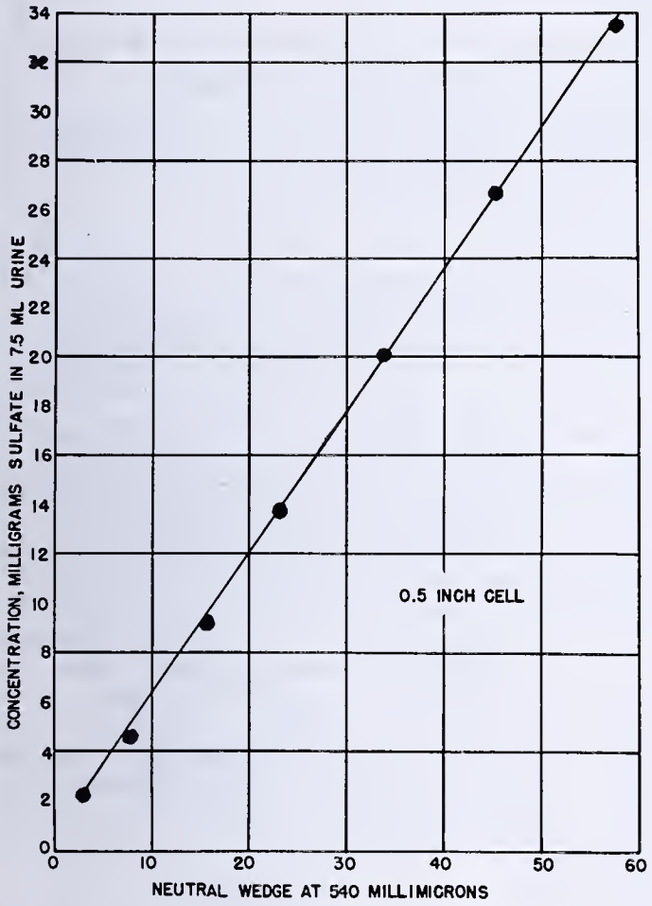

FIGURE 1. DIFFERENCES BETWEEN WEDGE READINGS FOR SAMPLES WITH VARYING CONCENTRATIONS OF SULFATE AND BLANKS. 0.5-INCH CELL USED

The authors first devised a colorimetric method (*4*), based on precipitation of the sulfate with benzidine and coupling of the benzidine with chromotropic acid to yield Dianil Blue R (colour index No. 390, *3*). Since it proved to be somewhat tedious, however, the simpler and shorter photometric method here reported was developed.

The advantages in this method over gravimetric methods are: (1) the speed of determination is increased; (2) a small aliquot may be used; (3) for the final measurement of the sulfates there is a choice of several instruments—namely, the neutral wedge photometer, the spectrophotometer, and the Leitz clinical photoelectric colorimeter.

Procedure

DETERMINATION OF INORGANIC SULFATES IN URINE. The urine is collected, the total volume is measured, and 7.5 ml. are pipetted into a small Büchner funnel equipped with Whatman No. 12 paper, and filtered by suction. As soon as the filtration is complete, the paper is washed three times with 15 to 20 ml. of distilled water. Three drops of alcoholic methyl red are added to the filtrate, which is made faintly alkaline with approximately *N* sodium hydroxide and then the acidity is regulated by making it just acid with hydrochloric acid (1 part of concentrated hydrochloric acid plus 4 parts of water). An excess of 1 ml. of hydrochloric acid (1 + 4) is added and the solution is diluted to 250 ml. with distilled water in a volumetric flask and shaken.

In order to eliminate any slight variation caused by the color of the urine, just enough solution is withdrawn for a blank (about 1.5 ml.) and read in a 1.25-cm. (0.5-inch) precision cell on the neutral wedge photometer, with a 540-millimicron filter (Aminco No. 54). To the remaining solution in the 250-ml. volumetric flask is added 1 gram of 20- to 30-mesh barium chloride. (This may be prepared from reagent-grade barium chloride, or obtained already screened from the Parr Instrument Company, Moline, Ill.) As a matter of convenience, a scoop may be made which will deliver 1 gram. The solution is shaken well, placed in the same cell, and read immediately on the neutral wedge photometer at 540 millimicrons. This reading is converted to the amount (in milligrams) of sulfate in 7.5 ml. of urine by reading from the standard curve (Figure 1).

HYDROLYSIS OF ETHEREAL SULFATES AND DETERMINATION OF TOTAL INORGANIC AND ETHEREAL SULFATE. A 7.5-ml. sample of urine is pipetted into a 150-ml. Erlenmeyer flask containing 2 ml. of water and 7.5 ml. of hydrochloric acid (1 + 4), and a cold-finger condenser is inserted into the top of the flask. The solution is boiled for 20 to 30 minutes, then cooled. Three drops of alcoholic methyl red are added to the sample. It is made just alkaline with approximately *N* sodium hydroxide and is filtered by suction through a Whatman No. 12 paper in a small Büchner funnel. The paper is washed three times with 15 to 20 ml. of distilled water. The filtrate is made just acid with hydrochloric acid (1 + 4). An excess of 1 ml. of hydrochloric acid (1 + 4) is added, and the solution is diluted to 250 ml. with distilled water

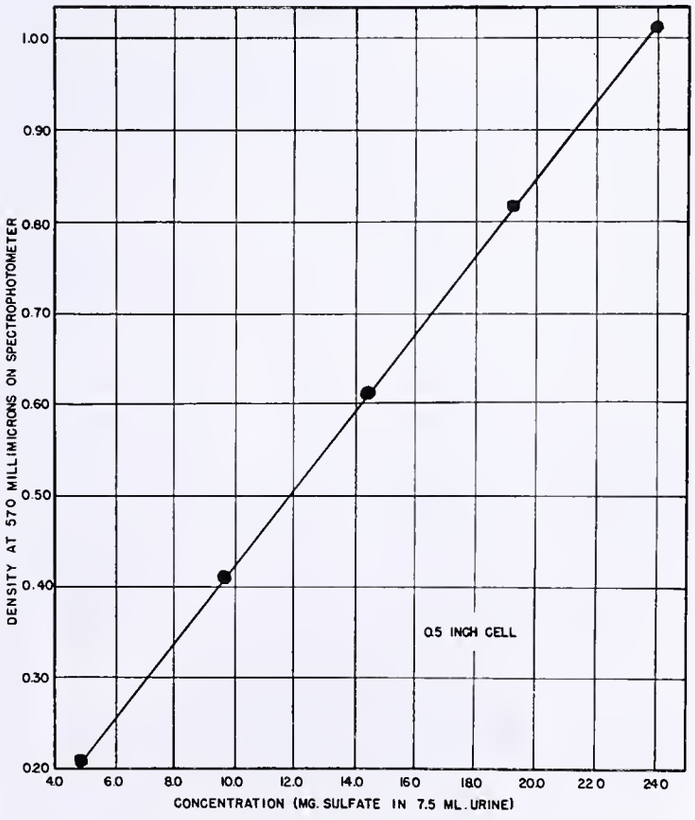

FIGURE 2. SPECTROPHOTOMETRIC DENSITY READINGS WITH VARYING CONCENTRATIONS OF SULFATE. 0.5-INCH CELL USED

in a volumetric flask and shaken, after which the procedure for the determination of inorganic sulfates is followed.

Preparation of Standard Curve. The standard curve is prepared from a series of readings on known amounts of sulfate in "synthetic urine" solutions prepared as described by Cholak (*1*), and containing approximately 5 mg. of sulfate per milliliter. The exact amount of sulfate is determined either by direct weighing of anhydrous sodium sulfate (Merck reagent) or by titrating diluted sulfuric acid (sulfate equivalent to acid) against standard alkali.

To 7.5 ml. of synthetic urine is added 1 ml. of the standard sulfate solution described above. In a similar manner solutions are made with 0.0, 2.0, 3.0, 4.0, 5.0, and 6.0 ml. of standard sulfate. They are made just alkaline to alcoholic methyl red with approximately *N* sodium hydroxide, and then just acid with hydrochloric acid (1 + 4), after which an excess of 1 ml. of hydrochloric acid (1 + 4) is added. The solutions are washed thoroughly into 250-ml. volumetric flasks, diluted to the mark, and shaken. The procedure is continued as described for determination of inorganic sulfates in urine.

The differences between the wedge readings for samples and blanks are plotted against the concentration (milligrams of sulfate in 7.5 ml. of urine) to give a standard curve (Figure 1).

Calculation of Per Cent of Inorganic Sulfates in Urine. The amount of inorganic sulfates in the entire sample is calculated as follows:

$$\text{Milligrams of sulfate as read from curve} \times \frac{\text{total volume of urine}}{7.5} = \text{quantity of inorganic sulfates (in milligrams)}$$

Inorganic plus ethereal sulfates in the entire sample:

$$\text{Milligrams of sulfate as read from curve} \times \frac{\text{total volume of urine}}{7.5} = \text{quantity of inorganic plus ethereal sulfates (in milligrams)}$$

Per cent of inorganic sulfates in urine:

$$\frac{\text{Milligrams of inorganic sulfate as read from curve}}{\text{Milligrams of inorganic + ethereal sulfate as read from curve}} \times 100\% = \% \text{ inorganic sulfates in urine}$$

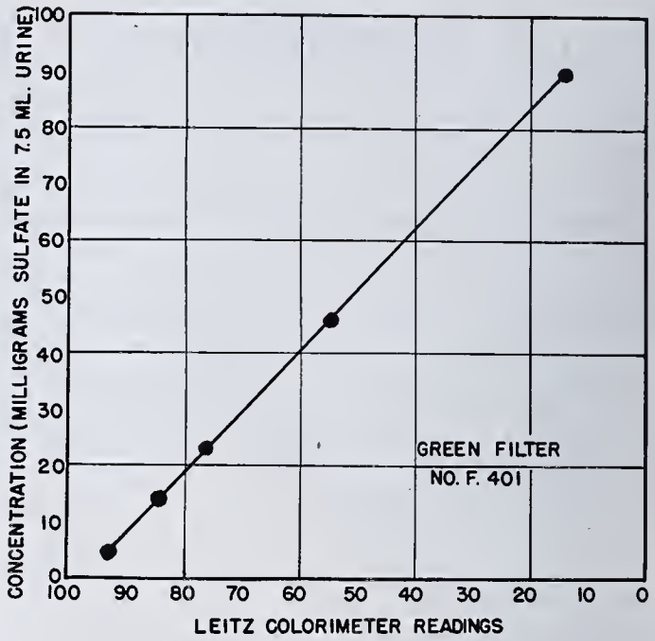

Figure 3. Readings with Varying Concentrations of Sulfate, Obtained with Leitz Colorimeter. Standard Leitz Cell Used

Table I. Comparison of Spectrophotometric and Gravimetric Methods

(Findings in 100 ml. of urine)

Source of Urine	Method Used	Inorganic + Ethereal $BaOS_4$	Inorganic $BaSO_4$	Inorganic + Ethereal SO_4^{--}	Inorganic SO_4^{--}	Inorganic SO_4^{--}
		Mg.	*Mg.*	*Mg.*	*Mg.*	%
Normal mixed human	Turbidimetric			148.7	139.3	93.6
	Gravimetric	356.8	327.2	147.8	134.7	91.1
Rabbit 243	Turbidimetric			98.0	89.3	91.1
	Gravimetric	227.6	202.6	93.7	83.4	91.5
Rabbit 244	Turbidimetric			156.0	134.6	86.3
	Gravimetric	385.0	332.0	158.4	136.3	86.0
Rabbit 245	Turbidimetric			106.6	95.3	89.3
	Gravimetric	240.0	212.0	98.8	87.0	88.0
Rabbit 246	Turbidimetric			157.3	153.3	97.4
	Gravimetric	416.0	363.0	171.2	149.4	87.0
Rabbit 241	Turbidimetric			192.0	157.3	81.9
	Gravimetric	445.0	343.5	183.1	141.1	77.2
Rabbit 242	Turbidimetric			78.0	73.3	94.0
	Gravimetric	155.2	141.2	63.9	57.7	92.0
Rabbit 248	Turbidimetric			174.0	157.3	90.4
	Gravimetric	430.4	378.0	177.1	155.6	87.9
Rabbit 249	Turbidimetric			189.3	170.6	90.1
	Gravimetric	429.2	400.4	176.6	164.7	93.3
Rabbit 245	Turbidimetric			157.3	134.6	85.6
	Gravimetric	406.5	350.5	167.3	144.0	86.0
Rabbit 246	Turbidimetric			133.3	108.0	81.0
	Gravimetric	309.0	273.0	127.2	112.3	88.0
Rabbit 268	Turbidimetric			117.3	106.6	90.9
	Gravimetric	265.0	236.5	109.1	97.3	89.0
Rabbit 269	Turbidimetric			91.9	86.0	93.6
	Gravimetric	208.0	189.5	85.6	78.0	91.0
Rabbit 270	Turbidimetric			132.7	117.3	88.4
	Gravimetric	274.0	243.2	112.8	100.1	88.7
Rabbit 271	Turbidimetric			212.0	200.0	94.3
	Gravimetric	599.2	561.2	246.5	230.5	93.6

or

$$\frac{\text{Total milligrams of inorganic sulfate}}{\text{Total milligrams of inorganic + ethereal sulfate}} \times 100\% = \% \text{ inorganic sulfates in urine}$$

As stated above, one of the advantages of this method is its flexibility. The spectrophotometer, the Leitz clinical photoelectric colorimeter, or even the Duboscq colorimeter may be employed. Separate standard curves must be prepared, however, for each instrument.

A standard curve is shown in Figure 2 in which densities, read on the spectrophotometer at 560 millimicrons, are plotted against the concentration (milligrams of sulfate in 7.5 ml. of urine). A standard curve for the Leitz instrument is shown in Figure 3. These solutions were read through the standard green filter No. F 401, supplied by E. Leitz, Inc., New York, N. Y.

Discussion

After the solution has been made just acid, the same results are obtained if 0.5 or 1.0 ml. of hydrochloric acid (1 + 4) is added in excess.

The wave length of the filter is not of particular importance since no selective color is being measured; however, wave lengths have been selected to which the eye is sensitive.

If aqueous barium chloride is used instead of 20- to 30-mesh crystalline barium chloride, the suspension is not lasting and very low and inconsistent results are obtained.

The results on urine samples obtained by the gravimetric method were compared with those obtained by the photometric method, the spectrophotometer being employed to measure densities (Table I). Figures for amounts and ratios were calculated on a basis of 100 ml. of normal urines. Although in some instances the amounts are not in good agreement, the ratios are.

Sensitivity. A difference of 0.25° in the spectrophotometric readings corresponds to about 0.25 mg. of sulfate in 7.5 ml. of urine. In the case of the neutral wedge photometer, a difference of 1 unit (1 mm.) corresponds to about 0.33 mg. of sulfate in 7.5 ml. of urine. Both instruments are calibrated for slightly finer divisions, but the readings given above may be duplicated without difficulty. Although determinations may be made over a greater range with the Leitz instrument, the sensitivity is little more than 1 mg. of sulfate in 7.5 ml. of urine for a difference of 1 unit.

Practical Application. The method works well in practice. The urine sulfates of rabbits treated with cyclic compounds were determined by the photometric method and the proportion of the inorganic sulfates was found to have fallen to 10 per cent or less. Upon cessation of the treatment, the inorganic sulfates returned to a normal of 85 to 95 per cent.

The presence of proteins does not interfere measurably with the method, since a solution containing 15 mg. of sulfate and 0.125 ml. of monkey blood serum yielded 15 mg. of sulfate.

Acknowledgment

The writers are indebted to W. Deichmann for carrying out the gravimetric sulfate determinations.

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Ceric Sulfate in the Determination of Iron Using the Molybdisilicic (Silicomolybdic) Acid Method

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THE authors have varied their method for the determination of iron (*3*) by substituting ceric sulfate for potassium dichromate, and have found that *o*-phenanthroline ferrous ion indicator is satisfactory in place of *N*-phenylanthranilic acid in the ceric sulfate runs. The method and general procedure for runs, blanks, and standardizations follow the ideas presented in the earlier paper.

The "stock ferric chloride" and "stock dichromate" were those used in the previous paper. The normality of 0.09733 for the former was taken from that paper. For the normality of the dichromate 0.10058 was used in place of 0.10073 being obtained from pure dichromate from volume ratios of the respective solutions used in titrating the stock ferric chloride by the molybdisilicic acid method. This method of substitution allows cancellation of errors in the procedure and blanks, giving an "absolute" normality.

From the dichromate the ceric sulfate was standardized by another substitution procedure, cancelling out any slight errors (*1*, *4*). The oxidizing solutions were in turn run into potassium iodide and acidified with sulfuric acid, and the iodine liberated was titrated with a sodium thiosulfate solution. From the relative volumes of the latter the normality of the ceric sulfate was obtained, after making a suitable correction for acidity differences.

The ceric sulfate was also standardized against sublimed arsenic trioxide, using osmium tetroxide catalyst and *o*-phenanthroline ferrous indicator. This method gave a normality of 0.09664 for the ceric sulfate, which is somewhat lower than the values of 0.09704 and 0.09697 obtained by the molybdisilicic (suggested by M. B. Mellon as a name preferable to silicomolybdic) acid method at the end and beginning of the work, using the stock ferric chloride whose stated normality was shown in the preceding paper to be absolute and so independent of the method used there. However, the thiosulfate method gave 0.09682 for the ceric sulfate normality. It appears that after application of the blanks the molybdisilicic acid method with ceric sulfate gives slightly too low results for iron, since the volume of ceric sulfate used seemed to be as much as 3 parts in a thousand too low, giving too high a normality above.

In Table I are comparisons of the volumes of ceric sulfate used for the titration of iron, with different indicator combinations. Unless otherwise noted, each figure represents the average of four runs from which no run varies by over 0.02 ml. In untabulated cases the blanks were positive but not over 0.02 ml. It will be noted that nonapplication of the blanks resulted in much better agreement than did application. The blanks for the molybdisilicic acid method and the volumes tabulated are those used in calculating the normalities given above. Had the blanks not been applied for the runs at the beginning of the work the normality would have been 0.09684 in place of 0.09697, a better agreement with the other methods. There is some question as to the real significance of the blanks, especially since their application in the earlier paper resulted in slightly low values for iron as compared with the value by weight. The use of *o*-phenanthroline ferrous indicator appears to be most satisfactory (Table I).

Table I. Titration of Stock Ferric Chloride with Ceric Sulfate[a]

Method	Ceric Sulfate per 45.00 Ml. of $FeCl_3$	Applied Experimental Blank	Corrected Volume
	Ml.	*Ml.*	*Ml.*
Molybdisilicic acid, *N*-phenylanthranilic acid (first runs)	45.23	−0.06	45.17
	45.23	−0.07	45.16
Same (at end of work)	45.22	−0.08	45.14
	45.21	−0.08	45.13
Mercuric chloride[b], *o*-phenanthroline, cold	45.23	Less than	...
	45.22	−0.02	...
Molybdisilicic acid, *o*-phenanthroline (same directions as with *N*-phenylanthranilic acid)	45.23[c]	−0.02	45.21
	45.20	−0.02	45.18

[a] 32 ml. of 1 to 1 H_2SO_4 added in all runs, in addition to equivalent of 8 ml. present in added ceric sulfate volume.
[b] No trouble (*2*) experienced with adsorption of indicator on calomel with small excess of stannous chloride used.
[c] Three runs only.

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Contribution 68 from the Chemical Laboratories of the University of Utah.

Determination of Phosphorus in Lubricating Oil

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THE increasing use of lubricating oil additives of the organically combined phosphorus type emphasizes the need of a method for phosphorus determination. These additives, which are predominantly organic phosphates (*3, 12*), phosphites (*13*), and phosphatides (*14*), are employed to improve the service behavior of the lubricating mineral oil with respect to oxidation stability, sludge formation, corrosiveness, and varnishing of engine parts.

Although it is occasionally desirable to know the precise nature of the phosphorus-type additive, this entails the usual lengthy and complex organic analysis which is rendered more difficult by the small amount present in lubricating oil. With samples of this kind one is generally content with detection and determination of the phosphorus, so that the quantity of additive can be calculated when its nature is known.

Of the methods available in the literature for determining phosphorus, the colorimetric and solid volumetric appeared most worthy of investigation because of their speed and simplicity.

The colorimetric methods of Denigès (*2*) and Fiske and Subbarow (*6*) for inorganic phosphorus compounds have been extended by Goodloe (*8*) to the photometric determination of added phosphorus in oils. This author ignites a gram of sample with a like amount of zinc oxide and after appropriate solution of the residue in dilute sulfuric acid, addition of molybdate, and reduction by stannous chloride, measures the intensity of the blue reduced molybdenum solution with an electric colorimeter whose scale is calibrated in terms of phosphorus. The present authors found that this procedure requires a considerable experience and is unreliable on used oils (for which its author, however, had not proposed it), since oils through use may accumulate impurities which, though not phosphorus, also reduce molybdenum. This lack of specificity gave preference to precipitation methods in which phosphorus is a constituent of the precipitate which, unlike the colorimetric method, serves at once for qualitative as well as quantitative determination.

Formation of yellow ammonium phosphomolybdate has long been used for the detection and determination of phosphorus in inorganic materials. Goetz (*4, 7, 10*) extended its application to steel by centrifuging the solution and precipitate and estimated the phosphorus from the volume of precipitate. This method appeared attractive because it involves apparatus commonly found in petroleum laboratories, and is the second step in the method finally adopted and used successfully in the authors' laboratories for three years. Recently Piercy, Plant, and Rogers (*11*) described a method for phosphorus in linseed oil and employed a similar centrifuging operation. To convert the phosphorus of the linseed oil to inorganic water-soluble form, these authors ignite 2 grams of oil in a standard Parr oxygen bomb. Though this, in the opinion of the present authors, is a dangerously large sample for ignition in such a bomb [the A. S. T. M. (*1*) recommendation for bomb sulfur being 0.6 to 0.8 gram], it is still much too small for the application of the centrifugal method to many commercial mineral lubricating oils because of their low concentration of phosphorus additives. For the same reason the classical Carius (*16*) method is unsuitable.

In the present method the phosphorus is converted to inorganic soluble form by ignition with sodium naphthenate which is soluble in oil and basic in nature. Ignition with oil-insoluble materials, such as potassium nitrate and potassium hydroxide (*5*) and calcium carbonate (*9*) recommended for other substances, yielded low and unreliable results when applied to lubricating oils, probably owing to loss of organic phosphorus because of lack of sufficiently intimate admixture of the oil and the reacting agent which is insoluble in it. The use of the relatively oil-insoluble sodium stearate likewise yielded low results.

Apparatus

An International Size 2 centrifuge, with special centrifuge tube (Figure 1), equipped with a capillary stem having a capacity of about 0.25 cc. The capillary has an inside diameter of approximately 2 mm., an inside length of about 4 cm., and seven graduations at each 0.030 cc. with subdivisions of five equal spaces.

No. 3 porcelain dishes.

Reagents

Ammonium molybdate prepared (*15*) by dissolving 90 grams of pure ammonium molybdate in 100 ml. of 6 *N* ammonium hydroxide plus sufficient water to effect solution, adding 240 grams of ammonium nitrate, and diluting to 1 liter.

Nitric acid, approximately 5 *N*.

Sodium naphthenate.

Procedure

NEW OILS. Into a No. 3 porcelain dish weigh 5 to 50 grams of sample (depending upon phosphorus content and limited by calibrated stem of centrifuge tube. This range of weight is satisfactory for samples containing 0.001 to 0.100 per cent of phosphorus. Samples of higher concentration have been analyzed by this method modified to use appropriately smaller samples) and approximately 2.5 grams of sodium naphthenate, and dilute to approximately 50 grams with ordinary phosphorus-free mineral oil.

Heat to 150° to 160° C. and keep at that temperature for not less than 2 minutes while stirring with a thermometer. Wipe the bulb of the thermometer with a piece of filter paper and add it to the dish. Ignite and burn until volatile matter is gone and a small amount of carbon is left.

Allow the dish to cool, add 20 ml. of 5 *N* nitric acid, boil gently for 5 minutes to convert pyrophosphates to orthophosphates, and filter into a 100-cc. special centrifuge tube (Figure 1). Wash the dish, sediment, and filter paper with 5 successive small washings of hot water, taking care not to use too much liquid, as the final volume must be 100 ml.

Bring the contents of the centrifuge tube to 50° to 55° C. by placing it in a water bath at that temperature, add 10 ml. of

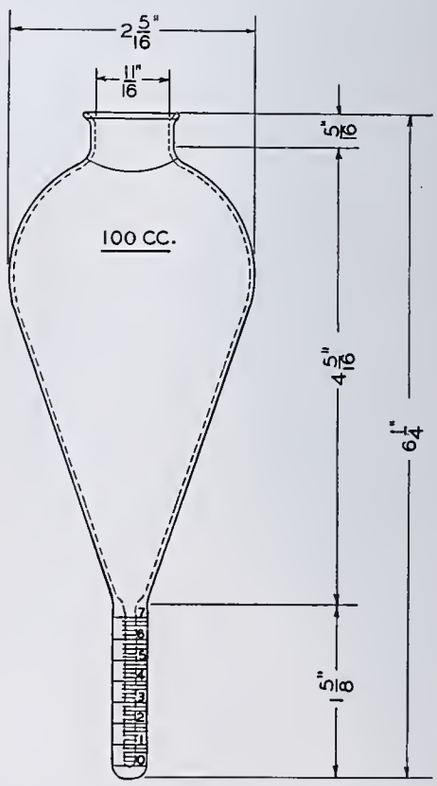

FIGURE 1. CENTRIFUGE TUBE

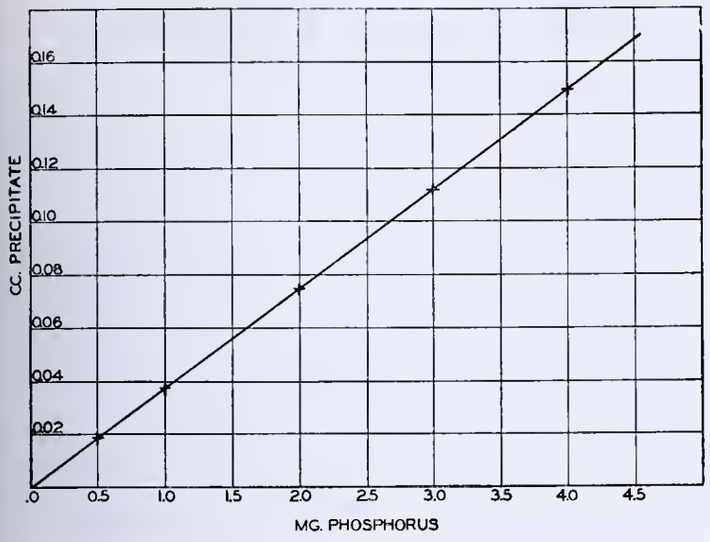

FIGURE 2. GRAPH FOR CALCULATION OF PHOSPHORUS CONTENT

ammonium molybdate reagent, and stir for 5 to 10 minutes. Permit to cool and adjust the volume to 100 ml. by adding water.

Centrifuge for 10 minutes at 2000 r. p. m. If the volume of precipitate is difficult to read, owing to uneven surface, stir the surface with a fine, clean platinum wire, recentrifuge the tube for 1 to 2 minutes, and read the volume.

Determine the amount of phosphorus by reference to Figure 2, which was prepared from tests on known solutions of lecithin in oil and confirmed by direct precipitation of standard solutions of disodium phosphate.

USED OILS. Through use, lubricating oils commonly become contaminated with metals, salts, and soaps of lead, copper, aluminum, iron, and tin. To determine the organically combined phosphorus still present in solution in the oil, a sample is clarified by centrifuging.

Some soluble heavy metal (lead, etc.) salts or soaps in solution in the oil used for phosphorus determination would cause high results, due to formation of insoluble heavy metal molybdates. Therefore, the ash resulting from burning the clarified used oil with sodium naphthenate is treated in the following manner:

Carefully add approximately 10 ml. of concentrated hydrochloric acid to the ash, covering the dish with a watch glass to prevent loss through spattering. After washing spattered material from the watch glass into the dish, evaporate the solution to dryness on a steam bath and add 20 ml. of 0.3 *N* hydrochloric acid. Warm the acid solution and saturate with hydrogen sulfide to precipitate the copper, lead, and tin. Eliminate the excess of hydrogen sulfide by heating the solution and then filter through paper (Whatman No. 44) and wash thoroughly with approximately 50 ml. of hot water. Evaporate the combined filtrate to dryness, add 20 ml. of 5 *N* nitric acid, and filter the solution into the special centrifuge tube. The presence of soluble iron and aluminum salts does not interfere. The procedure from this point is identical with that used for the determination of phosphorus in new oil.

When this method was applied to mineral lubricating oils to which known amounts of organic phosphorus compounds (additives) had been added, the results shown in Tables I and II were obtained, from which the accuracy and the precision of the method can be seen. The additives contained 2 to 10 per cent of combined phosphorus.

The last three samples (Table I) contain many times the phosphorus found in modified lubricating oils. Though good results were obtained, the method is not particularly recommended for products so high in phosphorus, because very small samples must be used.

Experience indicates that approximately 1 hour of a man's applied time and 2 hours of elapsed time are required for a single sample tested in duplicate. The method is very suitable for routine mass testing.

The qualitative value of the test is indicated by the fact that samples (25 grams) containing 0.00003 per cent of organically combined phosphorus consistently gave a precipitate though it was too little for quantitative purposes. Samples containing 0.00001 per cent of phosphorus did not consistently give a precipitate but the solution always was yellow compared to a blank test.

Summary

A simple accurate method for determining organically combined phosphorus in lubricating oil involves ignition of the sample with sodium naphthenate which serves to bind the phosphorus in inorganic water-soluble form. The water solution of the phosphate is reacted to form ammonium phosphomolybdate, which is centrifuged, and the phosphorus is calculated from the volume of precipitate.

The method has been used for over three years, during which it has been applied satisfactorily to new and used lubricating oils containing phosphorus organically combined as phosphate, phosphite, and phosphatide.

TABLE I. DETERMINATION OF PHOSPHORUS IN OILS

Sample	Phosphorus Present	Phosphorus Found	Sample	Phosphorus Present	Phosphorus Found
	%	%		%	%
1	0.001	0.001	10	0.025	0.025
2	0.002	0.002	11	0.025	0.025
3	0.003	0.003	12	0.025	0.024–0.024
4	0.005	0.005	13	0.050	0.048–0.048
5	0.010	0.010	14	0.050	0.049–0.051
6	0.010	0.010	15	0.092	0.093
7	0.011	0.011	16	3.00	2.98
8	0.011	0.012	17	4.00	3.98
9	0.022	0.023	18	5.00	5.00

TABLE II. DETERMINATION OF ADDITIVE (PHOSPHORUS TYPE)

Sample	Additive Present	Additive Found	Sample	Additive Present	Additive Found
	%	%		%	%
1	0.06	0.06	8	0.21	0.21
2	0.09	0.09	9	0.25	0.25
3	0.10	0.10	10	0.25	0.25
4	0.10	0.10	11	0.50	0.52
5	0.10	0.10	12	0.50	0.53
6	0.10	0.10	13	1.00	1.03
7	0.10	0.12			

Acknowledgment

The authors express their appreciation for the assistance of A. E. Burch and W. W. Johnson, who made many of the determinations.

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Determination of Moisture in Sugar Products

Corn Sugar "70" or "80"

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THE first paper of this series (*17*) dealt with moisture determination in corn sirup and corn sugar by the filter paper method. An average analysis of confectioners' corn sirup was given, since all manufacturers produce a product with essentially the same composition. However, the composition of crude corn sugar varies considerably, not only in dextrose equivalent (reducing sugars as dextrose, dry substance basis), but in residual constituents, such as acidity, protein, and ash, which are also present in greater amounts in corn sugar than in corn sirup. The composition of these crude sugars can be expressed best by tabulation of the range of values for the critical tests usually made on them (dry basis):

Reducing sugars as dextrose	82 to 92
Residual acidity as hydrochloric acid	0.02 to 0.04
Crude protein	0.06 to 0.15
Ash	0.8 to 1.5

The crude sugars, either chipped or in billet form, are generally sold in two grades: "70" corn sugar, containing not less than 70 per cent reducing sugars as dextrose, and "80" corn sugar, containing not less than 80 per cent reducing sugars as dextrose.

Although the filter paper method has been found satisfactory for numerous samples analyzed, occasional samples show apparent instability at 100° C. in vacuum. This is manifested by a tendency to lose weight very slowly, at a rate which would be neglected normally, but which would not be called a drying "plateau" as exhibited by corn sirup. This condition calls for an arbitrary definition of constancy, which is not regarded as a satisfactory basis for true moisture. Such a corn sugar has been the subject of many drying techniques, in an attempt to establish a method applicable to all corn sugars.

The following test made on an "80" sugar is an example of this slow "creep" or failure to reach a definite moisture plateau. The procedure was the Filter-Cel method (*9*) carried out at 100° C. in a Weber oven with a pressure of 1 mm. or less.

Elapsed Time	Sample Weight		Moisture	
Hours	*Grams*	*Grams*	*%*	*%*
4	20.5235	21.6140	50.00	50.02
19			50.16	50.15
24			50.18	50.18
29			50.27	50.27
73			50.85	50.87

Constancy of weight normally would be stated to occur in the period from 19 to 24 hours and such deviation usually would be passed over. However, there is no question that decomposition occurs later. This casts a shadow of doubt over the assumption that constancy occurs earlier, as a very slow decomposition might be taking place.

In order to eliminate any shadow of doubt, a series of tests was employed as follows:

A. 100° C., air oven, Filter-Cel method. To ascertain whether the vacuum was removing a possible volatile constituent.

B. 100° C., vacuum oven, Filter-Cel method. Short intervals of drying to see if constancy could be attained within reasonable limits.

C. 100° C., vacuum oven, Filter-Cel method. Extended period of drying to estimate the amount of decomposition.

D. 80° C., vacuum, Filter-Cel method. To find the effect of a lower temperature.

E. 38° C., modified Lobry de Bruyn, Filter-Cel method. To ascertain the effects of a still lower temperature, wherein, if decomposition is a factor, it is possibly going on at all times under normal storage.

F. 110–112° C., toluene distillation, Filter-Cel method. To measure the water directly and not a loss by weight.

G. 80–82° C., benzene distillation, Filter-Cel method. To measure water directly at a lower temperature.

H. 76–78° C., carbon tetrachloride distillation, Filter-Cel method. To measure water directly at a still lower temperature. So far as the authors know, this method has not been applied to sugar products, although developed and used for moisture in dynamite (*1*). The solvent here is heavier than water, necessitating a different type of trap. This type of trap offers mechanical disadvantages for similar development of the new bulb types of traps which have been successfully employed with benzene and toluene.

The data obtained on corn sugar for tests mentioned above appear in Table I.

With the exception of the benzene and carbon tetrachloride distillation methods, all have been described in previous papers of this series. Since the determination of true moisture in corn sugar has been obtained in part by correlation of distillation methods to gravimetric methods, a review of the development of the former will be given.

Historical

The literature on moisture determination reveals that a great deal of attention has been devoted to distillation methods. A large number of organic liquids, covering a wide range of boiling points and densities, have been proposed and used with many materials. For general purposes, the most useful ones appear to have been benzene and its homologs, toluene and xylene. Others extensively used are carbon tetrachloride and the lighter fractions of petroleum.

Hoffmann (*24*) in 1908 presented an interesting résumé of work on distillation up to that time. Attention was called to the fact that a German patent was granted in 1901 on a process for the determination of water in solids and liquids by distillation. The substance to be examined was either distilled with a liquid immiscible with water but with a higher boiling point or heated with such a liquid and then distilled with another liquid, likewise immiscible with water but with lower boiling point. In either case, the water collected in the distillation was measured. The method was originally designed for analysis of cereals and cereal products, and employed such liquids as petroleum fractions and toluene. It was quickly varied to make application to other substances by Gray (*23*), to other foods by Thorner (*41*), to cellulose products by Schwalbe (*36*), and to lignite by Gräfe (*22*). These investigators used a wide range of liquids immiscible with water.

Hoffmann presented data on hops, showing that the distillation process with turpentine and toluene gave results which very closely agreed with those obtained by older methods of drying *in vacuo* with phosphorus pentoxide or in drying chambers at 80° C. The author stated that for any substance in which water is to be determined, at least one of the ten variations of the method mentioned in the article will be found suitable.

Marcusson (*28*) worked with oils, fats, soaps, etc. (in 1905), and used xylene or benzene as his immiscible liquids. Testoni (*40*) made determinations on molasses by distillation in 1904.

Schwalbe (*36*) in 1908 reported the application of the distillation method to plant materials. "In examination of dense materials, it is important to select for the liquid of high boiling point one such that the tension of the aqueous vapor above 100° may be great enough to enable the bubbles of steam to overcome the resistance of passing through the mass." Many workers appear to have taken this view also, but it is not necessarily true with adequate dispersion.

In 1910 Sadtler (*35*) reported on the use of benzene for distillation of water from cheese, egg albumen, etc. Folpmers (*20*) used benzene, toluene, and xylene in his work on spices which he reported in 1916. Sindall (*39*) tried kerosene on spices previous to 1917 and Besson (*4*) used "petroleum spirit" on cheese and soap about the same time.

In 1917, van der Linden, Kauffman, and Leistra (*27*) published their results of distillation on molasses and other sugar factory products. They placed 50 grams of molasses or other product with 350 cc. of the immiscible liquid in a copper distilling flask connected to an upright condenser, the lower end of which discharged into a 250-cc. measuring cylinder graduated to 0.05 cc. Heating was so regulated that 100 cc. passed over in the first 0.75 hour, after which the rate was accelerated so that a second 100 cc. came over in the fourth quarter hour. When 200 cc. had been collected, the determination was assumed to be complete.

These workers observed that xylene gave very satisfactory results, provided a certain rate of distillation was observed, but that prolonged heating would cause decomposition of the product with formation of water. They further stated that other volatile immiscible liquids, such as benzene, toluene, kerosene, and mixtures of these gave less satisfactory results.

It would appear that the earlier workers failed to make the liquids of lower boiling point work satisfactorily on viscous materials because of apparatus limitations and no sample dispersion. The Dean and Stark (*12*) "distilling receiver tube" had not been developed nor had the improvements in dispersion due to Rice (*33*) and Fetzer, Evans, and Longenecker (*19*) made their appearance.

The development of the Dean and Stark device, which permits continuous refluxing and separation of the water, was a great forward step, as it provided means of removing the last traces of water efficiently. In 1925, Bidwell and Sterling (*5*) improved the design for precise work and their well-known "traps" are now standard equipment.

Norman (*32*) presented work on fats, oils, soaps, etc., in 1925 and drew attention to a special still head provided with a built-in reflux condenser of simple design to permit continuous refluxing. Accurate results were claimed and the use of benzene instead of xylene was recommended.

Yamada and Koshitaka (*43*) modified Norman's apparatus and recommended it for water determination of crude camphor in 1927. It was claimed that adhesion of condensed water on the wall of the still head can be completely avoided by a special still head with built-in reflux condenser having an inverted part at its end. The use of toluene or xylene was recommended instead of benzene for camphor.

In 1926, Dedlow and Smith (*13*) found that xylene distillation caused decomposition when applied to meat extract and successfully modified the method by conducting the distillation with xylene under vacuum. This is an interesting variation and gives a flexibility which should prove useful for many products, although it is a little complicated for routine work.

In 1936, Alexander (*1*) reported on an interesting variation in which a volatile liquid heavier than water—i. e., carbon tetrachloride—was used with a special tube adapted to the reverse of usual conditions. The paper stated that the method has long been in use in laboratories of the Hercules Powder Company for routine moisture determinations on dynamite. This liquid has some advantages, notably nonflammability. It has been investigated in this laboratory on corn sirup and corn sugar and results are reported below.

In 1929, Rice (*33*) contributed much to the successful operation of the distillation method for sugar products by placing Filter-Cel in the distillation flask and running the sirup in on top. This very useful technique was further developed in 1935 by Fetzer, Evans, and Longenecker (*19*) to apply to extremely viscous materials, such as high-gravity corn sirup. Trusler (*42*) in 1940 presented a very interesting study which reported on the use of benzene, toluene, and xylene with soaps.

The literature reveals that the method has been applied to mineral, vegetable, and animal oils, fats, sulfonated oils, camphor, tars, meat extract, cheese, eggs, cereals and cereal products, cellulose products, spices, hops, coal, fruits, sugar refinery products, corn products, tobacco, and many others, with varying degrees of success. Many ingenious improvements have been made and development has carried it to rather high precision but it appears to be ignored or neglected frequently under conditions where it might be of great use.

Among other contributions to the subject of moisture determination by distillation are:

Blythe (*6*), sulfonated oils; Barber (*3*), sulfonated oils by 100° oven, paraffin, benzene, toluene, gasoline, and xylene; Bakker and Stunhauer (*2*), foods and spices; Boller (*7*), determination of water with hydrocarbons; Cone and Davis (*10*), tobacco; Calderwood and Piechowski (*8*), technique; do Couto (*11*), oils and tar; Dohmer (*15*), case-hardening materials; Drefahl (*16*), wood preservatives; Fuchs (*21*), benzene method for mineral oils; Feder (*18*), methods proposed for sausages; Holtappel (*25*), foods with xylene; Jones and McLachlan (*26*), moisture by the toluene method; Myhill (*31*), coals with benzene, toluene, and xylene; Michel (*30*), foods; Marcusson (*28, 29*), lubricating greases (among earliest papers); Sindall (*38, 39*), distillation method recommended for spices; Rogers (*34*), leather with toluene; Schwalbe (*37*), further work on cellulose products; and DeLoureiro (*14*), improvements in technique.

Table I. Data on Corn Sugar

Test	Sample Weight		Elapsed Time	Moisture	
	Grams	*Grams*	*Hours*	%	%
A	10.8855	11.8616	16	49.58	49.61
			60	49.80	49.82
			84	49.90	49.91
			129	50.12	50.11
B	11.7250	10.4780	2	47.76	48.51
			4	49.53	49.55
			6	49.62	49.64
			8	49.65	49.67
			12	49.65	49.67
C	15.7759	13.9630	15	49.63	49.63
			60	49.85	49.96
D	14.0200	15.2040	15	49.63	49.65
			22	49.64	49.66
			37	49.65	49.67
			52	49.65	49.67
E	13.2823		100	49.50	
			300	49.60	
			500	49.62	
			600	49.64	
			700	49.64	
F	46.5593	46.5225	10	49.62	49.63
			15	49.63	49.64
			22	49.67	49.76
			52	50.41	50.40
G	35.6202	36.8530	12	49.66	49.68
			18	49.66	49.68
			50	49.66	49.68
			70	49.66	49.68

H — The common sample of sugar used for the above tests could not be used for carbon tetrachloride distillation, as the apparatus available was the standard Hercules distillation traps of 6-ml. capacity. Accordingly a heavier gravity of sugar was used. In the employment of dispersion for carbon tetrachloride distillation, the technique must be altered from that followed for benzene and toluene. The amount of Filter-Cel must be reduced, as the mass requires more space because of more vigorous agitation of carbon tetrachloride. In calibration of the traps, the amount of water recovered is never the full volume added. The blank is high, amounting to +0.14 ml. for the apparatus employed. Otherwise, the procedure followed was that of Alexander (*1*). With the common sugar products tested, water recovery was more rapid and completed in less time as compared to benzene distillation. The data obtained were:

80° C. Vacuum Oven		Carbon Tetrachloride Distillation	
%	%	%	%
20.19	20.21	20.18	20.23

The method may be said to suffer from two faults. One would seem to be inadequate appreciation of the importance of dispersion of viscous materials and the second, a tube design which limits size of samples in precise work.

Experimental

For this test, a corn sugar sirup, 90.7 per cent dextrose equivalent, was made, 26.45° Bé. (Bé. = Bé. 140°/60° F. + 1.00), adjusted to pH 5, carefully divided into samples in 120-ml. (4-ounce) bottles, fitted with screw caps lined with wax paper. These were placed in the refrigerator for subsequent analysis, one bottle being used for each analysis.

The moisture traps of the usual Bidwell and Sterling design have a capacity of 5 ml. in 0.1 ml. The outside diameter is approximately 10 mm. in order to obtain relatively large intervals for each 0.1-ml. division—essentially a microburet.

Larger traps (10-ml.) are obtained by increasing the diameter of the trap but with attendant sacrifice of precision in reading the meniscus; 10-ml. traps are the largest type practical for the usual apparatus. The small traps limit the test to materials containing comparatively small amounts of water, whereas it is de-

FIGURE 1. MOISTURE TRAPS

sirable in the interest of experimental precision to use large samples. Materials such as sirups, containing relatively large percentages of water, require a small amount of sample for 5- and 10-ml. traps, with a corresponding decrease in precision.

The usual 5-ml. traps were altered to include an ovoid bulb at the bottom (Figure 1). The bulbs chosen for sirup work were 15-, 20-, and 30-ml., blown on the usual 5-ml. (in 0.1-ml.) receivers, giving a total trap capacity of 20, 25, and 35 ml.

These traps have been used with a great deal of success. In order to enable the water to run freely into the bulb, special precautions must be observed in cleaning them: Scour with a cleanser powder (Dutch Cleanser), employing hot water and a small tube brush. Close the bottom opening and fill the trap with warm bichromate–sulfuric acid cleaning solution. Allow to stand a few hours, rinse well with distilled water, fill trap with 2 per cent sodium or potassium hydroxide solution (*14*), and allow to stand for a short period. Rinse thoroughly, drain, and dry by means of a radiant infrared lamp, which has been found more successful than an alcohol or ether wash.

Benzene Distillation

Benzene distillation presents a mixing problem when applied to a Filter-Cel sirup mass, particularly in the case of viscous corn sirup, where mixing must be thorough if the water is to be removed quickly and completely. The azeotropic binary mixture of benzene and water starts to boil around 69° C. It is therefore necessary to guard against evaporation during incorporation of Filter-Cel and sirup. This was accomplished by slipping a length of Gooch filter rubber tubing over the neck of the flask, then passing the stirring rod through and wiring the tubing to the stirring rod. The rubber tubing provides a flexible joint for stirring the Filter-Cel sirup mass and no vapor is lost from the flask.

In this work, all the traps were calibrated by distillation of weighed quantities of water as in an actual determination.

APPARATUS. Distillation flask, 250-ml. standard flask (*9*), used throughout this work for distillation and vacuum oven work.

Benzene, Mallinckrodt, analytical reagent grade.

Filter-Cel, prepared as previously described (*9*).

PROCEDURE. Approximately 30 grams of prepared Filter-Cel are run into a distillation flask, and brought to a constant weight in a vacuum oven (usually 4 hours). The sample is run on top of the Filter-Cel and reweighed for sample weight, then incorporated with the Filter-Cel. Then 100 ml. of benzene are run into the flask, which is fitted with rubber closure (described above), and Filter-Cel, sample, and benzene are thoroughly worked into a homogeneous mass. The flask is connected with the trap and condenser, and distillation is started gradually—2 drops per second. Ninety per cent of the water is removed within an hour, after which the rate of distillation is increased to 4 drops a second. After constancy has been attained, the trap water is brought to 20° C. and read. Large traps require considerable care in this operation, as do pycnometers, and the technique is much the same.

The data indicate that on a corn sugar which does admit of decomposition with prolonged heating, a value equal to or deviating by very little can be obtained by the Filter-Cel method, provided the time element is 8 to 12 hours from the air oven at 100° C., vacuum oven at 100° C., and toluene distillation. Vacuum oven at 80° C., Lobry de Bruyn at 38° C., and benzene and carbon tetrachloride distillations—all Filter-Cel methods—yield drying plateaus which are maintained for periods that extend beyond any anticipated moisture test and weight constancy can be regarded as true moisture.

The distillation methods as applied to sirups cannot be relied upon unless the material is dispersed over a large surface. Direct use of the solvents without dispersion will lead to erroneous results, either through failure to obtain the release of all the moisture, or by decomposition, which may be initiated in the mass by a delayed elimination of the water.

In order to complete the picture of moisture methods as applied to hydrolytic products of starch, a sample of corn sirup was rerun to include the benzene–Filter-Cel distillation, with results as follows:

			A		B	
° C.			%		%	
			(1)	(2)	(1)	(2)
100	Vacuum oven	Filter-Cel method	19.11	19.11	31.99	3.197
38	Lobry de Bruyn	Filter-Cel method	19.10	...	...	...
110–112	Toluene distillation	Filter-Cel method	19.12	19.11	...	...
80– 82	Benzene distillation	Filter-Cel method	19.09	19.10	...	...
76– 78	Carbon tetrachloride distillation	Filter-Cel method	...	...	31.96	31.97

Conclusions

Three methods have been recommended for determination of true moisture of crude corn sugar:

80° C. vacuum oven, Filter-Cel method.
38° C. Lobry de Bruyn, Filter-Cel method.
80–82° C. benzene distillation, Filter-Cel method.

The increased capacity of the type of receiver tube designed for this work permits much greater precision because of larger sample weights employed. The authors consider this practically essential in a research which aims at true moisture.

Dispersion is greatly facilitated by dilution and the use of large samples, where the purpose of the work is to determine stability of soluble materials such as viscous sugar sirups.

The 80° C. vacuum oven, Filter-Cel method, has been adopted for determination of moisture in "80" corn sugars by the Technical Advisory Committee of the Corn Industries Research Foundation.

The so-called "70" sugars are often difficult by oven methods and in these cases the value of benzene distillation is stressed. Some tanners' sugars ("70") decompose fairly rapidly at 100° C. and stubbornly retain lost traces of moisture at 80° C. In these cases a vacuum oven temperature of 90° C. is recommended as results at this level are most reproducible and check benzene distillation or the modified Lobry de Bruyn method at 38° C.

Stability of corn sugar on drying is dependent upon the pH of the material. Maximum stability is attained between pH 4.5 and 5.5. Precautions also should be observed in moisture tests on tanners' corn sugar. These bleached sugars often contain as high as 1500 p. p. m. of sulfur dioxide and are subject to "creep" in drying. Ash content is also often a factor in the stability of the corn sugar in drying. The above data have been obtained on standard hydrolytic products of starch with normal ash contents.

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Refiners' Sirup, Final Molasses (Blackstrap), and Hydrol

THE success of the new moisture methods, previously described for the hydrolytic products of starch (*1–4*), caused the investigation to be extended to more troublesome sugar refinery products, such as refiners' sirup, blackstrap molasses, and hydrol, the molasses from the manufacture of dextrose. It is impossible, of course, to cover the complete range of such products, and those used for testing were considered typical. It is recognized that a more thoroughly exhausted blackstrap or material which had been subjected to inimical manufacturing conditions might not be comparable to the materials studied.

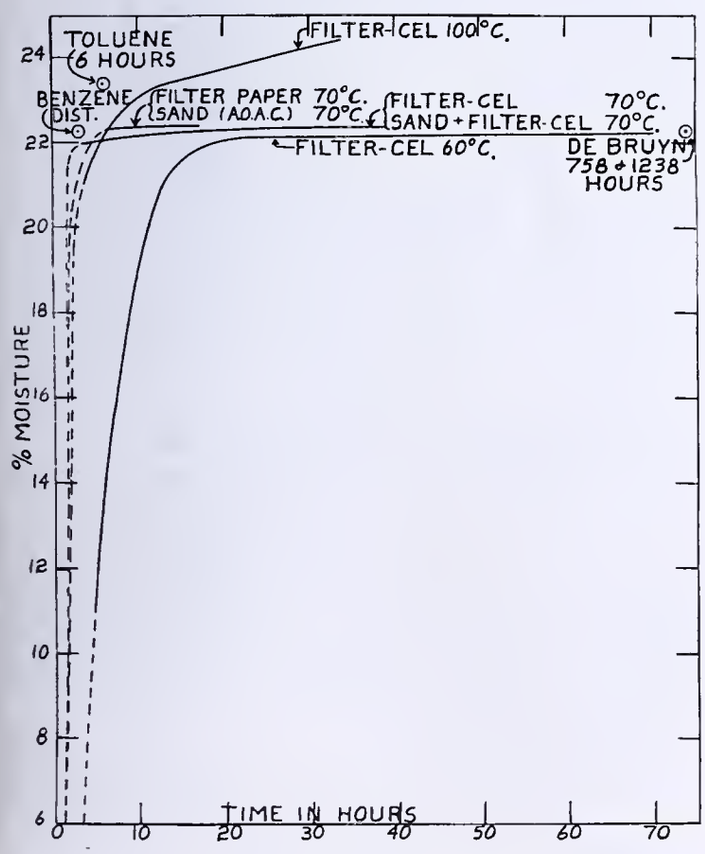

Figure 1. Data on Refiners' Sirup

The methods used have previously been discussed in detail (*1–4*). The A. O. A. C. sand method was used for comparison but altered in order to make it more workable. The moisture dish was aluminum with a tight-fitting cover. After the first weighing of the dried sample, the dish was closed tightly with the cover, inverted, and in order to loosen the sand, the bottom was tapped sharply with a spatula handle. Then the contents of the dish were shaken to assure a free-flowing material with a maximum exposure of surface. Constant weight was defined as a change of 1 mg. or less per 3-hour heating interval.

Refiners' Sirup

The sample used has the following analysis based on dry substance:

	%
Sucrose	44.20
Invert	47.10
Ash	2.70
Organic nonsugars	6.00

The data obtained are given in Table I and Figure 1.

At 70° C. in vacuum, the Filter-Cel method attained constancy. This value was checked by the following methods: filter paper, A. O. A. C., A. O. A. C. modified, A. O. A. C. sand and Filter-Cel mixture, modified Lobry de Bruyn Filter-Cel method, 38° C., and benzene Filter-Cel distillation. Data at 100° C., the Filter-Cel method in vacuum, and toluene distillation show evidence of decomposition.

Final Molasses (Blackstrap)

The molasses used in this test had the following analysis, dry-substance basis:

	%
Sucrose	44.08
Invert	22.62
Ash	12.93
Organic nonsugars	16.37

The data obtained appear in Table II and Figure 2.

Since the toluene distillation and 100° C. Filter-Cel method in vacuum gave decomposition on refiners' sirup, they were not tried on blackstrap, which is more susceptible to decomposition. The benzene distillation–Filter-Cel method; 70° C .

TABLE I. DATA ON REFINERS' SIRUP

Method	Temperature ° C.	Constancy	Time Hours	Moisture %	%
A. O. A. C. sand, vacuum	70	Doubtful	10	22.32	22.31
			14	22.30	22.32
			18	22.32	22.35
Filter paper, vacuum	70	Yes	8	22.32	22.31
			12	22.32	22.31
Filter-Cel, vacuum	60	No	70	22.22	22.23
Filter-Cel, vacuum	70	Yes	20	22.32	22.31
			25	22.33	22.34
			35	22.33	22.34
Filter-Cel plus sand, vacuum	70	Yes	15	22.30	22.32
			30	22.34	22.34
			39	22.34	22.35
Benzene	80–82	Yes	12	22.25	22.26
			20	22.29	22.31
			30	22.29	22.31
Lobry de Bruyn, vacuum	38	Yes	758	22.27	22.29
			1238	22.27	22.29
Filter-Cel, vacuum	100	No	8	22.92	23.04
			13	23.38	23.38
			18	23.66	23.71
Toluene	110–112	No	6	23.35	23.37
Filter-Cel, vacuum[a]	70	Yes	15	22.25	22.25
			21	22.25	22.26
			29	22.25	22.26
Carbon tetrachloride[a]	76–78	Yes	3	22.21	22.24
			18	22.21	22.24
			30	22.21	22.24

[a] The last two results were obtained months later and samples had lost some water during storage.

TABLE II. DATA ON BLACKSTRAP MOLASSES

Method	Temperature ° C.	Constancy	Time Hours	Moisture %	%
A. O. A. C. sand, vacuum	70	Yes[a]	20	28.50	28.51
			23	28.52	28.55
			26	28.52	28.55
Filter-Cel, vacuum	70	Yes[a]	21	28.57	28.57
			24	28.57	28.57
			27	28.57	28.57
Lobry de Bruyn	38	Yes	800	28.57	28.54
			900	28.57	28.54
Benzene	80–82	Yes	3	28.55	28.57
			18	28.55	28.57
Filter-Cel, vacuum	70	Yes	6	27.58	27.59
			12	27.60	27.60
			18	27.60	27.60
Carbon tetrachloride	76–78	Yes	3	27.62	27.61
			18	27.63	27.62

[a] Constancy was obtained by employing 3-hour heating intervals. Tests employing longer heating intervals failed to achieve constancy with final values exceeding Lobry de Bruyn values.

Filter-Cel vacuum; A. O. A. C. modified sand; and Lobry de Bruyn–Filter-Cel method at 38° C. all checked, indicating that the value obtained was probably the true moisture of the sample.

Hydrol

The hydrol which was tested had the following composition, dry-substance basis:

	%
Reducing sugars as dextrose	73.1
Dextrose (Sichert-Bleyer)	59.0
Ash	7.25
Crude protein	0.37

The data obtained are given in Table III. Again, close agreement was obtained. However, sufficient samples of hydrol have not been analyzed to state whether the moisture can be determined in all hydrol by the benzene–Filter-Cel method or by the 70° C. vacuum oven–Filter-Cel method.

Conclusions

Various moisture methods for determination of moisture in sugar by-products have been compared.

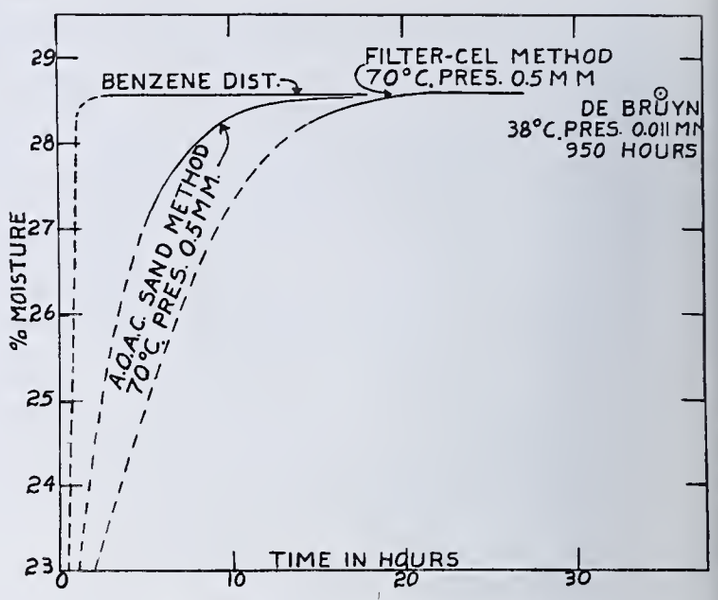

FIGURE 2. BLACKSTRAP MOLASSES

For determination of moisture, the 70° C. vacuum oven–Filter-Cel and the benzene distillation–Filter-Cel methods are recommended, except in possible cases where constancy cannot be attained by these. Recourse is had then to calibration by means of the modified Lobry de Bruyn–Filter-Cel method at 38° C.

The A. O. A. C. sand method, as modified, can be forced to give results comparable to those obtained by the other methods. It is not recommended by the authors, as much attention must be devoted to manipulation and a series of results may vary widely.

TABLE III. DATA ON HYDROL

Method	Temperature ° C.	Constancy	Time Hours	Moisture %	%
Filter-Cel, vacuum	70	Yes	15	21.90	21.88
			20	21.96	21.94
			25	21.98	21.96
			30	21.98	21.96
Lobry de Bruyn, vacuum	38	Yes	100	21.60	...
			300	21.75	...
			500	21.90	...
			600	21.91	...
			700	21.91	...
Benzene	80–82	Yes	20	21.89	21.92
			44	21.90	21.93
			68	21.91	21.93
			80	21.92	21.94
			100	21.94	21.92
Filter-Cel, vacuum[a]	70	Yes	15	26.18	26.18
			20	26.20	26.20
			25	26.20	26.22
			30	26.20	26.21
Carbon tetrachloride[a]	76–78	Yes	3	26.21	26.20
			18	26.22	26.20
			24	26.23	26.20

[a] Last two results were obtained on a different sample.

General Summary

In this series of five papers[1], a method of analytical procedure has been outlined for the determination of true moisture in those materials which, in the past, have been reported largely on a relative moisture basis. This technique consists of four essential points used in conjunction with the apparatus and procedures laid down:

1. Use of adequate dispersion of the sample, so as to expose maximum surface of the material in subsequent drying. By this means, water is removed quickly and completely. Quick re-

[1] Previous four can be found in IND. ENG. CHEM., ANAL. ED., **13**, 855, 858 (1941); *Ibid.*, **14**, 27 (1942); and this issue, page 124.

moval decreases the possibility of decomposition by reducing the material to the dry state in the minimum of time. Complete removal precludes the possibility of "slow creep", or inability to obtain constancy, which is defined as decomposition, loss of volatiles, etc.

2. Use of the modified Lobry de Bruyn technique of drying at 38° C., high vacuum, and extended time to obtain moisture and corresponding dry substance. Under these conditions, the temperature does not exceed that of normal storage conditions, and if decomposition is a factor, it may be expected to be present under storage conditions.

3. Use of the vacuum oven at 60° C. upwards, to determine a temperature constancy curve, whose final values correlate those obtained by the low-temperature drying method of 2. This technique involves high drying temperatures, high vacuum, and relatively short time. By it, the temperature limitations of the material are determined for this common laboratory drying practice.

4. Use of distillation methods to determine actual water. This procedure gives a range of temperature from 76° (carbon tetrachloride) to 140° C. (xylene), atmospheric pressure, and time limits which may be shorter than corresponding vacuum oven or, in any event, shorter than the modified Lobry de Bruyn technique. By distillation methods, the effect of vacuum in the removal of volatiles or other possible decomposition of the material should be substantially decreased if not entirely eliminated from the results. Thus, a drying curve should be obtained whose final values equal those of the Lobry de Bruyn or vacuum oven methods. If no correlation can be obtained, volatiles or decomposition may be considered a factor. In any event, a closer relative moisture value will be obtained.

Attention should be called to the interchanging features of the apparatus described. With use of standard taper joints, it is possible to oven-dry a sample and check the same sample further by distillation, or vice versa. Thus, it is often desirable, for example, to test a distillation sample upon completion by means of various oven temperatures for stability, etc.

The methods described in this series of papers have been tested and approved by various laboratories of the corn products industry through the Technical Advisory Committee of the Corn Industries Research Foundation, which have been most thorough in their criticism.

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Determining Liquid and Vapor Densities in Closed Systems

A Precise Method

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THE method proposed here for measuring densities makes use of a quartz bob suspended from a quartz helix. If the helix and bob are suspended in a homogeneous medium, the system will be buoyed up according to Archimedes' principle, causing a change in the length of the helix. Thus the length of the helix can be related to density by a calibration in a series of media of known density. Actually, it is possible to measure both liquid and vapor densities with the same bob and helix, using a separate calibration for each phase. Because of the limit imposed on the mass of the bob by the strength of the helix, this is best accomplished by using a bob with a density less than that of the liquid, so that the helix is stretched upward in the liquid when the helix assembly is totally immersed and the helix suspended from below. Total immersion in the phase whose density is being determined simplifies the buoyancy correction for the helix and eliminates errors due to surface tension.

The experimental arrangement for the measurement of both liquid and vapor density in the same system would then consist of a helix and bob suspended in a sealed glass tube, so that the helix assembly could be completely immersed in either phase, at will, by merely inverting the apparatus. This should serve as an excellent method for determining densities in closed systems where the absence of air and moisture is desired or where the pressure is above atmospheric. The method is also capable of high precision through the optimum choice of helix sensitivity, bob size, and measuring instrument. The chief drawback is that a considerable amount of labor is involved in the purification of the liquids and the measuring of the several lengths necessary for the calibration for each phase at each temperature; moreover, vapor densities are not accurately known over a wide range. However, by the extensive use of helices for density measurements in this laboratory, certain properties of quartz helices and methods of calibration have evolved which make the calibration much less laborious without sacrificing precision of measurement.

Theory and Method of Calibration

Relative Densities. The length to which a helix extends under the influence of gravity is a function of the temperature, T, and density, ρ, of the medium in which it is suspended, such that any change in length, ΔL, may be written

$$\Delta L = \left(\frac{\partial L}{\partial T}\right)_\rho \Delta T + \left(\frac{\partial L}{\partial \rho}\right)_T \Delta\rho \qquad (1)$$

The temperature and density coefficients of the helices used in this laboratory have been found to be constant over a 25° or greater temperature range, and over a considerable range of length, so that Equation 1 may be written for this range

$$\Delta L = a\Delta T + b\Delta\rho \qquad (2)$$

For an unloaded quartz helix, 4 × 0.9 cm., made from a fiber of 0.2-mm. diameter and consisting of 63 turns, $\left(\frac{\partial L}{\partial \rho}\right)_T$ is approximately 0.15 cm.[4] per gram. A 1-cc. quartz bob suspended from the helix increases this value to approximately 5 cm.[4] per gram. This sensitivity can be increased indefinitely (with a corresponding decrease in the range of densities which can be measured) by increasing the volume of the bob, and using a bob density only slightly different from that of the medium in order to avoid overloading the helix. By such means it is easily possible to measure small changes in density with a precision which cannot be justified by the possible precision of temperature control. Under normal load ($\simeq$ 1.1 gram in air) a helix of the dimensions given above will extend to approximately 9.0 cm. It should stretch to from 12 to 20 cm. before breaking. The temperature coefficient, a, is of a much lower order of magnitude than the density coef-

ficient, b, but it must be taken into account for precise work at different temperatures.

By measuring the length of the helix at different temperatures in media of known density, a and b can be found by solving the simultaneous equations resulting from Equation 2.

Equation 2 can be put into the useful form

$$\rho = \rho_s + \frac{a\,(T_s^0 - T^0) - (L_s - L)}{b} \qquad (2b)$$

Here L_s is the length of the helix in the standard medium of density, ρ_s, at the standard temperature, T_s^0, and L is the length in a medium of density ρ at a temperature T^0. Equation 2b expresses density in terms of the density of a standard medium and is applicable to both liquid and vapor density measurements, once a and b are known.

Absolute Densities. For the vapor, the calibration can be made practically independent of standard densities. The density coefficient, b, may be written

$$b = \left(\frac{\partial L}{\partial M}\right)_T \left(\frac{\partial M}{\partial \rho}\right)_T \qquad (3)$$

M is the apparent effective mass of the helix and bob as indicated by the length of the helix.

As with sorption balances (*2*), $\left(\frac{\partial L}{\partial M}\right)_T$ can be determined from the changes in length caused by the addition of small weights to a platinum bucket which is suspended from the helix in a medium of constant density and temperature. In this calibration the bob is removed and the calibration is extended over the range of length where $\frac{\partial L}{\partial M}$ is constant. The mass and volume of the bob should be such that all density measurements are made in this constant range.

$$\left(\frac{\partial M}{\partial \rho}\right)_T = \left(\frac{\partial M_b}{\partial \rho}\right)_T + \left(\frac{\partial M_s}{\partial \rho}\right)_T \qquad (4)$$

M_b and M_s are, respectively, the apparent effective mass of the bob and the spring and V_b and V_s are the corresponding volumes.

$$M_b = V_b\,(\rho_b - \rho) \qquad (5)$$

where ρ_b and ρ are the densities of the bob and the medium, and

$$\left(\frac{\partial M_b}{\partial \rho}\right)_T = -\,V_b \qquad (6)$$

This is the actual volume of the bob and its value can be determined by weighing in liquids of known density.

On the other hand $\left(\frac{\partial M_s}{\partial \rho}\right)_T$ is not the negative of the actual volume of the spring. The reason for this is that the apparent effective mass is obtained from L by means of a calibration which is made in terms of weights suspended from the lower end of the spring. The mass of the spring is not located at the lower end but is distributed over the entire length. If there are n turns in a perfect helix, any given element of mass located at turn t will stretch only the t turns which are above it, and the elongation produced will be only t/n times as great as was produced by the same mass in the calibration. If σ_s is the volume of quartz fiber in one turn of the helix, and ρ_s is the density of quartz, the element of mass is $\sigma_s\,(\rho_s - \rho)\,dt$ and the apparent effective mass of the spring is

$$M_s = \int_0^n (t/n)\,(\rho_s - \rho)\sigma_s\,dt = (\rho_s - \rho)\sigma_s\,(n/2) \qquad (7)$$

and therefore

$$\left(\frac{\partial M_s}{\partial \rho}\right)_T = -\,\sigma_s\,n/2 = -\,V_s \qquad (8)$$

This is one half of the actual volume of the helix. Thus V_s for a perfect helix may be determined from the weight of the helix and the density of fused quartz. It will be noticed that the factor $^1/_2$ will cause only one suspension end to be included in V_s. This is exactly what is desired, since the mass of the lower suspension end acts on all n turns, while the upper suspension end acts on none of the parts of the spring which is being measured. Although the helices used in this laboratory are slightly tapered, V_s as determined by this simple calculation has been found to agree within ± 0.002 cc. with the value V_s ($\simeq$ 0.025 cc.) determined experimentally. This corresponds to a 10 per cent error in the apparent effective volume of the helix, but introduces an error of only $0.2/V_b$ per cent in the measured density.

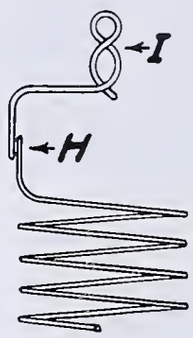

Figure 1

V_s is determined experimentally from length measurements on the unloaded helix in media of known density, and from Equations 3 and 4 is equal to $-\,b/\left(\frac{\partial L}{\partial M}\right)_T$ when the bob is absent. The value of $\left(\frac{\partial L}{\partial M}\right)_T$ for the unloaded helix must be determined by measuring the change in length caused by the addition of a small weight to the unloaded helix. This is necessary because the elongation per gram is different for small loads than for normal loads. A 0.02- to 0.03-gram piece of quartz fiber with a small hook for suspending from the suspension point of the helix (see Figure 1) makes a satisfactory weight. Any but a perfect helix will give two different values for V_s, depending on which end of the helix is up.

Combining Equations 2b, 3, 4, 6, and 8 and using the length of the helix loaded with bob in vacuum, L_s^0, at temperature T_s^0 as standard, we obtain for the density

$$\rho = \frac{a(T_s^0 - T^0) - (L_s^0 - L)}{-\left(\frac{\partial L}{\partial M}\right)_T (V_b + V_s)} \qquad (9)$$

Vapor or liquid densities calculated to the fourth significant figure from Equation 9 may be considered absolute, since the standard liquid densities necessary for determining V_b and V_s are known with this accuracy.

The size and density of the bob used will depend on the density of the material to be measured and the elastic properties of the helix. When helix and bob are to be used for both liquid and vapor density measurements, a bob density which is about the average of the densities of the two phases is preferred. This permits the use of a bob of larger volume, with correspondingly increased precision of measurement, without exceeding the load limit of the helix in either phase. The bob is blown in an oxyhydrogen flame from a quartz tube which has a diameter and wall thickness calculated to give the desired bob volume and weight, so that the extension in both phases falls within the constant range of $\left(\frac{\partial L}{\partial M}\right)_T$.

Precision

The validity of this method of calibration has been checked for both liquid and vapor. Using a helix of the dimensions given above, a bob having a volume of about 1.35 cc. and weight (in vacuum) of about 1 gram, and a measuring microscope (*3*) which could be read to 0.00006 cm., the density values for water from 10° to 35° C. were checked to 0.00003 gram per cc., using water at 25° C. as the standard liquid. With the same helix and bob assembly, vapor densities of sulfur dioxide in equilibrium with its solutions at 15°, 20°, and 25° C. checked with the densities calculated from vapor pres-

sure data to 0.00001 gram per cc., using the saturated vapor of pure sulfur dioxide as standard. Numerical data are reported in another paper (*4*).

For the precision with which these measurements were carried out, it was necessary to devise suspension points for both helix and bob so that the helix was never distorted when suspended, but hung freely. Precise indices which were out of the axis of the tube were also necessary for proper focusing. The cross point in a figure-eight loop of the helix fiber (*I*, Figure 1) was used to suspend the helix from a loop of 0.1-mm. (0.004-inch) platinum wire and likewise to suspend the bob from the helix. The indices (*H*, Figure 1) consisted of 0.5-mm. loops in the helix fiber at either end of the helix. They had sufficient curvature to permit the most accurate setting of the microscope cross hair at their outer edge and were in good enough alignment so that no change in the focus of the microscope was necessary when reading the two ends.

It is generally recognized that quartz helices exhibit no hysteresis after once being annealed. Quartz is also not easily corroded and has a very low thermal coefficient of volumetric expansion, 1.2×10^{-6} per ° C. For a 2-cc. quartz bob, and a change in temperature of 15°, the volumetric expansion amounts to 0.000036 cc. This is negligible for ordinary vapor density measurements but appreciable for precise measurements of liquid density, and will account for the small divergence which the authors have found in water. However, this effect can be eliminated by making the necessary correction for the change in the volume of the bob.

The volume of a hollow quartz bob of substantial wall thickness is also not greatly affected by pressures of the order of 4 atmospheres (approximate vapor pressure of sulfur dioxide at 25°). If the bob is sealed off at atmospheric pressure, the effect of 4 atmospheres is reduced to that of 3 atmospheres. Using Bridgman's (*1*) value for the compressibility of mineral quartz, the change in the volume of a 1-cc. bob would be 0.27×10^{-5} cc. per atmosphere. The error resulting from this cause can be reduced if necessary by determining the approximate compressibility of the bob before it is sealed off.

It is believed that this method will be found useful for the precise measurement of densities in many systems which are not accessible to measurements by the usual methods.

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Heats of Wetting for the Evaluation of Gas-Adsorbing Coconut Carbons

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THE use of heat of wetting as a means of evaluating gas-adsorbing carbons has been suggested by several authors in recent years (*1*, *7*), and restricted semianalytical methods have been developed by Macy (*8*) and Burstin and Winkler (*4*). This method has the distinct advantages, in comparison with other methods, of extreme rapidity and satisfactory accuracy, and by suitable correlation affords data as to other analytical characteristics of the carbon, as is shown below.

A large number of methods for evaluating the gas-adsorbing qualities of carbons have appeared in the literature, most of which have been critically discussed by Chaney, Ray, and St. John (*5*), and by Berl and Herbert (*3*). The majority of these methods are time-consuming and not overly indicative of the value of a carbon for a particular purpose. As has been pointed out by Chaney, Ray, and St. John (*5*), the most reliable analytical data pertaining to gas-adsorbing carbons are:

1. Service Time. Time required for the break-through of a vapor under standard conditions.
2. Adsorption Value. The weight of a vapor adsorbed by a carbon under conditions of saturation.
3. Retentivity Value. The weight of vapor held by a carbon through secondary valence bonds, under standard conditions.

All these data are more or less arbitrary, yet under suitable conditions they afford the most reliable evidence obtainable at present as to the gas-adsorbing characteristics of a given carbon. The chief criticism of these methods arises from the lack of definition of terms and of precise descriptions of the techniques which have appeared in the literature. It is only by a rigorous standardization of the analytical procedures that quantitative results can be achieved and duplicated. Toward this end, the authors have sought to standardize their methods as thoroughly as possible.

Macy (*8*) and later Bell and Philip (*2*) have shown the existence of a quantitative relation between the heat of wetting and the retentivity value of a specific carbon, but the possibility of a quantitative relation between adsorption value and heat of wetting has been a matter of considerable dispute. Burstin and Winkler (*4*) first suggested such a relation, but, unfortunately, their data were insufficient to warrant such a conclusion. Macy (*8*), as the result of the analysis of a large number of different carbons, found several rather large discrepancies, and, therefore, stated that no correlation between adsorption value and heat of wetting could exist. Other authors have either been undecided (*2*) or have confirmed in a degree (*3*) the data of Burstin and Winkler.

No correlation of heat of wetting with the service time of a carbon has been found in the literature.

In view of evidence which has been obtained by the authors, a definite relation between the heat of wetting of a carbon as determined under certain conditions as stated and the other analytical characteristics listed is postulated. This relation, however, is limited to specific types of carbon—that is, to carbons which have a common raw material source. While the present paper concerns itself with gas-adsorbing carbons derived from coconut, since this is the most efficient and most common type in use at present, nevertheless the procedure, with suitable modification, should be applicable to other common-source carbons.

The question of the effect of varying the method of activation on the correlation of these characteristics of common-source material carbons has been raised. Of the thirteen coconut carbons used in this work, five are known to have been activated by the same method. The other eight carbons were commercial carbons from various sources, whose method of activation was not known. Two of these latter carbons were prepared by high-temperature steam treatment

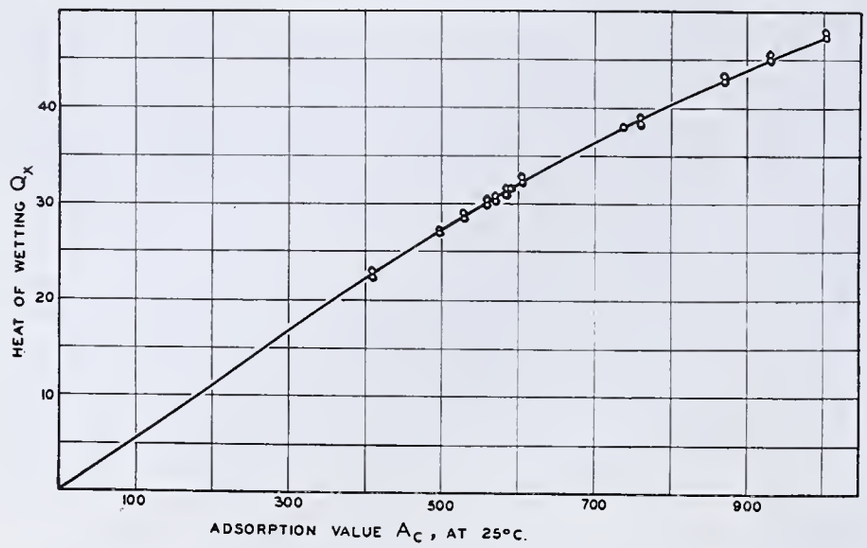

FIGURE 1. RELATION BETWEEN ADSORPTION VALUE FOR CARBON TETRACHLORIDE AND HEAT OF WETTING IN *m*-XYLENE

of a spent carbon which had been used to remove hydrocarbons from carbon dioxide. The temperatures of stripping were 425° and 535° C. The stripped carbons possessed a higher degree of activation than the original source carbon.

Pearce and McKinley (*9*) found the relation between adsorption value and the heat of adsorption (vapor phase) to be given by the expression:

$$Q = mX^n$$

where Q is the heat evolved in calories per gram of carbon, X is the milliliters of a given vapor adsorbed, and m and n are constants, depending upon the vapor used. Burstin and Winkler (*4*) found the following equation:

$$A_b = 19\,Q_b$$

where A_b is the adsorption value in milligrams of benzene adsorbed per gram of carbon, and Q_b is the heat of wetting for benzene in calories per gram of carbon. Both equations were derived from the analysis of a very limited number of carbons, and, therefore, their general applicability is slight.

Previous workers (*2, 4, 8*) have determined the heat of wetting of carbon by benzene, and compared these data with the adsorption value for benzene. The present writers have found the use of *m*-xylene for the determination of heats of wetting to offer several advantages—notably, a decreased volatility, a constant specific heat over the temperature range used, and approximately 50 per cent higher values for the heat of wetting. Because of the difficulties inherent in the use of the accelerated chloropicrin service time method, it was found desirable to use the accelerated carbon tetrachloride service time method (*6*) with suitable modifications. The use of this latter method makes possible a saving of time, since the service time and adsorption value determinations may be made successively.

Results of the analysis of thirteen samples of coconut carbons have led to the following empirical relations:

$$A_c^{25} = 17.9\,Q_x + 5.8 \times 10^{-6}\,(Q_x)^{4.43}$$
$$S_c^{20} = 1.34\,Q_x$$
$$S_c^{25} = 1.16\,Q_x$$
$$R_c = 9.38\,Q_x$$

In the above equations:

A_c^{25} = adsorption value for carbon tetrachloride (*4*) in mg. adsorbed per gram of carbon at 25° C.
S_c^{20} and S_c^{25} = service times for chloropicrin in minutes at 20° and 25° C., respectively
R_C = retentivity value for carbon tetrachloride in mg. retained per gram of carbon
Q_x = heat of wetting in *m*-xylene, in calories evolved per gram of carbon

Throughout this article, except as otherwise indicated, "chloropicrin service time" refers to the chloropicrin service time value as calculated from the carbon tetrachloride service time through the use of an integer factor. This factor has appeared in the literature with a value of 3.0 (*6*), but as the authors have used a 15.0-cm. column length of carbon in the service time determinations instead of 10.0 cm., the value of 2.0 has been used in the present work.

The results of the analyses are presented graphically in Figures 1, 2, and 3, and summarized in Table I.

Experimental

SERVICE TIME AND ADSORPTION VALUE. For the determination of these values, modifications of the methods of Chaney and of Fieldner (*5, 8*) are used. The apparatus is shown in Figure 4. The carbon tetrachloride is dried and redistilled before use, reserving the fraction boiling at 76–77.5° C.

TABLE I. ANALYSES OF COCONUT CARBONS

AD	A_c^{25}	R_c	S^{20}	S_c^{25}	Q_x
0.52	528	268	38.5	33.4	28.6
0.49	589	300	42.6	37.0	31.7
0.54	497	252	36.8	31.6	27.1
0.52	571	291	41.0	35.8	30.7
0.52	605	302	43.2	37.4	32.3
0.48	736	362	51.0	44.0	38.1
0.43	930	421	61.2	53.0	45.2
0.53	562	293	40.4	35.0	30.3
0.54	410	214	30.4	26.5	22.7
0.48	586	291	42.0	36.4	31.5
0.45	758	363	52.6	45.4	38.9
0.44	872	405	58.0	50.2	43.1
0.38	1004	454	64.2	55.4	47.5

AD = apparent density of 8- to 14-mesh carbon in grams per ml. Values for Q_x represent average of from 5 to 10 determinations, with a maximum deviation from the mean of 0.2 calorie.

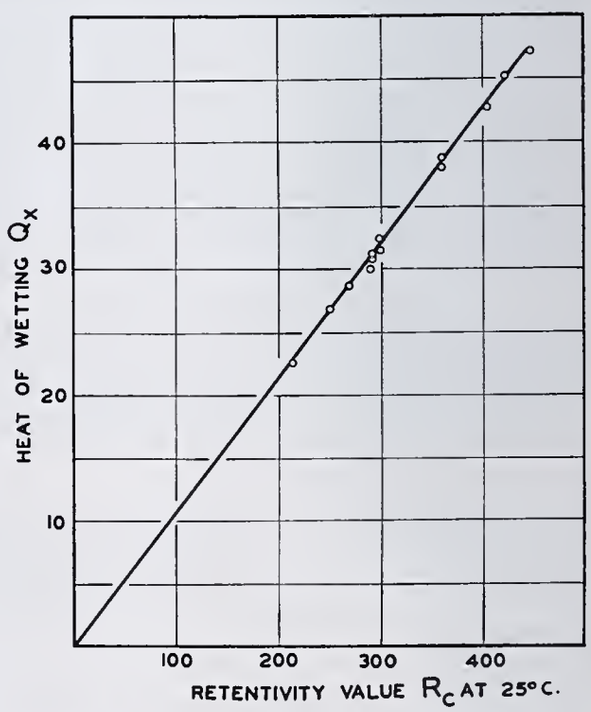

FIGURE 2. RELATION BETWEEN RETENTIVITY VALUE FOR CARBON TETRACHLORIDE AND HEAT OF WETTING IN *m*-XYLENE

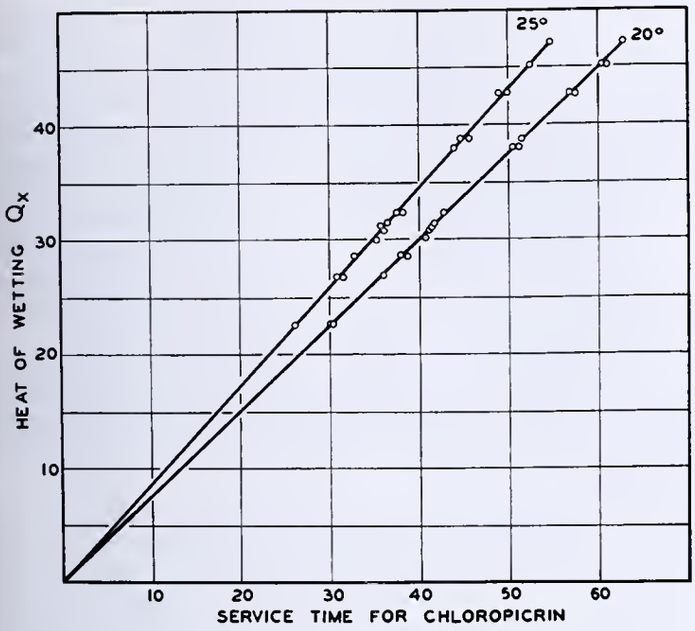

FIGURE 3. RELATION BETWEEN SERVICE TIMES AT 20° AND 25° AND HEAT OF WETTING IN *m*-XYLENE

A composite sample of the carbon is screened to 8- to 14-mesh on standard screens, dried 4 hours at 140° to 150° C., and cooled in a desiccator. A volume of the dried carbon (about 25 ml.), sufficient to give a tamped column length of 15.0 cm. in the adsorption tube, is transferred to the adsorption tube and the tube and contents are weighed to 1 mg. The carbon is then tamped by tapping lightly on a level wooden surface until no further diminution in volume is noted, at which point the column of carbon in the adsorption tube should measure exactly 15.0 cm. in length. This column length of 15.0 cm. should be reproducible upon inverting the adsorption tube and retamping the carbon. A simple method of achieving this result involves the use of a flat-bottomed test tube 1.3 cm. in inside diameter, and calibrated at 15.0 cm., for the measurement of the carbon before insertion into the adsorption tube. The adsorption tube is placed in position in the thermostat, which is controlled at 25.0°. Sufficient time is then allowed (about 30 minutes) for the carbon to reach temperature equilibrium with the thermostat.

An air-flow rate of 650 ml. per minute (490 ml. per minute, per sq. cm. of cross-sectional area of the carbon) is established through the apparatus, with the three-way stopcock turned so as to vent the carbon tetrachloride–saturated air to a hood. The stopcock is then turned to allow the carbon tetrachloride–air mixture to pass through the adsorption tube, the initial starting time is noted, and any necessary flow-rate adjustments are quickly made.

The first appearance of a greenish tinge in the burner flame (which is adjusted to a height of about 7.5 cm., 3 inches) is taken as the breakdown point, and the elapsed time recorded as "accelerated carbon tetrachloride service time". The adsorption tube-to-burner connection is then broken and the effluent carbon tetrachloride–air mixtures vented to a hood.

Passage of the carbon tetrachloride–air mixture is continued, with weighings of the adsorption tube each half hour, until no further gain in weight is evident (3 to 5 hours). The gain of carbon tetrachloride in milligrams per gram of carbon is recorded as the adsorption value.

The "accelerated carbon tetrachloride service time" as determined above is multiplied by 2.0 (*6*) to convert to the approximate corresponding "chloropicrin service time".

Precautions must be taken to ensure a constant 0° C. temperature of the carbon tetrachloride in the cooling bath, as well as a constant air-flow rate throughout the determination, since small variations in these factors will introduce relatively large errors.

RETENTIVITY VALUES. These values are determined by a modification of Macy's method (*8*).

A 3- to 4-gram sample of the saturated carbon from the adsorption value determination is transferred quickly from the adsorption tube to a glass-stoppered weighing bottle and weighed. The glass stopper is removed and the weighing bottle is placed in a vacuum desiccator lubricated with de Khotinsky cement which is in turn placed in an oven maintained at 90° to 100° C. The desiccator is connected to a vacuum pump and the carbons are evacuated at 2-mm. pressure, with periodical weighings, until the weight loss amounts to not more than 15 mg. over a period of 0.5 hour. The retentivity value is determined by graphing the percentage weight of vapor retained against time, and extending the portion of the curve representing low rate of vapor loss, which is approximately a straight line, back to the retentivity axis, as is shown in Figure 5.

The retentivity values may also be determined by evacuation at lower temperatures if wished, with achievement of comparable results. Lowering the temperature greatly extends the amount of time required to complete a determination, however—for example, evacuation at 25° requires 90 to 100 hours for the attainment of retentivity values, while at 100° 3 to 5 hours are required, as shown in Figure 5.

HEATS OF WETTING. A cylindrical silvered Dewar flask, 10 cm. high by 3.0 cm. in inside diameter, is used as a calorimeter. A 15° to 45° thermometer, graduated in tenths or twentieths of a degree is supported above the Dewar flask in such a manner that the thermometer bulb is about 5 mm. above the bottom of the flask. The thermometer is calibrated for 40-mm. immersion.

The heat capacity of the assembly is determined by the heat of neutralization of sodium hydroxide and hydrochloric acid using the value $H_N \cong 13{,}895$ calories at 20° (*10*). The authors' calorimeter, of the type outlined, had a heat capacity of 5.0 calories per degree.

A 25.00-ml. portion of dry *m*-xylene (boiling point 137–9°) is pipetted into the calorimeter, and the temperature of the cal-

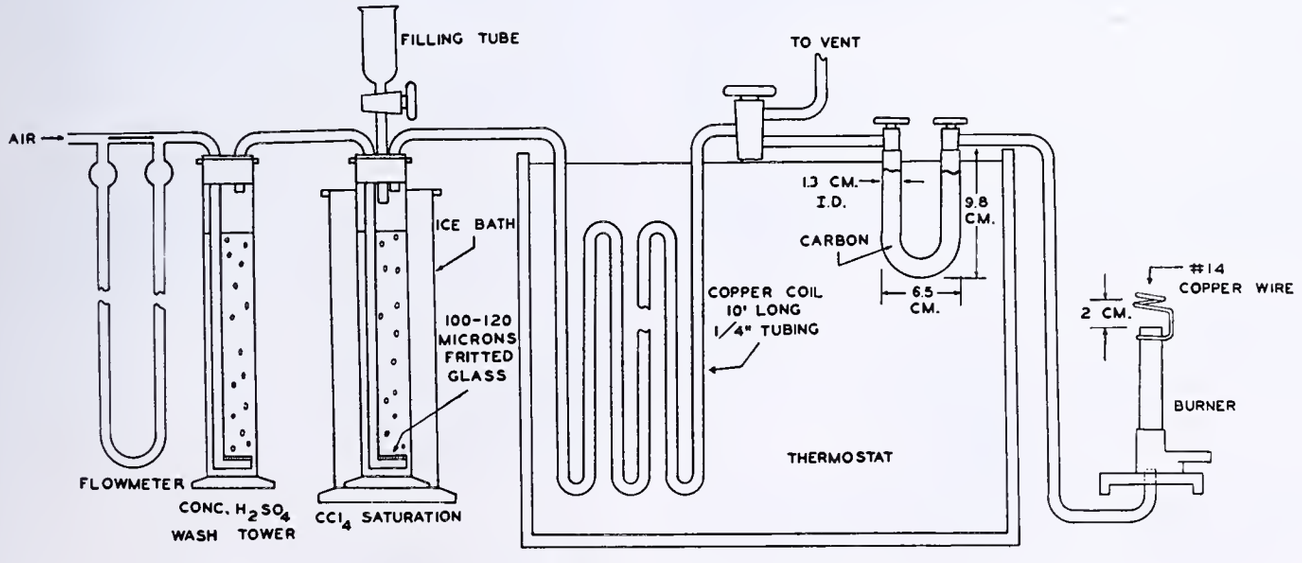

FIGURE 4. APPARATUS FOR DETERMINATION OF ADSORPTION VALUES AND SERVICE TIMES

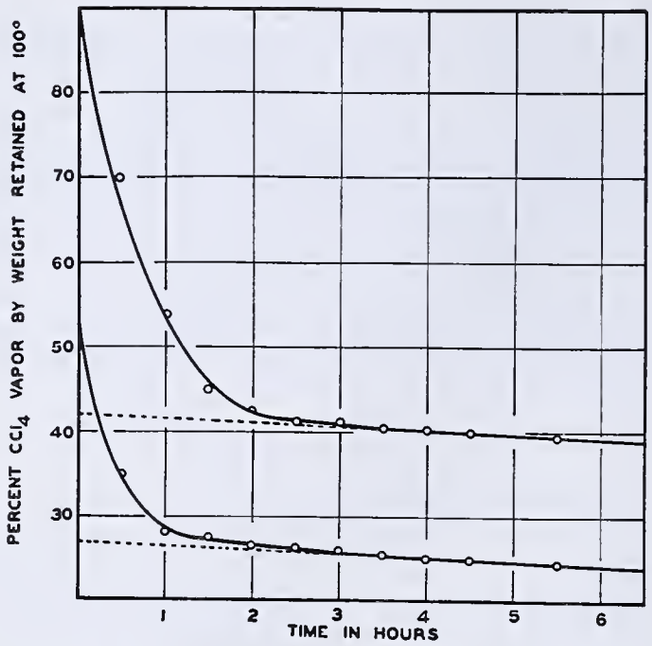

FIGURE 5. GRAPHICAL METHOD FOR DETERMINATION OF RETENTIVITIES

orimeter and contents is adjusted to a predetermined value, identical with that of the carbon sample to be tested, by means of small test tubes containing hot or cold water. This temperature is then recorded to within 0.02° as "initial xylene temperature".

A sample of the carbon to be tested (previously screened to 8- to 14-mesh and dried 8 hours at 140°) is weighed to the nearest milligram and added rapidly to the calorimeter. The contents of the calorimeter are mixed by a gentle rotation of the thermometer until a maximum temperature is reached. This temperature is read to within 0.02° and recorded as "final temperature".

Unused *m*-xylene may be recovered by transferring the contents of the calorimeter, at the conclusion of the run, to a No. 5 Whatman filter. The calorimeter should be wiped dry with a clean, lintless cloth between each run.

The temperature of the carbon should be known with an accuracy of 0.3° C. prior to its addition to the calorimeter. If it differs from the initial xylene temperature by more than 0.3°, the calculation of the initial mixing temperature of the carbon-xylene mixture from the known initial xylene temperature and the temperature of the carbon is then made, and this value subtracted from the final temperature and recorded as T_r, the temperature rise. From the known heat capacity of the calorimeter, H, and the weight of carbon used, m, the corresponding heat of wetting, Q_x, of the carbon for *m*-xylene in calories per gram of carbon is given by means of the formula:

$$Q_x = \frac{T_r}{m}(H + 8.32 + 0.17\,m) \text{ calories per gram of carbon}$$

The figure 8.32 is derived from the weight and specific heat of the xylene used, and 0.17 is the specific heat of carbon. This equation holds true within the temperature range of 18° to 35° C., since within this range variations in the specific heat and density of xylene are neglible.

The weight of dry carbon used in the above determinations is adjusted to give a temperature rise of from 3° to 4°. The room used for conducting the tests should be as free from drafts and rapid temperature changes as is possible.

Discussion

The authors have found that the proposed modified methods for the analysis of a gas-adsorbing carbon give more accurate and reproducible results than do the standard procedures. In the determination of adsorption values and service times, the use of fritted-glass bubbler tubes ensures saturation equilibrium between the air and the carbon tetrachloride. Similarly, by circulating the air–carbon tetrachloride mixture through a copper coil immersed in the thermostat prior to carbon exposure, and by the use of a thermostated adsorption tube, discrepancies due to heat effects are eliminated. The modified adsorption tube permits thermostating during a determination, and by means of the stopcocks prevents vapor loss during a weighing. Further, by making use of a longer carbon column for service time determinations (15.0 cm. instead of 10.0 cm.), the observed service time is lengthened and the effect of experimental error thus decreased.

The method of determining retentivity values has been modified by the use of weighing bottles and a vacuum desiccator in the procedure, to permit more ease of manipulation and the simultaneous analysis of a large number of samples.

Especially notable is the large increase in accuracy and reproducibility in the heat of wetting determinations, through the use of an improved calorimeter and the substitution of *m*-xylene for benzene as the wetting liquid. Heats of wetting obtained by the proposed method average about one and one-half times as large as those obtained by the standard Chemical Warfare Service method, on the same carbon. The comparison of these *m*-xylene heats of wetting with the corresponding service times, retentivity values, and adsorption values, as derived from the proposed procedures, leads to straight-line or smooth-curve functions. This statement, however, does not hold true for the standard methods of analysis when compared with the heats of wetting as determined according to C. W. S. specifications.

Using the modified procedures, duplication of results can be achieved in the determination of retentivity and adsorption values to within ±0.5 per cent. Constancy of service time values is dependent not only upon the temperature of the carbon tetrachloride saturation tower, and of the thermostat, but upon gas-flow rate per unit cross-sectional area of carbon and other factors such as the packing of the carbon and the observance of the first trace of carbon tetrachloride break-through. For this reason, some slight skill is required to duplicate results to within ±0.25 minute. The heats of wetting are readily duplicable to within 0.3 calorie. In accord with the proposed method of analysis for calculating service times, adsorption values, and retentivity values from the corresponding heats of wetting in *m*-xylene, the average mean deviation in the heats of wetting—namely, 0.2 calorie—will lead to a maximum error in the calculated values of the other analytical characteristics of ±0.25-minute service time, and less than ±5 mg. in adsorption and retentivity values. It is therefore apparent that the heat of wetting not only is more rapid and requires less skill than other analytical procedures for the evaluation of a carbon, but also yields results of equal accuracy.

Summary

An improved method has been developed for evaluating gas-adsorbing coconut carbons by means of the relation between the heat of wetting and other analytical factors. Equations enable calculation of service time, retentivity values, and adsorption values from the heats of wetting of coconut carbons. Modified methods for the determination of service times, adsorption values, retentivity values, and heats of wetting are given. Advantages of the heat of wetting method are its rapidity, its small requirements of equipment, and its quantitative characterization of a coconut carbon.

Acknowledgment

The authors are indebted to A. B. Ray of the Carbide and Carbon Chemicals Corporation for supplying six of the car-

bons used in this work, and for supplying certain data regarding these carbons.

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Turbidimetric Determination of Polymerized Hydrocarbons in Solution

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IN DEALING with unsaturated hydrocarbons subject to polymerization it is frequently necessary to determine the polymer content in a solution of the monomer. As the polymer is usually relatively high boiling, the monomer may be flash-distilled under vacuum and the residue weighed and considered polymer. This method is more or less inaccurate, especially for small quantities of polymer.

Some users of these materials have empirical specifications for maximum polymer content based on the insolubility of the polymer in methanol or petroleum naphtha. If a given dilution of the sample with one of these solvents does not produce a distinct cloud of precipitate, the polymer content may be assumed to be below the predetermined value. No mention has been found in the literature, however, of extending this principle to give an accurate analytical method.

To obtain a rapid and accurate analytical method for the analysis of polystyrene in solution of styrene and other aromatics, the dilutions for maximum sensitivity of the sample with methanol and petroleum naphtha were determined. It was found that two parts of absolute methanol added to one part of sample will give a distinct cloud when 0.0005 per cent of polymer is present in the original sample, and nine parts of Atlantic Refining Co.'s No. 44 naphtha added to one part of sample will give a distinct cloud when 0.005 per cent of polymer is present.

Furthermore, at these dilutions the effect on the polymer solubility of small changes in temperature is small. Analyses are normally performed at a room temperature of 20° to 25° C., but it was found that differences in results are negligible between 15° and 30° C.

Using these optimum dilutions, a set of standards was made up covering the range of 0.0005 to 0.3 per cent polymer directly. Higher polymer contents could be measured by first diluting the sample 10 to 1 or 100 to 1 with xylene. As had been feared, however, the standards containing the higher polymer concentrations deteriorated in a few weeks' time, owing to the settling of the precipitated polymer.

Some means of measuring the turbidity was then sought. Measurement of the height of a column of the liquid through which fine print could be read (as used in the kauri-butanol test) was tried, but great differences were found between different operators' ability to distinguish the fine print through the turbid liquid.

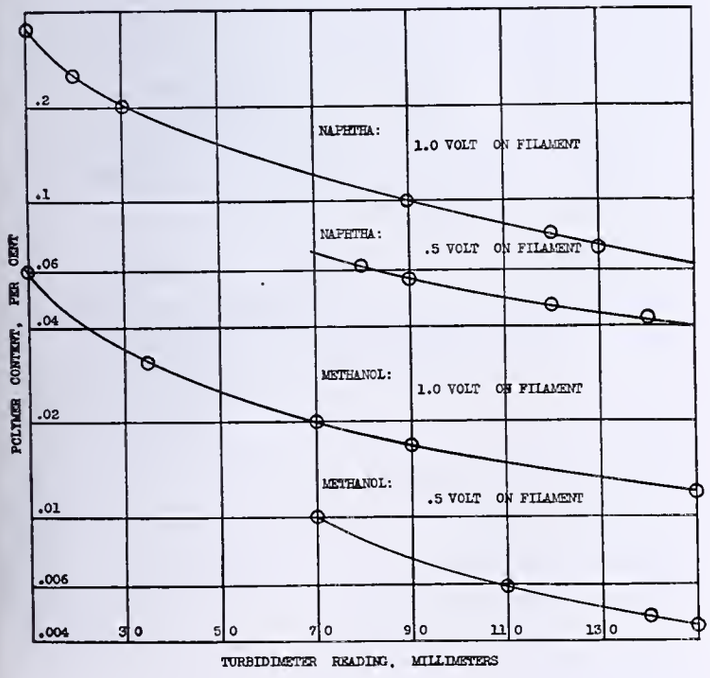

FIGURE 1

A Burgess-Parr sulfur turbidimeter (Arthur H. Thomas Co. catalog No. 9334) proved to be a satisfactory instrument for the measurement. A column of liquid is placed above an incandescent filament of standard (adjustable) intensity and a movable tube dipping into the liquid indicates the point of extinction of the filament by the turbid liquid.

Figure 1 shows per cent of polymer corresponding to turbidimeter readings for 2 to 1 methanol dilution and 9 to 1 No. 44 naphtha dilution. For low polymer concentrations, 200 ml. of absolute methanol are added to 100 ml. of sample in a stoppered cylinder and mixed by inverting several times. After 15 minutes the mixture is poured into the turbidimeter and a reading is taken using 0.5 or 1 volt on the filament, depending on the turbidity. For somewhat higher polymer concentrations, 180 ml. of No. 44 naphtha are added to 20 ml. of sample and the same procedure is followed. For polymer concentrations above the scale, 10 to 1 or 100 to 1 dilutions of the original sample with toluene or xylene may be used.

The turbidity increases during the first few minutes after adding the methanol or naphtha, but approaches a maximum after approximately 10 minutes. The 15-minute period was chosen so that time differences of a minute or two in making readings make no appreciable difference on the result.

A number of other polymers are known to be precipitated from a solution of the monomer by methanol and certain other solvents and it is believed that this method could be readily adapted to polymer analysis in such cases.

Polarographic Determination of Nitrates in Blood and Urine

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COLORIMETRIC methods have been used successfully for the determination of small amounts of nitrate in water and food extracts, but in the authors' experience the application of these methods to biological materials such as blood and urine has not been satisfactory. Whelan's method (*4*) was also tried, but the difficulty of preventing atmospheric oxidation of the reagent, diphenylbenzidine, rendered this procedure unsuitable. The use of the polarograph for the determination of nitrates has been reported by Tokuoka (*3*) and has been recently discussed by Kolthoff and Lingane (*2*). After a suitable procedure was developed for the removal of proteins and interfering substances, this method was used for blood and urine filtrates, with only slight modifications.

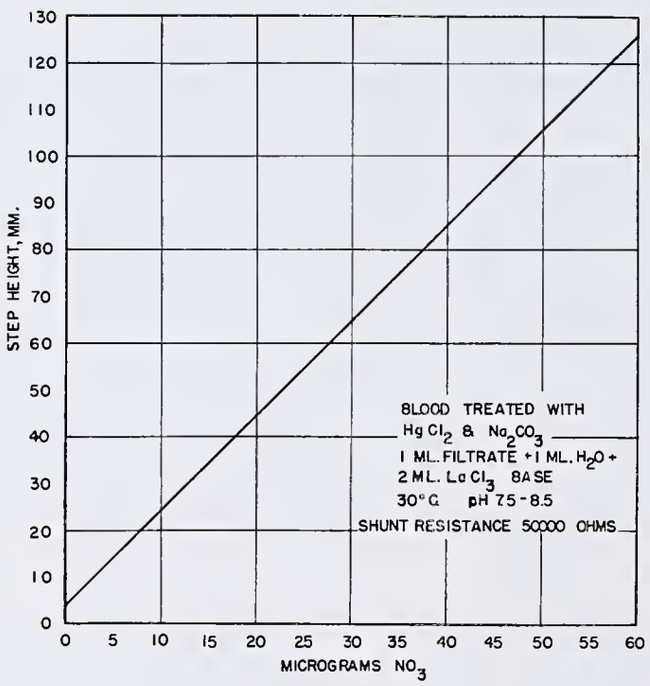
STEP HEIGHT, MM.
MICROGRAMS NO_3
BLOOD TREATED WITH
$HgCl_2$ & Na_2CO_3
1 ML. FILTRATE + 1 ML. H_2O +
2 ML. $LaCl_3$ BASE
30° C. pH 7.5 - 8.5
SHUNT RESISTANCE 50000 OHMS

FIGURE 1

The apparatus employed is a Leeds & Northrup Electro-Chemograph, equipped with a Micromax recorder and the dropping mercury electrode and electrolysis cell which were supplied with the instrument. Galvanometer sensitivity ranges of 0 to 80 and 0 to 8 microamperes are used. The estimations are made graphically by comparing the step height obtained with the samples to those obtained by adding known amounts of nitrate to blood and urine filtrates which previously showed no nitrate step. The standard charts prepared in this way are shown in Figures 1 and 2.

The blood filtrates are prepared by mixing 0.5 ml. of fresh blood with 3.5 ml. of water, and adding 0.5 ml. of mercuric chloride solution (5 grams per 100 ml.) and 0.5 ml. of sodium carbonate solution (1 gram per 100 ml.). After filtering, 2 ml. of the filtrate are acidified with 1 ml. of 0.4 *N* hydrochloric acid, heated to boiling, cooled, and made alkaline with 1 ml. of 1 *N* sodium hydroxide. One milliliter of this solution, which is equivalent to 0.05 ml. of blood, is placed in the electrolysis cell and mixed with 1 ml. of water and 2 ml. of 0.1 *N* lanthanum chloride, and nitrogen is bubbled through the solution for 5 minutes. The current set up by electrolyzing this solution at 30° over a range of 1.0 to 1.8 volts is recorded and the height of the nitrate step is measured from this chart. The method of measurement was that employed by Cholak and Bambach (*1*). The step height in the case of urine samples was slightly higher (3 to 5 per cent) for a given amount of nitrate. Separate standard curves were therefore used for urine and for blood.

The urine filtrate is prepared by mixing 0.5 ml. of fresh urine with 0.5 ml. of mercuric chloride solution (5 grams per 100 ml.) and 5.0 ml. of saturated barium hydroxide solution, diluting to 10 ml. with water, and filtering. One milliliter of this filtrate is used in the electrolysis cell under the conditions described above for the final blood filtrate.

The lanthanum chloride solution is prepared by dissolving lanthanum oxide (obtained by ignition of the acetate) in a slight excess of hydrochloric acid. A large excess of acid is avoided, since it has to be neutralized by the filtrate used. The nitrate step appears only in neutral or alkaline solutions. The half-wave potential of the nitrate step is 1.33 to 1.39 volts in the case of blood filtrates, and 1.47 to 1.53 volts in the case of urine filtrates, when referred to the standard calomel electrode. The difference in half-wave potential was probably due to a difference in supporting electrolytes.

When the higher sensitivity of the galvanometer is used, it is possible to determine as little as 0.5 microgram of nitrate in 1 ml. of the electrolysis mixture, which is equivalent to 40 micrograms of nitrate per milliliter of blood. The full sensitivity of the instrument cannot be employed because of the salts and other substances present in the filtrates. With pure solutions of nitrate it should be possible to determine as little as 0.1 microgram in 1 ml.

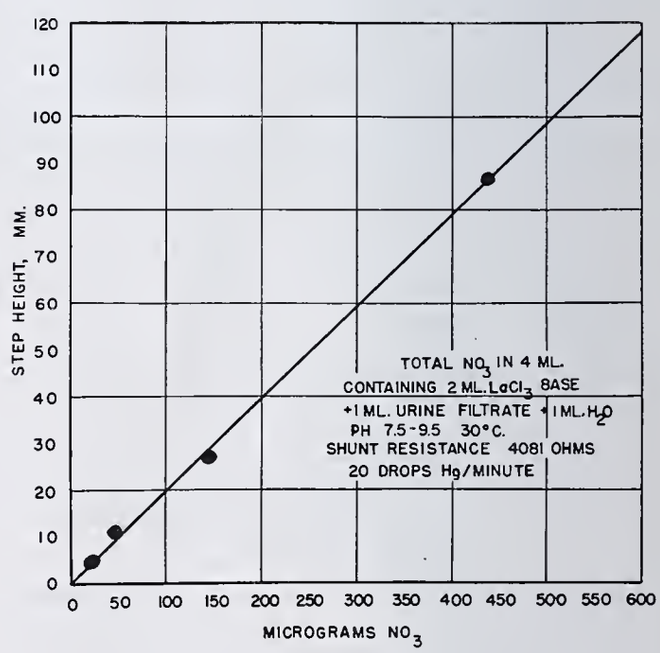
STEP HEIGHT, MM.
MICROGRAMS NO_3
TOTAL NO_3 IN 4 ML.
CONTAINING 2 ML. $LaCl_3$ BASE
+ 1 ML. URINE FILTRATE + 1 ML. H_2O
PH 7.5-9.5 30°C.
SHUNT RESISTANCE 4081 OHMS
20 DROPS Hg/MINUTE

FIGURE 2

Methods other than those described above for the preparation of the blood and urine filtrates were studied before these procedures were adopted. Most of the common protein precipitants were tried, but in every case the final solutions contained residual amounts of these precipitants, which interfered in the electrolysis. Urea does not cause interference but uric acid, which is present in human urine, does, unless it is removed.

The method of polarographic determination here reported has so far been applied only to rabbit blood and urine. The appearance and increased concentration of nitrate in both fluids after the oral or intravenous ingestion of certain aliphatic nitro compounds were successfully followed with this procedure.

Results obtained will be reported elsewhere.

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Determination of Combined Nitrogen in Steel

A Rapid Method

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A rapid method for determining combined nitrogen in steel utilizes a micro-Kjeldahl steam-distillation unit and a photoelectric spectrophotometer in conjunction with the well-known Nessler color reaction. The method is accurate and can be used for micro-, semimicro-, or macro-samples. The procedure is suitable for investigational work and has been in use for routine determinations for approximately one year with appreciable economy of labor and time. It is applicable to all steels containing acid- or alkali-soluble nitrogen compounds and may be modified to accommodate materials containing nitrogen in a difficultly soluble form.

NITROGEN is believed by many investigators to exercise a considerable influence on the physical properties of steel. The large number of determinations necessary for control purposes and to provide data for statistical studies make it essential to have an analytical method which, without sacrifice of accuracy, will reduce to the minimum the time and labor of a single nitrogen determination.

The Allen and the vacuum fusion methods are the most commonly used for determining nitrogen in steel. Neither is sufficiently rapid to use for control purposes nor to care adequately for the large numbers of routine determinations made necessary by present-day quality requirements. The experience of many industrial and research laboratories has indicated the value of micro and semimicro analytical methods where rapid accurate determinations are required. Using a micro-Kjeldahl unit designed to utilize steam-distillation in removing ammonia from alkaline solution, Klinger and Koch (*5*) developed a micromethod for determining the nitrogen content of steel surfaces. The method was sensitive and gave accurate results but, because of the large volume of reagents used, substantially the same time was required for a single determination as in the regular macroprocedure. Most of the micro-Kjeldahl units available commercially are not suitable for determining the nitrogen content of a series of samples in rapid sequence.

The purpose of this paper is to describe a rapid and accurate method developed in this laboratory for determining combined nitrogen in carbon steel. The method utilizes a standard micro-Kjeldahl unit which is suitable for rapid determinations either singly or in series. The ammonia in the distillate is determined by means of the color reaction introduced by Nessler. A photoelectric spectrophotometer is used to measure the intensity of the ammonia-Nessler color complex. The procedure may be applied to steels for which the usual macromethods are suitable and is also suitable for small quantities of material for which macroprocedures are not applicable. Modifications of the method are necessary for pig irons and alloy steels containing nitrogen compounds not readily soluble in acid or alkali.

The chemical reactions for the microprocedure are essentially the same as for the macroprocedure, except that the

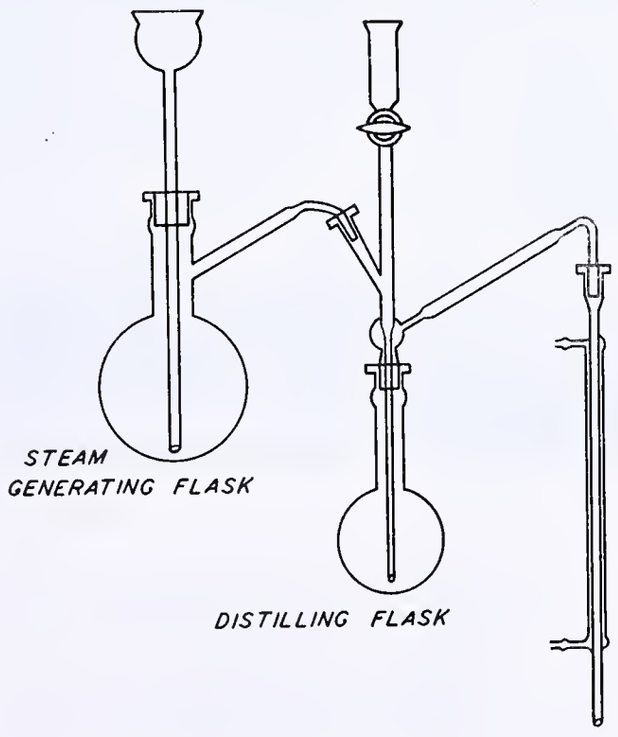

Figure 1. Diagram of Micro-Kjeldahl Unit

reagents are used in a more concentrated form. Nessler's reagent is used in place of standard solutions of acid and alkali.

Reagents

Hydrochloric acid, one part of acid, specific gravity 1.19, added to one part of ammonia-free water.

Sodium hydroxide, 600 grams dissolved quickly in 1 liter of ammonia-free water.

Nessler's reagent (*1*). Fifty grams of potassium iodide are dissolved in a minimum volume of cold water (approximately 35 ml.). A saturated solution of mercuric chloride is added slowly until the first slight precipitate of red mercuric iodide persists, and 400 ml. of a clarified 9 *N* solution of alkali (potassium or sodium hydroxide) are then added. The solution is diluted to 1 liter with ammonia-free water and allowed to clarify; the clear supernatant liquid is removed and used. The reagent does not deteriorate and can be stored indefinitely.

Standard ammonium chloride, 3.818 grams dissolved in water to make 1 liter of stock solution. Ten milliliters of this stock are diluted to 1 liter to give a solution, 1 ml. of which contains 0.01 mg. of ammonia nitrogen.

All reagents used are of C. P. grade. Solutions are prepared in an ammonia-free atmosphere and stored in carefully stoppered Pyrex bottles. When rubber stoppers are used they are first boiled for 30 minutes in a 10 per cent solution of sodium hydroxide and rinsed with ammonia-free water.

Apparatus

The micro-Kjeldahl apparatus illustrated in Figure 1 is simple in construction and is designed so that it can be attached to a single ring stand. It is built of Pyrex glass with all individual parts connected by standard-taper ground-glass joints. The steam-generating flask has a capacity of 500 ml. The distilling flasks generally used have a capacity of 50 ml. but other sizes are available and may be used when necessary; they are held in position during distillation by metal springs. Either a coiled tube or a West-type condenser is used for cooling the distillate; the delivery tip of the condenser is reduced to an inner diameter of approximately 0.31 cm. (0.125 inch), so that the condensate forms a liquid seal at this point. Standard low-form 50-ml. Nessler tubes are used as receiving flasks.

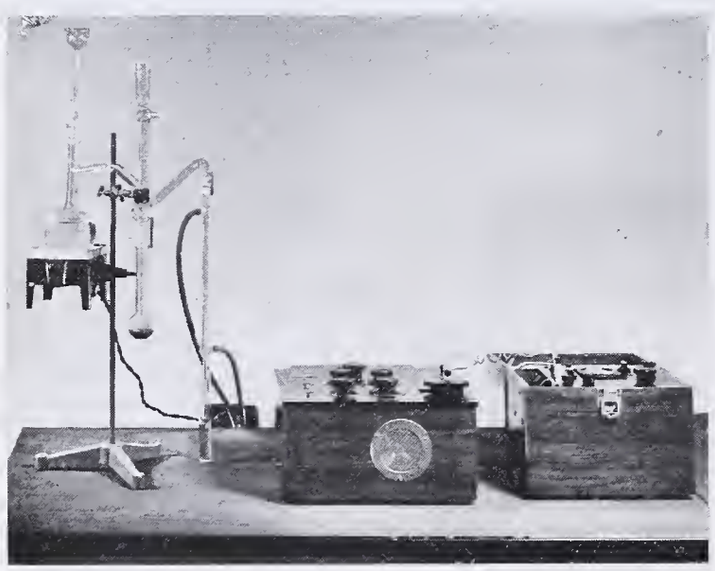

FIGURE 2. MICRO-KJELDAHL UNIT AND SPECTROPHOTOMETER

A water bath is used for dissolving the sample; it is arranged so that volatile products liberated from the sample during solution can escape. The bath is used in an ammonia-free atmosphere.

A Coleman photoelectric spectrophotometer equipped with a double monochromator is used for determining the concentration of the ammonia in the distillate. A spectral band width of 30 millimicrons is used. A photograph of the spectrophotometer is shown along with the distillation apparatus in Figure 2.

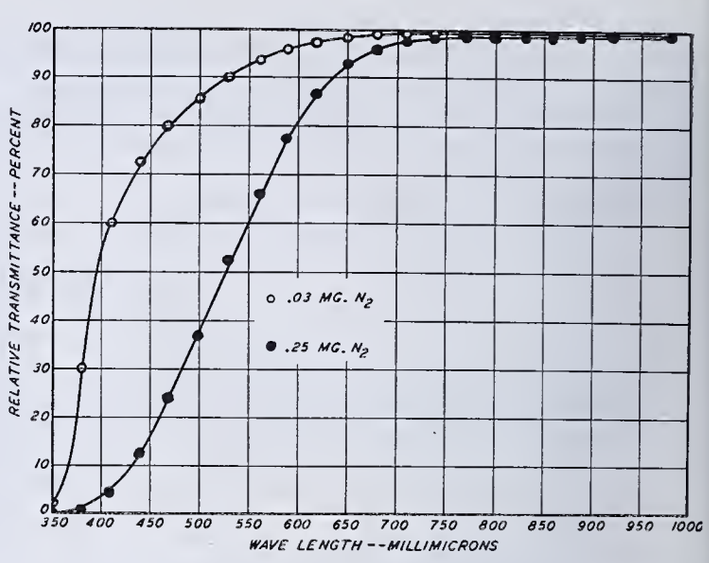

FIGURE 3. SPECTRAL TRANSMITTANCE CURVES FOR AMMONIA-NESSLER COLOR COMPLEX

Procedure

A weighed sample is placed in a 50-ml. distillation flask and covered with acid, and the flask and its contents are heated in a water bath until the sample dissolves. In general a 500-mg. sample is used with 10 ml. of hydrochloric acid as solvent. The quantities of sample and solvent may be varied, when necessary.

While the sample is dissolving, the steam-generating flask of the micro unit is filled with ammonia-free water. Heat is applied so that the steam generated passes through a distilling flask and into the condenser. Fifty-milliliter portions of the distillate are collected and tested with Nessler's reagent. When this test shows that the apparatus is free from ammonia the generating flask is refilled. The temperature is then maintained at slightly below the boiling point until a sample is ready for distillation. Then the temperature is increased sufficiently to cause a steady evolution of steam; an excess of sodium hydroxide (10 ml. are sufficient for 10 ml. of hydrochloric acid) is added from a buret to a dissolved sample in such a manner that the acid solution and the alkali remain in two separate layers. The flask containing the sample and the excess of strong alkali is connected to the micro unit in place of the empty distilling flask, without interrupting the steam generation. The steam passing from the generator into the distillation flask liberates and carries ammonia and steam into the condenser where the steam condenses. The solution is collected in a clean 50-ml. Nessler tube. The complete distillation requires about 5 minutes for a sample. The apparatus is then ready for the substitution and distillation of a second sample immediately. No delay is necessary for rinsing distillation flasks or transferring the sample to the apparatus. The capacity of the steam generator shown in Figure 2 is sufficient to distill the ammonia from a series of 6 samples without refilling.

The distillates are diluted to 50 ml. with ammonia-free water and 1 ml. of Nessler's reagent is added to each; the intensity of the resulting color is measured with the photoelectric spectrophotometer. The transmittance readings are then converted to per cent nitrogen by means of a standard conversion curve.

Precautions

A blank is determined on the reagents in exactly the same manner as for the steel except for omission of the sample. With carefully prepared reagents the blank correction is usually negligible; nevertheless the determination of a reagent blank should be made a daily practice.

The composition of the reagents should be reproduced closely from batch to batch. Each time a fresh stock of Nessler solution is used the calibration of the instrument should be checked. The most reliable and convenient means of checking or establishing the calibration curve is to dilute known quantities of standard ammonium chloride (or sulfate) to a volume of 50 ml., nesslerize directly, and determine the transmittance at the desired wave length.

The efficiency of the distillation procedure should be checked independently at least once each 8 hours by running a steel of known nitrogen content. In this manner possible errors in the determination are likely to be detected. Steels such as 55-a, 15-c, or 101-a certified by the National Bureau of Standards are suitable for this purpose.

After addition of the Nessler solution to the distillate the color develops rapidly and the intensity remains relatively constant for an hour. A minimum interval of 5 minutes between addition of Nessler reagent and the spectrophotometric reading is adequate. Readings should usually be completed on a series of samples within 30 minutes after addition of Nessler reagent to the first sample.

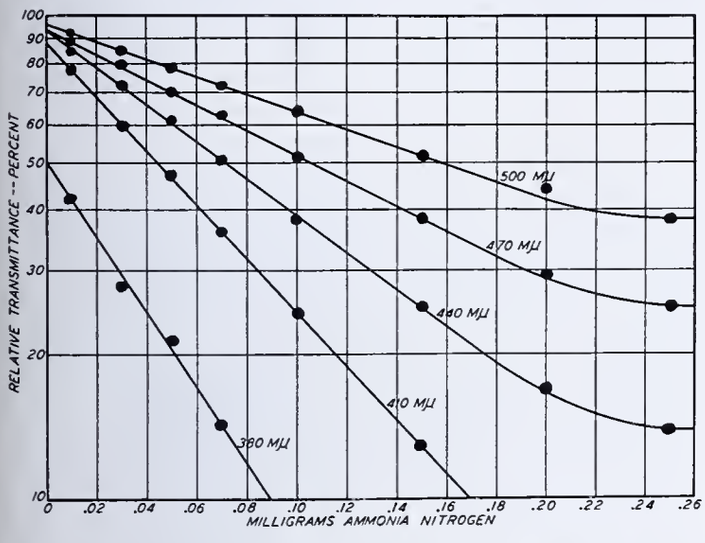

FIGURE 4. TRANSMITTANCE OF AMMONIA-NESSLER COMPLEX AT DIFFERENT WAVE LENGTHS

Consistently reliable results cannot be obtained unless the apparatus, including all distillation flasks and receiving flasks, is free from traces of ammonia. For this reason it is preferable to use a stock of clean Nessler tubes as receiving flasks and then nesslerize the distillate in them rather than to collect the distillate in a volumetric flask and transfer the solution to another flask. Such an arrangement also effects a worthwhile saving in time.

The size of sample may be varied as desired. In general, it is preferable to choose a sample of such size that it will contain from 0.02 to 0.12 mg. of ammonia nitrogen. By using a sample of either 500 or 250 mg. of steel this nitrogen content usually will be obtained. Sample solutions and distillates must be protected from possible contamination by atmospheric ammonia. The temperature of the distillate should be maintained constant, as near 25° C. as practicable.

Experimental

The spectral transmittance of the ammonia-Nessler complex was determined with the Coleman spectrophotometer, using a spectral band 30 millimicrons wide and distilled water as a reference standard. Two typical curves are shown in Figure 3. The transmittance of the ammonia-Nessler complex decreases sharply as the wave length is decreased below 650 millimicrons. It will be observed that the spectral region between approximately 390 and 500 millimicrons is the region giving a maximum difference in transmittance for a given difference in concentration. Wave lengths outside this region can be used for quantitative work only with considerable sacrifice of sensitivity. A band 30 millimicrons wide with a mean wave length of 410 millimicrons was chosen for a major portion of the work reported here because it gave a suitable sensitivity over a fairly wide concentration range.

Different solutions containing the same quantities of ammonium chloride were nesslerized and transmittance readings taken for three different concentrations. The maximum deviations from the mean transmittance values was 0.5, 0.3, and 0.2 per cent, respectively, for concentrations of 0.01, 0.03, and 0.05 mg. of ammonia nitrogen. These values indicated that the intensity of the ammonia Nessler complex could be reproduced consistently enough to permit the method to be used for determining nitrogen in steel by the micromethod.

Curves were constructed from the data obtained by measuring the transmittance of solutions prepared by adding 1 ml. of Nessler's reagent directly to 50 ml. of solutions containing different known quantities of ammonium chloride. The relationship between the logarithm of transmittance and the ammonia concentration is shown by the curves in Figure 4, which indicate that the ammonia-Nessler complex conforms to Beer's law up to a concentration of approximately 0.16 mg. Known quantities of ammonium chloride were placed in clean digestion flasks, 10 ml. each of hydrochloric acid and alkali were added, and the ammonia was recovered by a 5-minute distillation. The distillate was diluted to 50 ml., 1 ml. of Nessler's reagent added, and the transmittance determined after the color had developed. The agreement between the results of these experiments indicated that a 5-minute distillation was adequate for complete recovery of the ammonia.

A conversion curve was constructed for the instrument by using suitable quantities of carefully prepared standard ammonium chloride diluted to 50 ml. with ammonia-free water and nesslerized directly. Two points were sufficient to establish accurately the position and slope of the curve. The

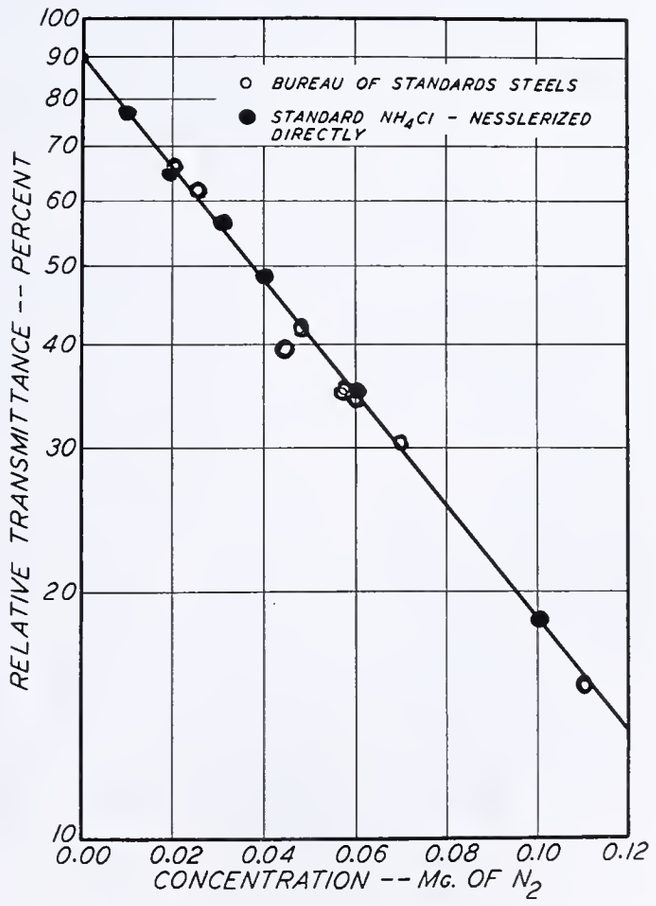
BUREAU OF STANDARDS STEELS
STANDARD NH4Cl - NESSLERIZED DIRECTLY
RELATIVE TRANSMITTANCE -- PERCENT
CONCENTRATION -- MG. OF N2

FIGURE 5. CALIBRATION CURVE FOR 410 MILLIMICRONS

curve shown in Figure 5 was prepared in this manner for a wave length of 410 millimicrons. The transmittance values obtained on steels supplied by the National Bureau of Standards are also shown in Figure 5; they are the averages of the values shown in Table I. The nitrogen values are those supplied by the National Bureau of Standards. The curve in Figure 5 indicates that a suitable calibration curve for nitrogen in steel can be established by direct nesslerization of a pure solution of ammonium chloride.

Table I. Determination of Combined Nitrogen in Bureau of Standards Steels

Standard Sample No.	Type of Steel	Wt. of Sample	Transmittance at 410 Mμ	Nitrogen Found		Nitrogen Present[a]
		Mg.		*Mg.*	%	%
55a	Open hearth iron, 0.01% C	500	64.5	0.021	0.0042	0.004
			68.0	0.018	0.0036	
			63.7	0.021	0.0042	
			64.7	0.021	0.0042	
			66.5	0.019	0.0038	
			63.6	0.021	0.0042	
15c	Basic open hearth steel, 0.1% C	500	61.4	0.024	0.0048	0.005
			61.0	0.024	0.0048	
			61.4	0.024	0.0048	
			59.0	0.026	0.0052	
8b	Acid Bessemer steel, 0.1% C	500	34.7	0.060	0.0120	0.0120
			31.7	0.066	0.0132	
			35.7	0.058	0.0116	
			36.0	0.058	0.0116	
10b	Acid Bessemer steel, 0.4% C	500	34.6	0.060	0.0120	0.0114
			34.8	0.060	0.0120	
22	Acid Bessemer steel, 0.6% C	500	40.6	0.050	0.0100	0.0095
			41.0	0.050	0.0100	
			42.5	0.047	0.0094	
23	Acid Bessemer steel, 0.8% C	500	34.3	0.061	0.0122	0.0116
			35.7	0.059	0.0118	
101	Stainless steel 18–8, 0.06% C	250	32.0	0.065	0.026	0.028
			29.8	0.070	0.028	
			28.9	0.072	0.029	
101a	Stainless steel 18–8, 0.05% C	250	15.6	0.111	0.044	0.044
			15.0	0.113	0.045	
			15.7	0.110	0.044	
106	Nitriding steel Nitralloy G, Cr-Mo-Al, 0.3% C	500	39.3	0.052	0.0104	0.009
			39.5	0.052	0.0104	
			39.0	0.053	0.0106	
			39.3	0.052	0.0104	

[a] Complete analyses of current standard samples may be obtained from their certificates of analysis prepared by National Bureau of Standards.

Accuracy of Method

To test the method, the nitrogen content was determined in multiple for a number of steels obtained from the National Bureau of Standards, using the procedure outlined here. The nitrogen content of these steels had been determined previously either by the solution-distillation procedure using the acid-alkali titration or by the vacuum fusion method. Both methods were used on samples 8b, 101, 101a, and 106. The results shown in Table I for the micromethod were obtained from a calibration curve based on standard ammonium chloride. With the exception of sample 106 the results are in excellent agreement with the recommended values certified by the Bureau of Standards. The values for this sample are well within the range of values determined by Thompson and Hamilton (*6*) on this steel. The results in Table I indicate that the method is capable of an accuracy and precision equal to that of the usual macroprocedure and confirm the data of Klinger and Koch (*5*) which indicate that the Nessler reaction in conjunction with steam-distillation affords a precise means for determining nitrogen in steel. They further indicate that the nitrogen content of steel can be determined with a rapidity that has not been attained heretofore. The carbon content of the steel appears to be without effect on the accuracy of the results for plain carbon steels. In this respect the data in Table I do not substantiate the conclusions of Tschischewski (*7*) and of Jordan and Swindells (*4*) that use of Nessler solution with high-carbon steels produces low results.

Samples 101, 101a, and 106 are low in carbon but contain appreciable quantities of chromium, nickel, molybdenum, and aluminum. Results indicate that the nitrogen in these steels is determined with essentially the same accuracy as in plain carbon steels. However, for pig irons or for materials high in such elements as silicon, titanium, vanadium, tungsten, or columbium or for materials containing these elements in combination with carbon and nitrogen, the routine procedure will not give accurate results for total nitrogen. In the macroanalysis of such steels special digestion treatments involving the use of hydrofluoric (*2*), sulfuric (*3*, *8*), perchloric (*3*), or phosphoric acids (*8*) have been developed. These, or other suitable preliminary treatments, may be used to obtain total nitrogen by the microprocedure with an appreciable saving in time and reagents.

Discussion

A number of advantages result from the use of steam distillation in determining nitrogen in steel. The necessary reagents can be used in concentrated form. Zinc, tartaric acid, or other materials are unnecessary to prevent bumping, foaming, or mechanical loss regardless of the quantity of sample used. The stirring effect of the steam facilitates removal of ammonia. Distillation flasks may be obtained in a variety of sizes for the micro-Kjeldahl unit shown in Figure 2. Consequently a distillation chamber can be selected in a size compatible with the quantity of sample used.

The micromethod has been used by the works laboratories in this corporation for over a year, during which time several thousand determinations have been made. The routine procedure has been applied mainly to low-carbon Bessemer and open hearth steels but has been applied successfully to high-carbon steels and, in modified form, to pig irons. The routine method appears capable of an accuracy substantially the same as that of the usual solution distillation or vacuum fusion procedure and permits an appreciable saving in labor and reagents. The technique of the method can be mastered by relatively inexperienced operators. The procedure is somewhat more exacting than that for direct distillation methods, but, in general, consistent results can be obtained with less difficulty.

Acknowledgment

The writer is indebted to H. W. Graham and H. K. Work, whose interest in nitrogen in steel made this work possible. In addition he acknowledges his debt to H. E. Slocum and his associates in the Aliquippa and Pittsburgh Works for their cooperation in establishing the method for use in the mill laboratories. To G. E. F. Lundell and his associates at the National Bureau of Standards, he is indebted for their cooperation in supplying steels of known nitrogen content.

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Volumetric Determination of Sulfur in Coal and Coke

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THE present accepted standard method (*1*) for the determination of sulfur in coal and coke specifies its oxidation and the recovery of the sulfur compounds in the form of soluble sulfates, followed by gravimetric determination as barium sulfate. The conversion of the sulfur compounds into sulfates is accomplished by combustion of the coal in an oxygen bomb, ignition with Eschka mixture, or fusion with sodium peroxide. The soluble sulfates are recovered in the bomb washings or by leaching the Eschka and peroxide residues. Complete conversion of the sulfur compounds to sulfate is ensured by a further wet-oxidation with bromine water.

Some fuel laboratories have found the gravimetric determination as barium sulfate time-consuming and costly, especially where a large number of coal samples are tested daily, and have studied volumetric methods using internal indicators. The methods receiving most consideration employ either tetrahydroxyquinone (*4, 5, 6*) or sodium rhodizonate (*4*) as indicator.

The American Society for Testing Materials Committee D5 on Coal and Coke has sponsored during the past year, through its Subcommittee I on Methods of Testing, a cooperative study of the comparative accuracy and reproducibility of the standard method of sulfur determination and of volumetric methods using tetrahydroxyquinone and sodium rhodizonate as indicators. Samples of four coals ranging in sulfur content from approximately 0.6 to 3.3 per cent were collected from large commercial deliveries, air-dried, reduced, and ground to pass through a 250-micron (No. 60) sieve in accordance with the standard A. S. T. M. methods (*1, 3*). Representative portions of each coal were distributed among the following eight laboratories cooperating in the study:

Commercial Testing and Engineering Company, Chicago, Ill.
Consolidated Edison Company of New York, Inc., New York, N. Y.
Fuel Engineering Company of New York, New York, N. Y.
State of Illinois, State Geological Survey Division, Urbana, Ill.
The Koppers Coal Company, Pittsburgh, Penna.
The Textor Laboratories, Cleveland, Ohio
United States Department of Interior, Bureau of Mines Central Experiment Station, Pittsburgh, Penna.
Western Electric Company, Kearny, N. J.

Experimental

The coal samples were oxidized in an oxygen bomb or by the Eschka method. Separate portions of each sample were used for each determination.

Gravimetric determinations (*1*) were made on the bomb washings by seven of the cooperating laboratories and on the leachings from the Eschka mixture by all eight laboratories. Volumetric determinations using tetrahydroxyquinone as internal indicator were made by the seven laboratories, and six of the laboratories used sodium rhodizonate as indicator.

A total of 380 individual determinations was made.

The procedures employed in the volumetric methods were as follows:

TETRAHYDROXYQUINONE AS INDICATOR. *Reagents.* Sodium carbonate solution, 18.3 grams per liter. Methyl orange indicator, 0.1 per cent aqueous solution. Bromine water, saturated solution. Phenolphthalein indicator, 0.5 per cent solution in 50 per cent by volume neutral isopropyl alcohol. Approximately 0.02 *N* sodium hydroxide solution. Approximately 0.02 *N* hydrochloric acid solution. Hydrochloric acid solution, 50 per cent by volume concentrated hydrochloric acid, 50 per cent by volume water. Isopropyl alcohol, 99 per cent, ethyl alcohol, 95 per cent, or denatured alcohol, formulas 30 or 3A. Tetrahydroxyquinone indicator. Barium chloride solution gravimetrically standardized, 1 ml. equivalent to 0.0005 gram of sulfur.

Procedure. Bomb-Washing Method. Weigh approximately 1 gram of the coal or coke sample into the capsule in which it is to be burned. Samples which are likely to be blown out of the capsule should be briquetted. Place the capsule in the bomb and proceed as outlined in Section 21 (*1*) finally collecting the bomb washings in a 250-ml. beaker. Titrate the bomb washings with the sodium carbonate solution using methyl orange indicator. Heat the solution and filter. Proceed as indicated below under Eschka method.

Eschka Method. Proceed as outlined in Sections 17 to 20, inclusive (*1*), finally collecting the Eschka leachings of about 250 ml. in a beaker.

Make the bomb washings or Eschka leachings slightly acid with 1 to 1 hydrochloric acid and add 2 ml. of bromine water. Evaporate to about 75 ml. or less and cool to below 35° C. Add 2 or 3 drops of phenolphthalein indicator and then add carefully approximately 0.02 *N* sodium hydroxide until solution is faintly pink. Transfer the solution to a 100-ml. volumetric flask and dilute to 100 ml. with distilled water. Pipet a 25-ml. aliquot into a 125-ml. Erlenmeyer flask and neutralize by adding carefully approximately 0.02 *N* hydrochloric acid until the pink color just disappears. Add 25 ml. of isopropyl alcohol and about 0.4 to 0.5 gram of tetrahydroxyquinone indicator. Swirl the flask until the indicator is dissolved and titrate slowly with standard barium chloride solution, continually swirling the flask until a permanent rose color is imparted to the mixture.

Calculation. The barium chloride solution gives a blank titration of 0.1 ml. and this value should be deducted. The per cent of sulfur in the sample equals (ml. of barium chloride chloride solution − 0.1) × 0.2. When the Eschka method is used, a further correction must be applied either by running a blank or preferably by determining a known amount of sulfate added to a solution of the reagents after these have been put through the prescribed series of operations (*1*, Section 20, *f*).

Notes. The titration is best accomplished over a white surface. A sharper end point may be obtained by adding about 1.0 ml. of a 1 per cent silver nitrate solution to the titration mixture after about 2.0 ml. of the standard barium chloride solution has been added, and then continuing the titration with the barium chloride to the rose colored end point. The most distinct end points are obtained with titrations requiring up to about 15 ml. and the concentration of the barium chloride has been so selected as to give satisfactory results up to 3 per cent sulfur by weight. If the sulfur content of the sample should exceed 3 per cent by weight, it would be desirable either to select a smaller aliquot such as 10 ml. or to use a stronger barium chloride solution (1 ml. equivalent to 0.001 gram of sulfur).

SODIUM RHODIZONATE AS INDICATOR. *Reagents.* Approximately 0.02 *N* sodium hydroxide. Phenolphthalein indicator, 0.5 per cent solution in 50 per cent by volume neutral isopropyl alcohol. Barium chloride solution, either 0.1 *N* or 1 ml. equivalent to 0.001 gram of sulfur, gravimetrically standardized. Ammonium sulfate solution, either 0.1 *N* or 1 ml. equivalent to standardized barium chloride solution. Sodium rhodizonate indicator, prepared by grinding together in a mortar 1 part by weight of sodium rhodizonate to 400 parts by weight ammonium chloride until thoroughly mixed. Isopropyl alcohol, 99 per cent, ethyl alcohol, 95 per cent, or denatured alcohol, formulas 30 or 3A. Hydrochloric acid solution, 50 per cent by volume concentrated hydrochloric acid, 50 per cent by volume water.

Procedure. Bomb-Washing and Eschka Methods. Proceed as outlined under Tetrahydroxyquinone Method.

Make the bomb washings or Eschka leachings slightly acid with 1 to 1 hydrochloric acid and add 2 ml. of bromine water. Evaporate to about 35-ml. volume and add 2 or 3 drops of phenolphthalein indicator. Add carefully approximately 0.02 *N* sodium hydroxide until solution is faintly pink. Add a volume of isopropyl alcohol equivalent to the volume of the solution. Add a measured amount of the standard barium chloride solution sufficient to precipitate the sulfate plus an excess of 3 to 5 ml. This excess can be estimated readily with a little experience. After allowing a minute or two for completion of the reaction, add 0.2 gram of the sodium rhodizonate indicator and thoroughly mix by swirling.

Table I. Determination of Sulfur in Coal

(Comparison between A. S. T. M. standard gravimetric methods and selected volumetric methods)

Laboratory	A. S. T. M. Gravimetric Methods D-271-40				Volumetric Methods				Average
	Bomb Washings		Esehka		Tetrahydroxy-quinone		Sodium Rhodizonate		
	Determination	Max. diff.	Determination	Max. diff.	Determination	Max. diff.	Determination	Max. diff.	
	Per cent by weight				*Per cent by weight*				
	Sulfur Found in Coal A								
A	1.17		1.20		1.24		1.22		
	1.18	0.06	1.18	0.05	1.20	0.04	1.25		
	1.21		1.23		1.24		1.26		
	1.23		1.21		1.20		1.26	0.04	
							1.26		
							1.25		
							1.25		
Av.	1.20		1.21		1.22		1.25		1.22
B	1.15		1.22		1.20		1.23		
	1.20	0.05	1.21	0.01	1.18	0.06	1.24	0.10	
	1.19		1.22		1.24		1.14		
Av.	1.18		1.22		1.21		1.20		1.21
C	1.18		1.28		1.26		1.17		
	1.28	0.10	1.31	0.03	1.26	0.00	1.25	0.16	
	1.23		1.30		1.26		1.33		
Av.	1.23		1.30		1.26		1.25		1.26
D	1.25		1.23		1.24		1.20		
	1.26	0.02	1.18	0.05	1.22	0.04	1.21	0.03	
	1.24		1.20		1.20		1.23		
Av.	1.25		1.20		1.22		1.21		1.22
E	1.18		1.31		1.18		1.15		
	1.24	0.06	1.22	0.09	1.21	0.08	1.22		
	1.22		1.22		1.26		1.26		
			1.24				1.30	0.16	
							1.31		
							1.13		
							1.22		
Av.	1.22		1.25		1.22		1.23		1.23
F	1.14		1.39		1.18		..		
	1.15	0.06	1.36	0.03	1.15	0.07	..	..	
	1.20		1.37		1.22		..		
Av.	1.16		1.37		1.18		..		1.24
G	..		1.20		..		1.21		
	..	..	1.26	0.06	..	..	1.23	0.03	
	..		1.26		..		1.24		
Av.	..		1.24		..		1.23		1.24
H	1.23		1.24		1.32		..		
	1.20	0.03	1.22	0.08	1.30	0.04	..	..	
	1.23		1.30		1.28		..		
Av.	1.22		1.25		1.28		..		1.25
No. of tests	22		26		22		26		
Grand av.	1.21		1.26		1.23		1.23		1.23
Max. diff. between determinations		0.14		0.21		0.17		0.30	

Laboratory	A. S. T. M. Gravimetric Methods D271-40				Volumetric Methods				Average
	Bomb Washings		Esehka		Tetrahydroxy-quinone		Sodium Rhodizonate		
	Determination	Max. diff.	Determination	Max. diff.	Determination	Max. diff.	Determination	Max. diff.	
	Per cent by weight				*Per cent by weight*				
	Sulfur Found in Coal B								
A	1.64		1.68		1.65		1.67		
	1.64	0.04	1.70	0.02	1.70	0.05	1.66	0.04	
	1.64		1.68		1.68		1.70		
	1.68		1.69		1.66		1.69		
Av.	1.65		1.69		1.67		1.68		1.67
B	1.66		1.64		1.66		1.65		
	1.66	0.07	1.64	0.01	1.69	0.06	1.62	0.03	
	1.73		1.65		1.63		1.65		
Av.	1.68		1.64		1.66		1.64		1.66
C	1.68		1.61		1.60		1.61		
	1.58	0.10	1.61	0.01	1.69	0.09	1.58	0.03	
	1.60		1.62		1.62		1.61		
Av.	1.62		1.61		1.64		1.60		1.62
D	1.54		1.68		1.55		1.54		
	1.55	0.01	1.66	0.02	1.54	0.04	1.56	0.04	
	1.54		1.66		1.58		1.52		
Av.	1.54		1.67		1.56		1.54		1.58
E	1.65		1.64		1.56		1.66		
	1.64	0.02	1.67		1.60	0.04	1.56		
	1.63		1.60	0.08	1.59		1.72		
			1.66			1.71		0.14	
			1.68				1.61		
							1.58		
							1.60		
Av.	1.64		1.65		1.58		1.63		1.63
F	1.60		1.70		1.60		..		
	1.62	0.02	1.73	0.03	1.63	0.03	..	..	
	1.61		1.73		1.66		..		
Av.	1.61		1.72		1.63		..		1.65
G	..		1.69		..		1.63		
	..	..	1.70	0.02	..	..	1.72	0.09	
	..		1.68		..		1.68		
Av.	..		1.69				1.68		1.69
H	1.61		1.67		1.64		..		
	1.64	0.03	1.68	0.01	1.66	0.08	..	..	
	1.61		1.67		1.72		..		
Av.	1.62		1.67		1.67		..		1.65
No. of tests	22		27		22		23		
Grand av.	1.62		1.67		1.63		1.63		1.64
Max. diff. between determinations		0.19		0.13		0.18		0.20	

Table II. Determination of Sulfur in Coal

(Comparison between A. S. T. M. standard gravimetric methods and selected volumetric methods)

Laboratory	A. S. T. M. Gravimetric Methods D271-40				Volumetric Methods				Average
	Bomb Washings		Eschka		Tetrahydroxyquinone		Sodium Rhodizonate		
	Determination	Max. diff.	Determination	Max. diff.	Determination	Max. diff.	Determination	Max. diff.	
	Per cent by weight				*Per cent by weight*				
	Sulfur Found in Coal C								
A	0.69		0.71		0.68		0.67		
	0.68	0.03	0.71	0.01	0.66	0.06	0.69	0.07	
	0.71		0.70		0.68		0.74		
	0.71		0.71		0.70		0.74		
					0.72				
					0.70				
Av.	0.70		0.71		0.70		0.71		0.71
B	0.73		0.69		0.69		0.79		
	0.74	0.01	0.67	0.01	0.66	0.08	0.71	0.08	
	0.73		0.68		0.74		0.73		
Av.	0.73		0.68		0.70		0.74		0.71
C	0.70		0.66		0.74		0.68		
	0.68	0.02	0.65	0.04	0.76	0.05	0.65	0.02	
	0.70		0.69		0.71		0.66		
Av.	0.69		0.67		0.74		0.66		0.69
D	0.63		0.67		0.66		0.65		
	0.64	0.03	0.65	0.02	0.68	0.03	0.70	0.05	
	0.66		0.66		0.65		0.69		
Av.	0.64		0.66		0.66		0.68		0.66
E	0.74		0.70		0.66		0.68		
	0.69	0.05	0.69		0.68	0.02	0.70		
	0.71		0.66	0.05	0.68		0.70	0.10	
			0.71				0.63		
			0.69				0.64		
							0.60		
Av.	0.71		0.69		0.67		0.66		0.68
F	0.68		0.73		0.70		..		
	0.71	0.05	0.74	0.01	0.66	0.04	..	..	
	0.66		0.73		0.66		..		
Av.	0.68		0.73		0.67		..		0.69
G	..		0.71		..		0.66		
	..	..	0.71	0.02	..	..	0.72	0.08	
	..		0.69		..		0.64		
Av.	..		0.70		..		0.67		0.69
H	0.67		0.70		0.78		..		
	0.70	0.03	0.65	0.05	0.80	0.02	..	..	
	0.69		0.68		0.78		..		
Av.	0.69		0.67		0.79		..		0.72
No. of tests	22		27		24		22		
Grand av.	0.69		0.69		0.70		0.69		0.69
Max. diff. between determinations		0.11		0.09		0.15		0.19	

Laboratory	A. S. T. M. Gravimetric Methods D271-40				Volumetric Methods				Average
	Bomb Washings		Eschka		Tetrahydroxyquinone		Sodium Rhodizonate		
	Determination	Max. diff.	Determination	Max. diff.	Determination	Max. diff.	Determination	Max. diff.	
	Per cent by weight				*Per cent by weight*				
	Sulfur Found in Coal D								
A	3.16		3.26		3.15		3.18		
	3.12	0.12	3.26	0.06	3.15	0.05	3.14		
	3.22		3.20		3.20		3.12	0.13	
	3.24		3.24		3.15		3.14		
							3.25		
							3.23		
Av.	3.19		3.24		3.16		3.19		3.20
B	3.18		3.20		3.11		3.14		
	3.18	0.06	3.20	0.00	3.18	0.08	3.13	0.06	
	3.24		3.20		3.19		3.27		
Av.	3.20		3.20		3.16		3.18		3.19
C	3.20		3.37		3.17		3.02		
	3.21	0.09	3.36	0.02	3.16	0.03	3.07	0.02	
	3.29		3.38		3.19		3.04		
Av.	3.23		3.37		3.18		3.04		3.21
D	3.14		3.16		3.09		3.12		
	3.17	0.03	3.22	0.07	3.10	0.04	3.11	0.04	
	3.16		3.23		3.06		3.08		
Av.	3.16		3.20		3.08		3.10		3.14
E	3.13		3.20		3.14		3.18		
	3.15	0.07	3.19		3.18	0.05	3.20		
	3.20		3.18	0.09	3.13		3.22		
			3.27				3.14	0.12	
			3.24				3.09		
							3.10		
							3.10		
Av.	3.16		3.22		3.15		3.15		3.17
F	3.06		3.27		3.26		..		
	3.12	0.07	3.31	0.04	3.21	0.08	..	..	
	3.13		3.29		3.18		..		
Av.	3.10		3.29		3.22		..		3.20
G	..		3.27		..		3.22		
	..	..	3.24	0.04	..	..	3.18	0.10	
	..		3.23		..		3.12		
Av.	..		3.25		..		3.17		3.21
H	3.16		3.25		3.18		..		
	3.21	0.05	3.26	0.01	3.18	0.02	..	..	
	..		3.26		3.16		..		
Av.	3.19		3.26		3.17		..		3.21
No. of tests	21		27		22		25		
Grand av.	3.18		3.25		3.16		3.14		3.18
Max. diff. between determinations		0.23		0.22		0.20		0.25	

Table III. Summary

Coal	Over-all Average Sulfur, % by Wt.	Maximum Differences between Over-all Averages and Averages Reported by Each Laboratory — A. S. T. M. Gravimetric Methods: Bomb washing	Eschka	Volumetric Methods: T. H. Q.	Sodium rhodizonate	Permissible A. S. T. M. Difference between Different Laboratories
A	1.23	0.07	0.14	0.05	0.04	0.10
B	1.64	0.10	0.08	0.08	0.10	0.10
C	0.69	0.05	0.04	0.10	0.05	0.10
D	3.18	0.08	0.19	0.10	0.14	0.20

mine the fraction of the total number of tests falling within certain limits—namely, 68.3 per cent of all determinations should fall within the band, mean $\pm \sigma$. The larger the value of the standard deviation, the greater the spread about the mean value. The probable error, r, by definition is the value of the limit from the average

Table IV. Summary

Coal	A. S. T. M. Gravimetric Methods — Bomb Washing: Max. diff. between detns.	Bomb Washing: No. of labs. exceeding A. S. T. M. permissible difference	Eschka: Max. diff. between detns.	Eschka: No. of labs. exceeding A. S. T. M. permissible difference	Volumetric Methods — T. H. Q.: Max. diff. between detns.	T. H. Q.: No. of labs. exceeding A. S. T. M. permissible difference	Sodium Rhodizonate: Max. diff. between detns.	Sodium Rhodizonate: No. of labs. exceeding A. S. T. M. permissible difference	Permissible A. S. T. M. Difference between Determinations by Same Laboratory
A	0.10	4	0.09	3	0.07	3	0.16	3	0.05
B	0.10	2	0.08	1	0.09	3	0.14	2	0.05
C	0.05	0	0.05	0	0.08	2	0.10	4	0.05
D	0.12	1	0.09	0	0.08	0	0.13	2	0.10

At this point the solution should have acquired a distinct red color, indicating an excess of barium chloride. If the color is not red, add more of the standard barium chloride solution and another portion of the indicator. It is important that the indicator be added after the reaction is complete and that an excess of barium chloride be present.

Titrate the excess barium chloride with standard ammonium sulfate solution, adding about one drop per second with constant swirling until there is a slight fading of the original red to a light pink. Continue the titration at a rate of 5 to 7 drops per minute until one drop produces a light orange tint. Allow to stand a minute or two. A change to a pronounced canary yellow indicates the end point; if this does not occur, one more drop is usually sufficient. Direct titration to the yellow color invariably means an overrun end point introducing a significant error.

Calculation. Ml. of $BaCl_2$ solution − ml. $(NH_4)_2SO_4$ solution × factor in grams of S per ml. × 100 = % by wt. of S.

When using the Eschka method, the additional correction previously referred to must be applied.

Discussion of Results

The results obtained are presented in Tables I and II.

The maximum differences between the over-all average sulfur content of each coal and the averages reported by each laboratory for each method are summarized in Table III.

These data show that the average results reported by all the laboratories for all the methods are well within the permissible A. S. T. M. differences for different laboratories established for the present gravimetric methods, with the single exception in the case of the Eschka determination for Coal A. This rather large difference was caused by one laboratory obtaining abnormally high results on this coal by the Eschka gravimetric method as compared with the results of the other laboratories, and hence is not considered significant. Eliminating the average of this laboratory, the maximum difference would be reduced from 0.14 to 0.07.

The maximum differences between individual determinations reported by any laboratory for each coal and method, and the number of laboratories reporting maximum differences between individual determinations which exceed the permissible A. S. T. M. differences for the same laboratory, are summarized in Table IV.

Table V summarizes the average sulfur contents by the several laboratories and presents the standard deviation and probable error. The standard deviation has been calculated in accordance with the A. S. T. M. method (*2*) and measures the spread or dispersion of the individual determinations about the mean value. The standard deviation, σ, is the square root of the average of the squares of the individual results from their arithmetical average. It is used to determine within which 50 per cent of the results will fall and is indicated to be $0.6745 \times \sigma$. Since the average standard deviations are relatively of the same order of magnitude, the average sulfur contents as determined by any of these methods will check each other closely, and will be within the present A. S. T. M. permissible differences between determinations. Also the variation of successive determinations about the mean value will be about the same for each of the methods.

On the basis of the results reported herewith, it is concluded that the volumetric methods using either tetrahydroxyquinone or sodium rhodizonate as indicators will yield results of the same degree of reproducibility and accuracy as the present standard gravimetric method.

Comments received from the several cooperating laboratories did not indicate any serious difficulties in carrying

Table V. Comparison of Results, Standard Deviations, and Probable Errors by Various Methods

Method	A. S. T. M. Gravimetric: Bomb washings	Eschka	Volumetric: T. H. Q.	Sodium rhodizonate	Average
Coal A					
No. of tests	22	26	22	26	96
Average sulfur content, % by wt.	1.21	1.26	1.23	1.23	1.23
Standard deviation, σ	0.045	0.063	0.038	0.044	0.040
Probable error, r	0.030	0.043	0.026	0.030	0.032
Coal B					
No. of tests	22	27	22	23	94
Average sulfur content, % by wt.	1.62	1.67	1.63	1.63	1.64
Standard deviation, σ	0.045	0.048	0.061	0.061	0.054
Probable error, r	0.030	0.032	0.041	0.041	0.036
Coal C					
No. of tests	22	27	24	22	95
Average sulfur content, % by wt.	0.69	0.69	0.70	0.69	0.69
Standard deviation, σ	0.030	0.029	0.044	0.061	0.041
Probable error, r	0.020	0.019	0.030	0.041	0.028
Coal D					
No. of tests	21	27	22	25	95
Average sulfur content, % by wt.	3.18	3.25	3.16	3.14	3.18
Standard deviation, σ	0.063	0.025	0.036	0.088	0.053
Probable error, r	0.042	0.017	0.025	0.059	0.036
Coals A, B, and C					
Average standard deviation, σ	0.040	0.046	0.048	0.055	0.047
Average probable error, r	0.027	0.031	0.032	0.037	0.032

out the procedures, even though analysts unfamiliar with these volumetric methods were employed. The principal difficulty in applying the methods seems to have been in determining the sodium rhodizonate end point. However, two laboratories apparently solved this problem by doubling the amount of indicator.

The principal merit of the volumetric methods is the saving in time and cost in laboratories testing a considerable number of samples each day. Laboratories determining calorific value can use the oxygen bomb calorimeter and a volumetric determination of the sulfur in the bomb washings to considerable advantage. The saving in time by use of the volumetric methods instead of the present standard gravimetric method is not appreciable in laboratories making an occasional determination.

As a result of this study, the subcommittee is recommending the tetrahydroxyquinone and sodium rhodizonate methods for adoption as A. S. T. M. tentative standards as alternatives to the present standard gravimetric method.

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Determination of Copper Reduced by Sugars

Use of Ceric Sulfate as a Volumetric Reagent

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IN PHYSIOLOGICAL studies on the tung tree a reliable method was desired for the determination of reducing sugars in various tissues. Several methods (*3*, *8*, *10*) are reported in the literature on the use of ceric sulfate in the determination of reducing sugars, but none involves the determination of cuprous oxide through the reduction of ferric ammonium sulfate with subsequent titration of the ferrous ion with ceric sulfate. The volumetric permanganate method (*1*) is reliable and has been employed in the determination of reducing sugar in plant tissue. However, the authors have found that ceric sulfate can be substituted for potassium permanganate in this method with the following advantages: It gives a sharper end point with *o*-phenanthroline ferrous complex indicator (*11*); the solution of this salt is exceedingly stable (*2*); the reduced form (cerous ion) is colorless, offering no interference with indicators; and there is only one change in valence, Ce^{IV} to Ce^{III}, and hence no intermediate products are possible.

Table I. Determination of Reducing Sugars

(Expressed in milligrams of dextrose)

Munson-Walker	Volumetric Permanganate	Shaffer-Hartman	Volumetric Ceric Sulfate
	50 Ml. of Dextrose[a]		
49.5	49.5	49.9	49.6
49.3	49.6	50.1	49.8
49.8	50.0	49.8	49.6
49.5	49.5	49.7	49.9
49.9	49.3	49.9	50.0
49.7	50.0	50.0	49.7
50.0	49.5	50.2	49.9
49.5	49.3	49.5	50.0
49.2	49.6	49.6	50.0
49.5	49.7	50.0	49.6
Av. 49.6	49.6	49.9	49.8
	50 Ml. of Diluted Extract of Tung Leaves		
49.6	50.5	50.5	50.4
49.6	50.1	50.3	50.7
49.7	50.4	50.3	50.4
49.5	50.3	50.3	50.5
49.6	50.8	50.6	50.7
49.7	50.4	50.7	50.3
49.4	50.1	50.3	50.4
50.0	50.5	50.7	50.3
49.9	50.1	50.5	50.7
49.8	50.2	50.7	50.3
Av. 49.7	50.3	50.5	50.5
	25 Ml. of Dextrose and 25 Ml. of Diluted Extract of Tung Leaves		
48.8	49.2	50.1	49.7
49.7	49.8	50.6	49.9
50.0	49.4	50.8	49.8
49.4	49.9	50.2	49.9
48.9	48.8	50.3	49.6
48.9	49.1	50.3	49.4
49.3	49.8	49.9	49.4
49.2	49.0	50.1	49.4
48.8	48.8	50.6	49.4
49.8	49.9	50.2	49.9
Av. 49.3	49.4	50.3	49.6

[a] Dextrose solution contained 1 mg. of dextrose per ml.

Experimental

The methods of preparation of tung tissue and the analytical procedure used for the precipitation of cuprous oxide are similar to those of the Association of Official Agricultural Chemists (*1*) with the exception of a few changes to facilitate certain operations.

In the clarification of the aqueous sugar solution, which is obtained from the alcoholic extract of tung tissue, the flocculent precipitate formed by the addition of saturated lead acetate is removed by centrifugation rather than by the tedious process of filtration. The cuprous oxide is precipitated as directed in the Munson-Walker procedure (*1*, *5*), except that an electric heater is substituted for a Bunsen burner.

The cuprous oxide is dissolved in ferric ammonium sulfate as in the volumetric permanganate method (*1*), with the exception that 50 ml. of hot ferric ammonium sulfate are used instead of 10 ml. of cold solution. Great care must be exercised to ensure complete solution (*4*); otherwise erratic results will be obtained. The solution is made to a final volume of approximately 150 ml. and then titrated at once at a temperature between 50° and 60° C. with 0.05 *N* ceric sulfate (*7*). One drop of *o*-phenanthroline ferrous complex indicator is added at the beginning of the titration and another near the end point. The color change is from

orange to a light green. Solutions having a high temperature cause the indicator to fade (*9*).

Results

The volumetric ceric sulfate method compared with the volumetric permanganate (*1*), Munson-Walker (*1*, *5*), and Shaffer-Hartman (*6*) methods gave essentially the same results on a known dextrose solution, an aqueous extract of tung leaves, and a mixture of the extract and a known dextrose solution (Table I). It is evident that ceric sulfate can be used successfully in this procedure.

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Content of *l*-Pimaric Acid in Pine Oleoresin

Improved Methods for Its Determination

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THE *l*-pimaric acid content of pine oleoresin has been a matter of considerable speculation for a long time.

Dupont (*4*) advanced a theoretical basis for predicting the amount of individual resin acids present in pine oleoresin. He estimated the amounts of these acids present by observation of the optical rotation of mixtures (*2*) and by isomerization of the *l*-pimaric acid present with mineral acid (*3*). This type of analysis was further developed by Sandermann (*6*) for *l*-pimaric acid present in oleoresin by change in optical rotation when this acid of $[\alpha]_D$ −280° is isomerized with mineral acid to *l*-abietic acid $[\alpha]_D$ −104°. The method is subject to the objection that, in addition to *l*-pimaric acid, other unidentified acidic constituents are also isomerized, under the condition of this determination, to *l*-abietic acid.

The recent discovery that *l*-pimaric acid readily forms a Diels-Alder addition compound in the cold with maleic anhydride (*1*, *7*) has provided a new basis for analytical determinations of *l*-pimaric acid and possibly other conjugated double bond acids that might be present. The mild conditions of this reaction and the technique for quantitative removal of the addition product, without altering the other unstable acids present in the complex, make possible a new approach to the problem of the "sapinic acids". Sandermann (*5*) used this reaction with maleic anhydride to replace the isomerization of *l*-pimaric acid with mineral acid. The change in optical rotation was observed and used as a basis for calculating the amount of *l*-pimaric acid present.

Another method developed by Sandermann (*5*, *6*) also made use of the addition product of maleic anhydride with *l*-pimaric acid, but depended upon acidimetric titration and not rotation measurements. In this method a known weight of resin acids was reacted with an excess of maleic anhydride, the excess maleic anhydride was then extracted, and the resin acids plus the *l*-pimaric acid addition product was titrated with alkali. The per cent of *l*-pimaric acid present was calculated from the increased alkali needed to neutralize the reaction product over that needed for the original acids.

In the present work this maleic anhydride procedure was modified so that a known amount of maleic anhydride was added to a known amount of resin acid mixture in *n*-pentane solution. After reaction had taken place the excess maleic anhydride was extracted quantitatively and the aqueous extract titrated with alkali. Since the addition compound of *l*-pimaric acid is not soluble in water, this makes possible the determination of the amount of maleic anhydride reacted and from that the per cent of *l*-pimaric acid can be calculated.

While no reaction between maleic anhydride and gum turpentine constituents is to be expected at room temperatures, nevertheless to eliminate the possibility of abnormal behavior blank determinations with turpentine were made. These showed that the reaction of maleic anhydride with turpentine at a temperature around 20° C. for a period of 4 hours was smaller than the limits of experimental error. This makes possible the determination of *l*-pimaric acid present in pine oleoresin without the necessity of first extracting the acidic portion of the oleoresin from the turpentine.

In this method any unknown compound, present in the oleoresin, that reacts with maleic anhydride at room temperature will give too high a value for the *l*-pimaric acid content of oleoresin.

A new method for the determination of *l*-pimaric acid makes use of the insolubility of the *l*-pimaric acid addition product of maleic anhydride in *n*-pentane. At 20° C. the solubility of this addition product was found to be 0.03 gram in 100 cc. of *n*-pentane.

For analysis the pine oleoresin was dissolved in *n*-pentane and filtered free from dirt, chips, and water. The concentration of the acidic constituents was determined by titration with alkali and then a known volume of *n*-pentane solution was reacted with an excess of maleic anhydride in acetone solution. The addition product crystallized from solution and was filtered off, washed, dried, and weighed. This addition product was found to be of high purity. All samples melted above 220° C. The purified material melts at 226–229° C. (*7*).

The completeness of the removal of this addition compound from *n*-pentane solution is illustrated by the reaction of pure *l*-pimaric acid with maleic anhydride. Thus, of a 3-gram sample of *l*-pimaric acid, less than 0.05 gram of noncrystalline material was removed from the mother liquor after the addition compound had been removed by filtration.

The quantitative nature of this reaction at room temperature was not disturbed by the presence of other nonreacting resin acids and turpentine. This was shown by addition of pure *l*-pimaric acid to an *n*-pentane solution of oleoresin to increase the *l*-pimaric acid content from 32.7 to 35.9 per cent. *l*-Pimaric acid found in the fortified oleoresin was 36.2 per cent.

The solubility of the addition product is increased somewhat by the small amount of acetone added and also by the resin acids of unknown identity that are present in the oleoresin.

These factors would produce too low a value for *l*-pimaric acid of pine oleoresin.

These two methods for the analysis of pine oleoresin were applied to a series of longleaf oleoresin samples collected during the season of 1940 near Olustee, Fla. The samples were stored at −5° C. and all determinations were made at the end of the turpentining season.

These results (Table I) are in fair agreement with those reported by Sandermann (*5*) and show that *l*-pimaric acid does not account for the major portion of the primary acids present in pine oleoresin. The straight-line decrease of *l*-pimaric acid content of longleaf oleoresin with the season indicates that the controlling factor in the decrease of *l*-pimaric acid is the length of the face on the tree over which the oleoresin flows, exposed to the air, to reach the cup. Evidently *l*-pimaric acid is not appreciably isomerized by rise in temperature during the hot part of the season, as is indicated by Sandermann (*5*). If temperature were a governing factor, the *l*-pimaric acid content of oleoresin should reach a minimum at midsummer and rise again with the cooler weather of fall.

Table I. *l*-Pimaric Acid Present in Resin Acid Fraction of Pine Oleoresin

Month, 1940	*l*-Pimaric Acid: Volumetric method, %	*l*-Pimaric Acid: Gravimetric method, %
April	38.4 38.7	36.0
June	36.3 36.6	34.2 33.7
August	35.4 35.1	32.4 31.7
November	33.2 33.7	31.3 30.5

Quantitative Reaction of *l*-Pimaric Acid with Maleic Anhydride

A solution of 3.00 grams of *l*-pimaric acid, $[\alpha]_D^{20}$ −274° (2 per cent absolute alcohol), in 150 cc. of *n*-pentane was stirred while a solution of 1.3 grams of maleic anhydride in 10 cc. of absolute ether was added. Stirring was continued for 2 hours, during which time the addition compound crystallized from solution. It was removed by filtration and the filtrate was extracted with water until the aqueous extracts reacted neutral to Congo red. The *n*-pentane was then distilled and the noncrystalline residue weighed less than 0.05 gram.

Description of Methods

Approximately 100 grams of well-mixed pine gum were dissolved in 200 cc. of *n*-pentane, and the mixture was filtered through a fluted filter paper into a glass-stoppered flask. This filtration removed dirt, chips, and water found in the pine oleoresin.

To determine the total resin acid content of this solution 10 cc. were pipetted into 25 cc. of an alcohol-benzene mixture (500 cc. of alcohol, 500 cc. of benzene, and 0.2 gram of phenolphthalein). This solution was then titrated with 0.5 *N* alcoholic potassium hydroxide.

Volumetric Determination of *l*-Pimaric Acid. Twenty-five cubic centimeters of the above standardized *n*-pentane solution of pine oleoresin were placed in an iodine flask, and to it were added 20 cc. of standardized 10 per cent maleic anhydride in acetone solution. The flask was shaken and then allowed to stand at 20° C. for 4 hours.

At the end of this time the contents of the flask were transferred into a separatory funnel with the aid of 50 cc. of benzene. The maleic anhydride was then extracted as maleic acid with three 100-cc. portions of water. These aqueous extracts were united in a second separatory funnel and then extracted with 25 cc. of benzene. The aqueous layer was drawn off into the titration beaker and the benzene extract was washed by shaking with 15 cc. of additional water. This wash was also added to the titration beaker. The amount of maleic acid in the aqueous extracts was determined by titration with aqueous 0.5 *N* sodium hydroxide, using phenolphthalein as an indicator. The difference between the amount of maleic anhydride originally added and that accounted for in the aqueous extracts represents the amount of maleic anhydride reacted with *l*-pimaric acid.

The per cent of *l*-pimaric acid present in the acidic portion of the pine oleoresin was calculated by the formula:

$$\% \; l\text{-pimaric acid} = \frac{a \times 3.082 \times 100}{b}$$

where *a* equals the weight in grams of maleic anhydride reacted with *l*-pimaric acid, and *b* equals the weight in grams of resin acids present in the 25 cc. of *n*-pentane solution used.

Gravimetric Determination of *l*-Pimaric Acid. Two hundred cubic centimeters of the standardized *n*-pentane solution of pine oleoresin were placed in a three-necked distilling flask fitted with a thermometer and a stirrer. The stirrer was connected into the stoppered reaction flask through a bearing which was not gas-tight.

The *n*-pentane solution was stirred and a solution of 7 grams of maleic anhydride in 4 cc. of acetone was added rapidly. Stirring was continued for 2.5 hours. During the first half hour the temperature of the reaction mixture increased and cooling with water was necessary to hold the temperature at 30° C.

At the end of the first hour crystallization had taken place and at the end of 2 hours the reaction was packed in ice and stirring was continued for a half hour longer. The crystalline material was then filtered into a Büchner funnel using mild suction. A portion of the filtrate was used to make the transfer as quantitative as possible. Suction was then cut off entirely, 25 cc. of *n*-pentane were added to the filter cake, and the cake was puddled to ensure efficient washing. The *n*-pentane wash was drawn off slowly with very mild suction. The crystalline material was transferred to a weighed crystallizing dish and dried in vacuum at 100° C. for 2 hours and then weighed.

The per cent of *l*-pimaric acid present in the acids of pine oleoresin was calculated by the formula:

$$\% \; l\text{-pimaric acid} = \frac{c \times 0.755 \times 100}{d}$$

where *c* equals the weight in grams of the dried addition product of *l*-pimaric acid and maleic anhydride and *d* equals the weight in grams of the resin acids present in the 200 cc. of *n*-pentane solution used.

The amount of maleic anhydride added was adjusted so as to have approximately 1 gram excess at the end of the reaction. Larger excess was avoided in order to keep to a minimum the amount of acetone added. A large excess also causes maleic anhydride to crystallize from the reaction mixture.

Determination of *l*-Pimaric Acid Added to Pine Oleoresin. A solution of 200 grams of longleaf pine oleoresin in 400 cc. of *n*-pentane was filtered as outlined above. This solution was titrated with alcoholic potassium hydroxide to determine the concentration of resin acids present.

A solution of 6 grams of maleic anhydride in 3 cc. of acetone was then reacted with 200 cc. of the *n*-pentane solution which contained 44.10 grams of resin acids. The dried addition product weighed 19.10 grams, which corresponds to 32.7 per cent of *l*-pimaric acid present in the acidic portion of the oleoresin.

To a second 200-cc. portion of the standardized resin acid solution were added 2.20 grams of pure *l*-pimaric acid. A solution of 7 grams of maleic anhydride in 4 cc. of acetone was added as outlined above. The dried addition product weighed 22.20 grams, which corresponds to 36.2 per cent of *l*-pimaric acid.

Based on the control run and the amount of pure *l*-pimaric acid added, the calculated value should be 35.9 per cent.

Summary

Determination of the *l*-pimaric acid content of longleaf pine oleoresin throughout the turpentining season showed a constant decrease in *l*-pimaric acid content with the progress of the season. The progressive change in *l*-pimaric acid content was followed by means of a volumetric and a gravimetric method based on the Diels-Alder reaction with maleic anhydride.

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Colorimetric Determination of Silver with 2-Thio-5-keto-4-carbethoxy-1,3-dihydropyrimidine

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SHEPPARD and Brigham (*3*) reported the preparation of a compound to which they assigned the name 2-thio-5-keto-4-carbethoxy-1,3-dihydropyrimidine and which gave reactions with a number of metallic ions. The reaction with silver was observed to be especially sensitive and selective in nitric acid solution. Further studies by the present authors have shown that the compound gives the following reactions in neutral solution in addition to those reported (*3*): Co^{++}, Mn^{++}, Ni^{++}, pink colors; Hg^{++}, pink precipitate; Tl^{+}, blue precipitate. In nitric acid solution Au^{+++} and Hg_2^{++} give pink precipitates and Pd^{++} a slight red precipitate. In the nitric acid–sodium acetate buffer used for the determination of silver, the following reactions were observed: Ag^{+}, purple color and precipitate; Au^{+++} and Pd^{++}, orange color and precipitate; Hg_2^{++} and Hg^{++}, pink color and precipitate.

Schoonover (*2*) described a method for the colorimetric determination of silver using *p*-dimethylaminobenzalrhodanine and noted that adsorption of silver on glassware may introduce appreciable error at low silver concentrations. The authors have found that the purple colored compound given by silver with 2-thio-5-keto-4-carbethoxy-1,3-dihydropyrimidine is also applicable to the colorimetric determination of small amounts of silver. A comparison of these silver reagents under the conditions recommended in this paper showed that their sensitivity is approximately the same, that solutions of the silver compound of 2-thio-5-keto-4-carbethoxy-1,3-dihydropyrimidine are more stable, especially at higher silver concentrations, and that the colors given by *p*-dimethylaminobenzalrhodanine with gold and palladium are more intense than those given by the former reagent.

The effect of a number of factors on the reaction of silver with 2-thio-5-keto-4-carbethoxy-1,3-dihydropyrimidine has been studied and is reported in this paper. Results of a study of the colorimetric determination of silver with this reagent are also given.

Reagents

All reagents used were of the highest purity obtainable and were further purified, if necessary, to remove any chloride present.

2-Thio-5-keto-4-carbethoxy-1,3-dihydropyrimidine. One sample was obtained from the Eastman Kodak Company and another was prepared in this laboratory. A solution containing 0.01 per cent of the reagent in acetone was used. The solution has a dark orange color when first prepared but slowly changes to a yellow color. The freshly prepared solution is slightly more sensitive and for this reason fresh solutions should be prepared at least once a week.

Silver. A solution containing 1 mg. of silver per ml. was prepared from silver nitrate and was diluted to the desired concentration.

Sodium acetate–nitric acid buffer was prepared by adding 320 ml. of 1 *M* nitric acid to 200 ml. of 1 *M* sodium acetate and diluting to 1 liter with water. The buffer had a pH of 1.1 as prepared but on diluting 10 times with water the pH changed to 2.0.

Gelatin. A 0.2 per cent aqueous solution was used.

Experimental

The reagent has a pink color in basic solution, orange in neutral solution, and yellow in acid solution. The intensity of the yellow color is only slightly affected by the pH, the intensity being nearly the same in 0.01 or 2 *M* nitric acid.

The intensity of the colored silver compound, as well as the rate of its development, is markedly influenced by the pH. The reaction rate is greatest and the color development most intense in approximately neutral solutions having a low electrolyte concentration. The reaction rate decreases with decreasing pH and the color formed may vary from purple to blue, depending on the pH. Despite the decreased reaction rate, a buffer with a low pH was used because less interference and a greater stability of the color resulted. Even at the low pH, however, the reproducibility and stability of the color are unsatisfactory for colorimetry, unless a small amount of gelatin is present to stabilize the colloidal suspension of the silver compound. Under the conditions recommended, 40 to 50 minutes are required for the development of maximum color intensity. The color is not sufficiently stable to permit the use of a set of standards over an extended period of time. Consequently, the unknowns and standards should be prepared at as nearly the same time as possible and the comparison should be completed within an hour.

All experiments were performed at room temperature, except for several at 50° C. to determine the temperature effect. The sensitivity was less at 50°, undoubtedly because of an adsorption of silver by the glassware.

General Procedure

Transfer the silver solution to a 100-ml. volumetric flask, add 10 ml. of the sodium acetate–nitric acid buffer and 0.5 ml. of the gelatin solution, and dilute almost to the mark with water. Add 0.6 ml. of the reagent, make up to the mark with water, and mix thoroughly. Color comparison may be made 30 to 40 minutes after addition of the reagent, either in 50-ml. Nessler tubes (220 mm.) or in 100-ml. tubes (160 mm.) in a roulette comparator (*4*). Using a volume of 100 ml., the following increments in the series of standards were used: 0 to 6 micrograms, 2 micrograms; 7 to 30 micrograms, 1 microgram. With a volume of 50 ml.: 0 to 7 micrograms, 1 microgram; 8 to 12 micrograms, 0.5 microgram; 13 to 16 micrograms, 1 microgram.

The method may be applied to smaller volumes, using smaller amounts of the reagents and suitable standards. Precautions must be taken, however, at very low silver concentrations to

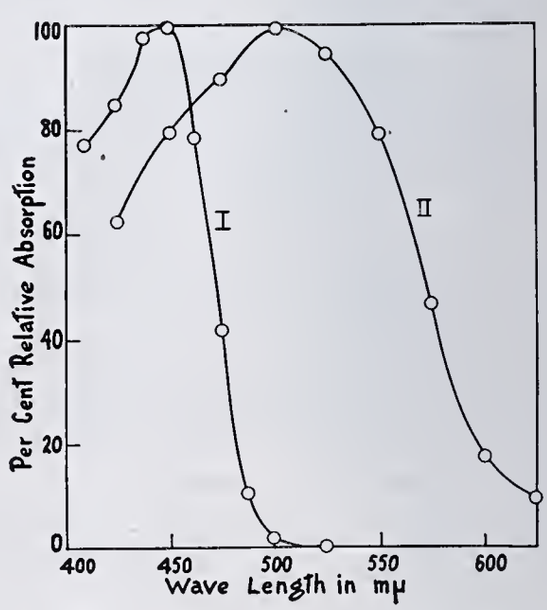

FIGURE 1. RELATIVE ABSORPTION OF 2-THIO-5-KETO-4-CARBETHOXY-1,3-DIHYDROPYRIMIDINE AND ITS SILVER COMPOUND

I. Reagent. II. Silver compound

avoid error due to adsorption of silver by the glassware. All vessels must be either Pyrex or fused silica. If Pyrex is used, heating must be avoided. Pyrex test tubes are satisfactory for making the color comparisons.

Interference

As previously noted, a number of ions give reactions with the reagent in neutral or very weakly acid solutions but fail to react in a stronger nitric acid solution. In addition to the observations on the spot plate, the possible interference of a number of ions was determined under the conditions recommended in the general procedure, using a silver concentration of 20 micrograms. Mercurous and mercuric mercury interfere and must be absent. The following colored ions interfere only if present at concentrations exceeding that required to produce the color due to the ion itself. The limiting concentrations are: Co^{++}, 0.5 mg.; Cu^{++}, 1 mg.; Fe^{+++}, 2 mg.; Ni^{++}, 1.5 mg. The following ions are without effect at a concentration of 10 mg.: Cd^{++}, Mn^{++}, Pb^{++}, and Zn^{++}.

The limiting concentrations are: nitric acid, 0.10; sodium nitrate, 3; sodium sulfate, 0.1; ammonium nitrate, 0.5 millimoles. Larger amounts of nitric acid or sodium nitrate cause a decrease in color intensity. Sulfate and ammonium ions have the reverse effect—i. e., they increase the color intensity.

Sensitivity

On the spot plate using 0.05 ml. of silver solution, 0.05 ml. of buffer, and 0.03 ml. of reagent, the minimum concentration of silver detectable is 5 p. p. m. (0.25 microgram); in aqueous solution the minimum is 3 p. p. m. (0.15 microgram).

The sensitivity at the various silver concentrations using 50-ml. Nessler tubes (220 mm.) is as follows: 0 to 2 micrograms, 2 micrograms; 3 to 7 micrograms, 1 microgram; 8 to 12 micrograms, 0.5 microgram; 13 to 16 micrograms, 1 microgram. The sensitivity falls off sharply above 15 micrograms, and the amount of silver should not exceed this value for 50-ml. volumes.

With the roulette comparator using 100-ml. tubes (160 mm.) the sensitivity is: 0 to 6 micrograms, 3 micrograms; 7 to 30 micrograms, 1 microgram. Above 25 micrograms the sensitivity decreases and little increase in the intensity occurs above 30 micrograms. The most sensitive range is from 15 to 20 micrograms.

Although the reaction is sensitive to about 1 part in 30,000,000 (3 micrograms) at the lowest silver concentrations, a sensitivity of 1 part in 100,000,000 (1 microgram) may be attained by working in the most sensitive range.

Relative Absorption Curves

The relative absorption curves for the reagent and for the silver compound obtained from measurements with a photoelectric spectrophotometer (*1*) are given in Figure 1. Curve I, the reagent curve, was obtained for a solution prepared from 10 ml. of buffer, plus 0.5 ml. of gelatin, plus 1 ml. of reagent, plus water to make 100 ml. Curve II, the compound curve, was obtained for a solution prepared as above, using 120 micrograms of silver and 1.2 ml. of reagent. At 500 mμ the absorption by the reagent is very slight, whereas that of the compound is approximately at the maximum, a condition favorable for the elimination of absorption due to excess reagent, without sacrificing the sensitivity. The reaction, however, is not especially suitable for spectrophotometric studies because of the slow reaction rate and the limited stability of the colored solutions.

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This investigation was supported partly by a grant-in-aid from the Carnegie Corporation of New York. It is one of a projected series based upon studies of organic reagents in inorganic analysis, conducted as a cooperative effort in which ten institutions are participating under the direction of John H. Yoe. Those cooperating with the University of Virginia are: Hampden-Sydney, Mary Baldwin, Randolph-Macon (Ashland), Virginia Military Institute, Virginia Polytechnic Institute, Washington and Lee, William and Mary, University of North Carolina, and Tulane University.

Rapid Estimation of Bromides in the Presence of Chlorides

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DURING the course of work in this laboratory, it became necessary to find a rapid method for the determination of low concentrations of bromides in the presence of equivalent or excess amounts of chlorides. Since none of the available methods proved suitable, a method for higher bromide concentrations (*1*, *2*) was simplified and adapted for low concentrations by the use of a photoelectric colorimeter. The method is based upon the liberation of free bromine by the addition of chlorine water and measurement of the developed color directly in the aqueous solution. Previous methods require extraction of the liberated bromine by carbon tetrachloride.

Apparatus. The photoelectric colorimeter was a Klett-Summerson Model 900-3, in which was used a KS-42 filter (transmission range 400 to 465 millimicrons). (This instrument is available from the Klett Manufacturing Company, 179 East 87th St., New York, N. Y.) A buret was mounted in position to discharge into the colorimeter cell.

Procedure. Fifty milliliters of bromide-containing solution were treated with 10 ml. of 1 to 1 sulfuric acid, and the whole was diluted to 75 ml. and mixed thoroughly. About 50 ml. were placed in the colorimeter cell and the indicator was adjusted to read zero. Saturated chlorine water was then added dropwise, stirring the cell contents well after each addition and observing the scale reading. The maximum scale reading was used for reading the bromide concentration from the calibration curve.

Data from which the calibration curve was plotted are given in the following table:

Colorimeter Scale Reading	Bromide Concentration *Milliequivalents/l.*
17	0.02
70	0.80
85	1.0
139	1.6
174	2.0

In order to obtain reproducible results, it was essential to measure the maximum developed color. Under these conditions, blindfold tests consistently gave results within 0.02 milliequivalents per liter in the range 0.02 to 2.0 milliequivalents per liter of bromide concentration, in the presence of chloride concentrations ranging from an equivalent concentration to 1000 times the bromide concentration. Higher concentrations may be measured by diluting an aliquot to bring the concentration within the above range. Obviously, the presence of iodides or color will interfere with this determination.

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Determination of Sulfur

Accuracy and Precision of Several Methods

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IT IS well known that the gravimetric determination of sulfur as barium sulfate is subject to rather large errors, due chiefly to coprecipitation. Many methods for the precipitation, filtration, and drying of the barium sulfate have been recommended. The author of each method generally gives experimental data on the determination of sulfur in a pure compound, such as sodium or potassium sulfate, to indicate the accuracy of the method. Frequently data are also included for the analysis of a mixture of the alkali sulfate with about an equal quantity of some other salt, such as sodium chloride or nitrate. But data for the determination of known amounts of sulfate in the presence of much larger quantities of other salts are exceedingly scarce in the literature.

This paper describes the results of determinations of sulfur (*2*) in known mixtures of sodium sulfate and sodium chloride by several well-known methods. Sodium chloride was chosen as the foreign salt because it is often present in solution when barium sulfate is precipitated for the determination of sulfur. The fusion of inorganic substances with sodium carbonate and subsequent acidification with hydrochloric acid introduce quantities of sodium chloride much larger than the sulfur that may be in the sample. The sodium peroxide bomb method for the determination of sulfur in organic substances also introduces large quantities of sodium chloride—for example, when 0.5 gram of sodium benzene sulfonate is oxidized by 14 grams of sodium peroxide, the resultant mixture after acidification contains 130 moles of sodium chloride for each mole of sodium sulfate.

The following methods were investigated: The method of Kolthoff and Sandell (*4*) was tested because it is typical of those found in most textbooks of quantitative analysis. The procedure of Fales and Thompson (*1*) was investigated because of the unusual treatment of the precipitate—drying at 110° to 120° C. instead of the customary ignition. This method was found to be very inconvenient because of the digestion period of 12 hours; therefore, a modification with a digestion period of 21 hours was also investigated. Fales and Thompson found that nitrate ion introduced less error in their method than chloride ion. In the determination of sulfur following a fusion with either sodium carbonate or sodium peroxide, the nitrate ion can be introduced as easily as the chloride by using nitric acid for the acidification. Therefore the modified method of Fales and Thompson was also tested in the presence of sodium nitrate instead of sodium chloride. The method of Popoff and Neuman (*6*) was investigated because it has been found better than the usual methods. Hintz and Weber (*3*) claimed an unusual accuracy for their method in the presence of a large quantity of sodium chloride. The method of Njegovan and Marjanovic (*5*) was also tested and discarded because difficulty was experienced in filtering the very fine-grained precipitate.

Reagents

Reagent grade sodium sulfate was purified by recrystallization and dried at 110° C. No further loss was incurred by heating to a dull red heat. The sodium chloride was purified by treating the saturated solution with hydrogen chloride. In some cases, reagent grade sodium chloride was used without purification; then appropriate blank corrections were applied to the results. The other compounds were of reagent grade.

Procedure

An appropriate quantity of pure sodium sulfate was taken, either by direct weighing or by weighing a standard solution with a weight buret, and to it were added known quantities of pure sodium chloride (or nitrate). The mixture was then analyzed for sulfur according to the procedure under investigation. Blank corrections were applied in all cases.

The important features of the various methods are listed in Table I.

TABLE I. IMPORTANT FEATURES OF VARIOUS METHODS

Method	Na_2SO_4 Taken	HCl Taken	Total Volume	Excess $BaCl_2$	Order of Mixing	Time of Mixing	Digestion	Temperature of Drying
	Millimoles		*Ml.*	*Millimoles*		*Min.*	*Hours*	*° C.*
K and S	2.1	12	350	2.9	Usual	4	1	800
F and T	3.5	3	450	1.5	Usual	8	12	115
F and T (modified)	3.5	3	450	1.5	Usual	8	21	130
P and N	3.5	18	350	0.5	Reverse	4	1	800
H and W	7.5	27	550	1.5	Usual	Fast	1	800

Results

Table II gives the relative errors of the methods tested. The values for no foreign salt and for the maximum quantity of foreign salt are the mean of four determinations. The other values generally represent single determinations.

The precision of the methods is indicated by the relative mean deviation of the values with no foreign salt and with the maximum quantity of foreign salt (Table III).

Discussion

Table II reveals that the method of Hintz and Weber is by far the most accurate when the entire range of ratios of sodium chloride to sodium sulfate is considered. It is un-

TABLE II. RELATIVE ERRORS OF METHODS

Method	Molar Ratio of NaCl (or $NaNO_3$) to Na_2SO_4										
	0	1	2	5	10	20	40	60	110	190	309
	Parts per thousand										
K and S	−3	−8	−8	−9	−11	− 9	−12	−16	−17	−16	..
F and T	−2	...	...	...	..	..	..	−12	−13	..	−12
F and T (modified)	−5	−6	−6	−7	− 8	−10	..	−13	−15	..	−15
F and T ($NaNO_3$)	−5	+2	+1	+2	+ 4	+ 6	..	+12	+ 9	+13	+14
P and N	+3	+3	+4	+4	+ 4	+ 3	+ 4	+ 7	+ 9	..	..
H and W	+2	+2	+1	+4	+ 4	+ 3	+ 3	+ 4	+ 4	+ 4	..

TABLE III. PRECISION OF METHODS

Method	Relative Mean Deviation	
	No foreign salt	Maximum foreign salt
	Parts per thousand	
K and S	1.5	1.3
F and T	0.1	0.2
F and T (modified)	0.5	1.4
F and T ($NaNO_3$)	0.5	0.2
P and N	0.3	1.1
H and W	0.3	0.6

fortunate that this method is used so little and that it is not mentioned in the textbooks of analytical chemistry, particularly because it is the quickest and easiest of all. The method of Popoff and Neuman stands second in accuracy.

Four determinations are not enough to establish the precision of a method, but they give a valuable clue regarding the reproducibility of results. In this respect, the original method of Fales and Thompson is by far the best, with Hintz and Weber's procedure second.

It is interesting to consider the causes of the errors. When barium chloride is added slowly to sodium sulfate, the crystals of the precipitate grow in an excess of sulfate ions. Thus they acquire a negative charge and attract the sodium ions, which are then buried beneath the surface of the crystals by deposition of barium sulfate. This coprecipitation of sodium accounts for the low results in the method of Kolthoff and Sandell.

This negative error is partly compensated by incomplete drying in the method of Fales and Thompson. Nitrate ion is extensively coprecipitated by barium sulfate and introduces a positive error. This accounts for the increasing positive errors with addition of sodium nitrate in the method of Fales and Thompson.

The procedure of Popoff and Neuman decreases the coprecipitation of sodium by the reverse precipitation. This method of precipitation, however, increases the usually small coprecipitation of chloride ion until it becomes the major error. This introduces a positive error.

When the precipitant is added all at once, as recommended by Hintz and Weber, the growth of the crystals occurs chiefly after the addition of the precipitant when there is a comparatively small excess of barium ion. Thus neither the positive error due to the coprecipitation of chloride ion nor the negative error due to the coprecipitation of sodium ion is great, and the method yields good results. It might be expected that the rapid precipitation would produce very fine-grained barium sulfate, but no difficulty was experienced in this filtration. Perhaps the rather high acidity of the method accounts for the filterability by increasing the solubility slightly.

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Conductometric Assay of Inorganic Salt in the Presence of Wetting Agents

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A method is described for the rapid assay of active ingredient in commercial wetting agents by a conductometric method. It is not necessary for the chemical nature of the material to be known so long as a small amount of material free of inorganic salt can be isolated for the calibration curve. The method may be applied wherever the components of a mixture have materially different specific conductivities in solution.

DAWSON (*5*) suggested that electrical conductivity of aqueous solutions might be used as a physical constant for evaluation of mixtures with the help of a calibration curve, just as specific gravity, refractive index, or viscosity is used. Relatively few applications of this principle have been made, most analyses by conductometric means having been carried out by titration methods (*3*, *6*).

References have been made to the use of conductivity for controlling the purity of a liquid such as distilled water (*1*, *7*), evaluating the concentration of sulfuric acid (*2*), or determining the concentration of a single electrolyte in aqueous solution (*4*). Šandera (*8*) determined the composition of a mixture of organic liquids by determining the conductivity of a saturated solution of a suitable electrolyte in the mixture. However, no use has apparently been made of the method in the rather frequently occurring problem of determining the composition of a mixture of two water-soluble materials which have different specific conductivities in aqueous solution.

It is known that at concentrations below the point where appreciable errors are introduced by concentration effects, electrical conductivity is an additive property, so that when a mixture of two water-soluble solids is dissolved in water and diluted to a fixed concentration, the conductivity is a straight-line function of composition by weight in the original mixture. It is possible to apply this relationship very generally. For example, it may be employed in connection with a second constant, such as refractive index or analytical composition with respect to a single element, for the evaluation of three-component systems.

This principle has here been applied to the rapid assay of sulfonated and sulfated surface-active agents for inorganic salt and active ingredient content. In this case the surface-active compounds are organic sulfonates or sulfates of high molecular weight which have specific conductivity in water solution approximately one seventh to one tenth that of sodium sulfate; in general, commercial products contain mixtures of several or many related compounds as active ingredients.

The method gives results which compare favorably with those obtained by solvent means or by precipitation of the usual analytical precipitates in the presence of the surface-active material, and can be carried out in very much shorter time.

Experimental

The measuring bridge used was Model RC-1 of Industrial Instruments, Inc., Jersey City, N. J., a 60-cycle alternating current bridge using a cathode ray tube as null-point indicator. The cell was of the dip type, with a constant of 1.108.

Variations in pH within reasonable limits made little or no difference in the results obtained. The materials examined had normal pH values in dilute solution of 7.0, 6.5, and 5.5, respectively. In all three cases a variation of one full pH unit from the normal in the very dilute solution used gave no detectable error.

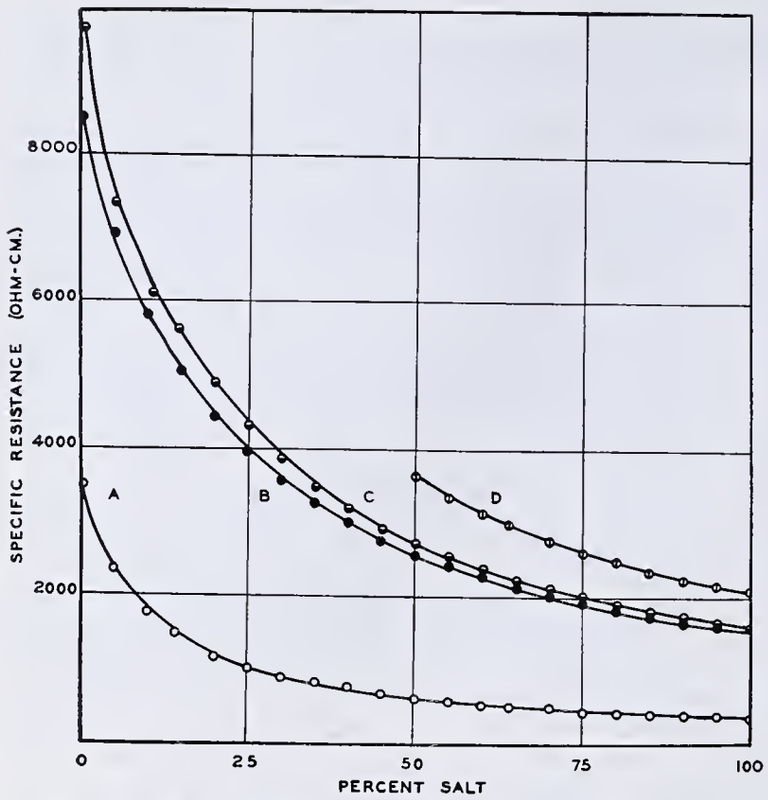

FIGURE 1. SPECIFIC RESISTANCE *vs.* PER CENT SODIUM SULFATE IN DRY SOLID

(Curves *A* and *D* were determined by A. C. Bell.)

A. Sodium sulfate and sodium salt of sulfated coconut oil monoglyceride, 0.20% solution
B. Sodium sulfate and sodium salt of commercial sodium lauryl sulfate, 0.04% solution
C. Sodium sulfate and sodium salt of mixed alkyl aromatic sulfonic acids, 0.04% solution
D. Sodium sulfate and sodium salt of sulfated coconut oil monoglyceride, 0.03% solution

SALT-FREE MATERIALS. In preparing the calibration curve, inorganic salt-free active ingredient was used. Three methods of preparation were employed.

When the inorganic material present was insoluble in hot alcohol—e. g., sodium sulfite or sodium sulfate—two extraction methods were used. In the first method, the oven-dried material was extracted in a Soxhlet extractor with absolute ethanol and the extract dried in a vacuum oven at 100° C. In the second method, a concentrated aqueous solution of a small amount of the material was extracted twice with butanol. The extracts were combined and diluted with several volumes of butanol; then the solvent was fractionally distilled off to remove water until the boiling point was constant (116°) and the solution was filtered hot to remove the salt which crystallized during the distillation. Evaporation of the filtrate gave material free of inorganic salt.

The material was not considered salt-free unless a qualitative test for sulfate ion was negative.

In cases not reported here, when the inorganic salts were soluble in alcohol, dialysis has been used for isolation of the active ingredient.

With all methods care was taken that no fractionation of the organic material occurred during the separation; more soluble fractions will in general have higher specific conductivity.

CALIBRATION CURVE. With salt-free material obtained by the methods described above, the calibration curve was made by the following method: Exactly 200 ml. of 0.040 per cent solution of salt-free material in distilled water were prepared and the conductivity was read. (In all determinations distilled water having a specific resistance of more than 300,000 ohm-cm. was used.) Without removing the electrodes, 10.53 ml. of a 0.040 per cent solution of sodium sulfate were added from a buret and the mixture was gently agitated with the electrodes until a constant reading was obtained on the bridge. This gave the point of the calibration curve for 5 per cent (10.53/210.53) salt content. More salt solution was added and the procedure was continued down to the 50 per cent point. For the other half of the calibration curve the procedure was reversed, with salt solution in the beaker and salt-free active ingredient solution added from the buret. For each point the temperature was read with an accuracy of 0.2° and the reading was corrected to 25° by the formula

$$R_{25} = R_t + 0.02\,(t - 25)\,R_t$$

R_t and R_{25} being the observed and corrected resistances, respectively.

When several conductivity cells are used for the work, the values should also be corrected for cell constant before the calibration curve is drawn.

Calibration curves for three typical materials are shown. Curves are plotted in Figure 1 for specific resistance *vs.* composition and in Figure 2 for specific conductivity *vs.* composition. Since the latter is an additive property, the curves for conductivity are straight lines. Each point represents a single determination, and the deviations from the straight line in the conductivity curves are a measure of the error of the method.

Points are reproducible to 1 per cent salt content or better, and the maximum error is probably less than 2 per cent salt content.

If another concentration than 0.040 per cent is used, it should be so chosen that the range of composition most often dealt with gives specific resistances of 2000 to 5000 ohm-cm. These are low enough to eliminate errors due to variations in the distilled

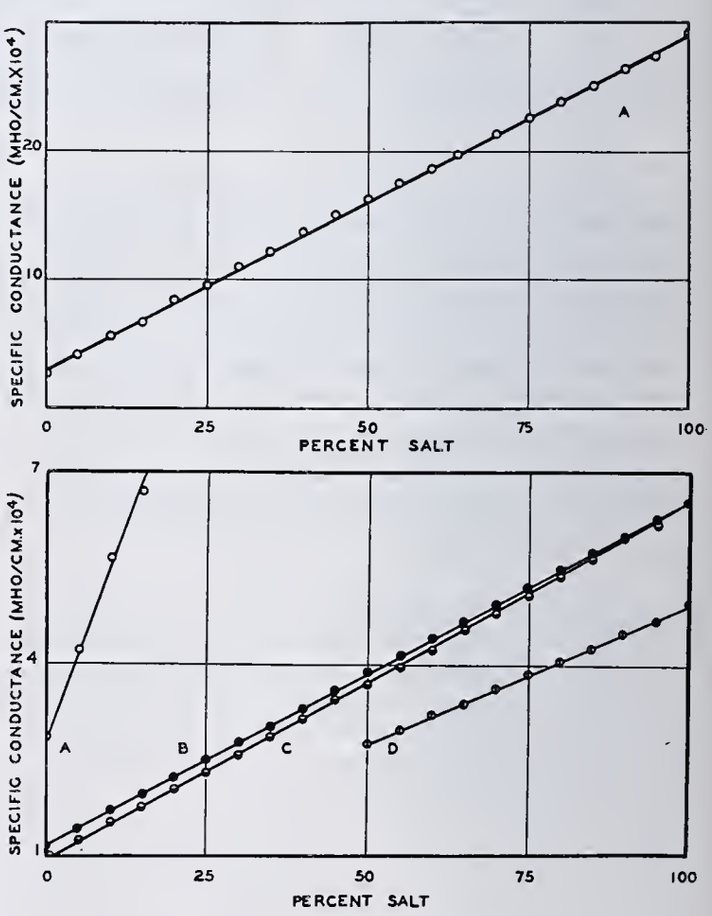

FIGURE 2. SPECIFIC CONDUCTIVITY *vs.* PER CENT SODIUM SULFATE IN DRY SOLID

(Curves *A* and *D* were determined by A. C. Bell.)

A. Sodium sulfate and sodium salt of sulfated coconut oil monoglyceride, 0.20% solution
B. Sodium sulfate and sodium salt of commercial sodium lauryl sulfate, 0.04% solution
C. Sodium sulfate and sodium salt of mixed alkyl aromatic sulfonic acids, 0.04% solution
D. Sodium sulfate and sodium salt of sulfated coconut oil monoglyceride, 0.03% solution

water and high enough to avoid errors due to polarization of electrodes.

Analytical Procedure

In operation, a sample of the material was dried in a vacuum oven, and a solution of 0.040 per cent concentration made up in distilled water. The solution was allowed to stand 15 minutes to reach thermal equilibrium, and was then transferred to a short specific gravity cylinder large enough to hold the dipping electrode. The solution was agitated gently with the electrode, which was inserted to a fixed mark, and the bridge was balanced. The temperature of the solution was read and the specific resistance corrected to 25° by the formula given above, and for cell constant when necessary.

To ensure clean electrodes, it was found better to make another reading on a fresh sample of the solution and accept the values only if the two readings agreed within 1 per cent.

The salt content was read directly from the calibration curve.

Any nonvolatile water-insoluble material that is present will affect the result by decreasing the apparent amount of salt present. This may be corrected for by determining the amount of water-insoluble material by a suitable method (ether extraction for fatty material, filtration of aqueous solution for solids), and correcting for this in making up the solution for conductivity reading, so that the concentration is 0.040 per cent with respect to soluble inorganic salt plus soluble active ingredient. The result is then obtained as per cent salt on a moisture-free and water-insoluble–free basis.

It is undoubtedly possible to apply this procedure when more than one inorganic salt is present. In the particular case of sodium sulfite–sodium sulfate mixtures, when the sodium sulfite is present in variable small percentages, little error is introduced by considering the sulfite present as sodium sulfate, since the specific conductivities of the pure salts are almost equal. In other cases it would probably be necessary to determine the concentration ratios of the inorganic salts present by some other means and use a calibration curve for the mixture actually worked with.

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An Improved Sublimation Apparatus

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PROBABLY the most common type of sublimation apparatus to be found in chemical laboratories consists of two test tubes, one within the other. The substance to be sublimed is placed in the larger test tube, which is then heated in a bath or with a free flame, while the inner tube, which functions as a condenser for the sublimate, is kept cold with running water. The capacity of such an apparatus is very limited, as only a small quantity of substance will adhere to the surface of the condenser, or will drop off when the inner tube is removed.

The apparatus shown in Figure 1, *A*, has been found to give excellent results with a number of compounds. The condenser may be a straight tube or made with a bell-shaped lower end, as shown. Heating the end of this tube with a soft brushy flame to the softening point of the glass and applying a gentle suction to the other end produce an even convex or cuplike surface.

The most important feature of this apparatus is a screen to catch any crystals of the sublimate that may break away from the condenser. This screen is supported by three glass rods fused into the edge of the condenser cup and bent inward at right angles (*B*). If a bath is used for heating, the surface of the liquid should be at about the level of the screen or a trifle above, to prevent sublimation on the screen and clogging of the meshes. The screen must be resistant to action by the sublimate, and of such mesh as to retain all crystals that may fall on it. A 40-mesh German-silver screen was used in this laboratory. For sublimation under vacuum, a tube may be sealed into the side of the outer jacket, as shown in Figure 1, or run through the rubber stopper supporting the condenser. Cooling water may be run in and out of the condenser through a two-hole stopper at the top, instead of through the one-hole stopper and side tube as shown in the diagram.

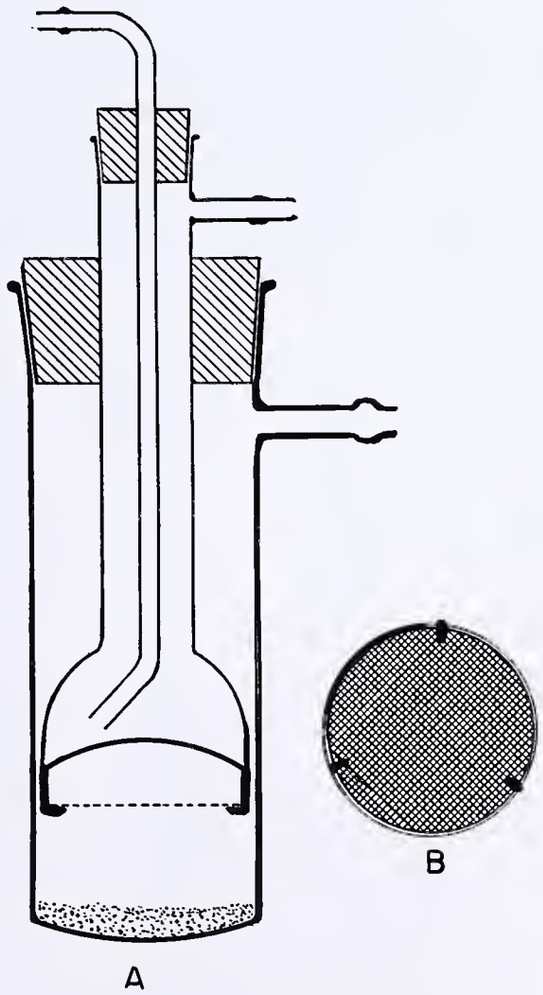

Figure 1. Sublimation Apparatus

Assay of Benzaldehyde

Use of Hydroxylammonium Sulfate and Aqueous Sodium Hydroxide

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THE strength of benzaldehyde, either pure or in solution, can be estimated with varying degrees of accuracy by several methods. The official methods of the Association of Official Agricultural Chemists (*1*) recommend determining the benzaldehyde as the phenylhydrazone. Though this method gives the most reliable and constant results, it is time-consuming, and the phenylhydrazine must be freshly distilled (though if stored in the refrigerator it will keep for about 3 weeks); the precipitate must stand overnight, the filtration requires considerable time, and *in vacuo* at 70° C. the precipitate requires 3 hours to dry. Furthermore phenylhydrazine can cause a very disagreeable allergy, resulting in swelling and intense itching of the fingers or other parts, and eventual peeling of the skin.

The bisulfite method originally proposed by Ripper (*8*) has been discussed by Kolthoff (*5*, *6*) and Donnally (*3*). Benzaldehyde is added to a known excess of sodium bisulfite, and the sodium bisulfite not used in forming the benzaldehyde addition compound is titrated with standard iodine solution. This method is not, in the authors' experience or according to the literature, satisfactory even for pure material. It is awkward and inconvenient, and the authors have found it to be decidedly unreliable.

The third method, recommended by the United States Pharmacopoeia (*9*), involves the titration of the hydrochloric acid liberated by benzaldehyde from a solution of hydroxylammonium chloride. The reaction of aldehydes with hydroxylammonium chloride was first studied by Cambier and Brochet (*2*), who established the quantitative relationship between the liberation of the acid and the moles of aldehyde present. Since then, wherever the method has been applied, the chloride has always been used. In dilute solution there is an error of 7 to 9 per cent (*7*). Besides being expensive, the chloride is unstable, dissociating into free hydrochloric acid and hydroxylamine. The acid liberated from the hydroxylammonium chloride by benzaldehyde is titrated using alcoholic potassium hydroxide with bromophenol blue as indicator (*4*, *9*). It was claimed (*4*) that unless the alkali was added drop by drop, results would be low.

The authors first compared the results obtained by the phenylhydrazone and the hydroxylammonium salt methods and found that they checked. Next preliminary experiments using aqueous sodium hydroxide instead of alcoholic potassium hydroxide showed that the aqueous alkali was entirely satisfactory. They then found that it was not necessary to add the alkali drop by drop. Finally, as a result of the search for a better indicator, they found that tetrabromophenol blue gave an end point much more quickly perceived, although it does not affect the extreme deviation in the results.

Two years ago the Commercial Solvents Corporation put hydroxylammonium sulfate on the market, and as this salt is stable and cheap, the authors immediately decided to test its usefulness in the assay of benzaldehyde. The hydroxylammonium sulfate as furnished by the Commercial Solvents Corporation is entirely satisfactory, and can be used without further purification, though it may be necessary to filter the solution to make it brilliantly clear. If it is desired to recrystallize the reagent, boiling in 50 per cent methyl alcohol with a little Darco G60 gives excellent results. The authors' experiments may be summarized as follows (see Table I):

Hydroxylammonium sulfate can be used in place of the unstable and expensive chloride.

Aqueous sodium hydroxide can be used in place of alcoholic potassium hydroxide.

The sodium hydroxide can be run in rapidly to within 2 to 3 cc. of the end point in less than one minute. A single titration, after sufficient practice in catching the end point, requires only 1.75 minutes.

Tetrabromophenol blue gives an end point more quickly perceived than in the case of bromophenol blue (*9*), though the error is about the same.

The extreme deviation in the results obtained depends largely on how frequently such titrations are run. When run daily, the extreme variation may be, on an average, only 0.2 to 0.3 per cent, while weekly titrations immediately raise the error to as much as 0.6 to 0.7 per cent. The extreme deviation has thus been reduced from 1 to 1.5 per cent (alcoholic potassium hydroxide and bromophenol blue) to a maximum of 0.6 to 0.7 per cent.

TABLE I. ASSAY OF BENZALDEHYDE

Batch No.	Benzaldehyde Found: Pharmacopoeia method[a]	A. O. A. C. method	Authors' method
	%	%	%
242	84.3	86.0	..
	85.8	85.8	..
243	92.6	92.6	..
	92.2	92.6	..
244	95.0	95.3	..
	95.5	..	..
233	98.3	99.1	..
	97.0	..	..
	97.3	..	..
	99.2	..	..
PL12	..	91.5	91.8
	..	91.8	92.1
	..	91.7	92.2
PL13	..	97.8	97.6
	..	98.0	98.1
	..	97.7	97.5
151	..	94.2	94.2
	..	94.4	93.8
	..	..	94.0
152	..	95.9	95.5
	..	96.0	95.8
	..	..	96.0
153	..	96.5	96.5
	..	..	96.3
	..	..	96.5
154	..	96.4	96.6
	..	96.5	96.4
	..	..	96.3

[a] $NH_2OH.HCl$, 96% C_2H_5OH, 0.1 *N* alcoholic KOH, bromophenol blue.

Procedure

Solutions required: 1.25 per cent hydroxylammonium sulfate in 50 per cent methyl alcohol, 0.1 *N* standard aqueous sodium hydroxide, and 0.1 per cent tetrabromophenol blue in 50 per cent methyl alcohol.

The 50 per cent methanolic solution of hydroxylammonium sulfate is acid and must be neutralized. For six batches of benzaldehyde which are assayed in triplicate, 600 ml. of solution are made. Three milliliters of tetrabromophenol blue indicator are added, turning the solution a clear green. About 12 to 15 ml. of 0.1 *N* sodium hydroxide are required to change the color to blue. Since the indicator is dichroic, the color in bulk is different from that of a small quantity; therefore alkali is added until the color of a 30-ml. sample poured into a 75-ml. Erlenmeyer-shaped flask is a clear pale blue. The solution should be neutralized immediately before use, because if it stands for more than 2 hours, sufficient acid is liberated by hydrolysis of the hydroxylammonium sulfate to change the color of the indicator and so make the results of the benzaldehyde assay high.

A 5-ml. Lunge weighing pipet is used to measure out the sample. In order further to reduce the danger of oxidation, the pipet is filled nearly full. The taper is fine enough so that about 10 drops give 0.2 gram. The weight of the pipet after weighing out the first sample can safely be used as the weight of the pipet be-

fore the next sample, and so on, without introducing an appreciable error. The benzaldehyde is dropped into a 75-ml. Erlenmeyer-shaped glass-stoppered flask containing 30 ml. of neutralized hydroxylammonium sulfate solution, and the flask is gently swirled. Samples lower than 90 per cent benzaldehyde usually go into solution with difficulty.

Titration

Standard 0.1 *N* sodium hydroxide is run rapidly into the solution until within about 2 to 3 ml. of the end point, then a few drops at a time until the color is green-blue, and finally dropwise until the color is again the clear, pale blue. The absence of direct sunlight is desirable. This part of the assay is almost exclusively responsible for any error.

The results obtained will be more truly representative if the samples are kept in glass-stoppered Pyrex bottles whose lips can be thoroughly cleaned.

The benzaldehyde content is calculated from the expression

$$\frac{\text{Ml. of } 0.1000\ N \text{ NaOH} \times 0.0106}{\text{weight of sample}} \times 100 = \% \text{ benzaldehyde}$$

Acknowledgment

The authors gratefully acknowledge the cooperation of the Commercial Solvents Corporation in furnishing samples of hydroxylammonium sulfate.

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Colorimetric Determination of Phosphorus in Biological Materials

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MISSON'S (*3*) method for the estimation of phosphorus has recently been adapted to the determination of this element in iron, steel, and iron ores (*4*, *5*). During use of the method in food analyses in this laboratory, the fact became evident that its range, sensitivity, and precision could be increased considerably in this application, where limiting interferences are rare. For this purpose the effect of time on the development of the yellow phosphovanadiomolybdate at various concentrations has been studied, the optimum acid concentration has been found, the range over which Beer's law holds precisely has been determined at three wave lengths of light, and spectrophotometric calibration data have been obtained with nine series of standard solutions. The results of these experiments have been condensed in a convenient procedure for the rapid and precise determination of phosphorus in foods and other biological materials, and this procedure has been tested by comparison with official gravimetric methods.

Apparatus and Reagents

A Coleman Model 10-S spectrophotometer was used to measure transmittance values. The spectral band width was 30 mμ. Matched square cuvettes were used to hold the reference liquids and test solutions. The dark current adjustment was frequently checked during the measurements, which were made at temperatures between 25° and 30° C.

Two independently prepared sets of reagents were used. 15 *N* nitric acid, 6 *N* hydrochloric acid, 9 *N* perchloric acid, and 4 *N* aqua ammonia were purified by distillation methods. Standard potassium dihydrogen phosphate solution containing 0.1000 mg. of phosphorus per ml. was made by dissolving 0.4393 gram of twice recrystallized and vacuum-dried material in distilled water and making to 1 liter. Another standard solution containing 0.1000 mg. of phosphorus per ml. was made by dissolving 1.351 grams of silver phosphate (*2*) in 5 ml. of 15 *N* nitric acid and diluting to 1 liter. Ammonium vanadate solutions were made by dissolving 2.345 grams of vacuum-desiccated ammonium metavanadate in 500 ml. of hot water, adding 10 ml. of 15 *N* nitric acid, and diluting to 1 liter. One sample of this salt was prepared by double recrystallization of reagent grade material with centrifugal draining; the other was a sample of atomic weight purity. One ammonium molybdate solution was prepared as described by Willard and Center (*5*), using vacuum-dried molybdic acid purified by precipitation with 6 *N* hydrochloric acid, followed by two crystallizations from water, with centrifugal draining. A second solution of this reagent was a 10 per cent solution of ammonium molybdate shown to be free of phosphate and chloride by blank tests; this was used with the systems containing silver. Fresh molybdate solutions were prepared frequently, as required.

Summary of Calibration Experiments

In obtaining calibration data, opportunity was taken to study the effect of various factors on the precision and accuracy of the method. Thus, while test solutions for the calibrations were made by the same method described below for the analyses, standard phosphate solutions were used instead of digested samples, various concentrations and mixtures of nitric and perchloric acid were tried, and each system was kept under observation for a much longer time. Different combinations of solutions from the two sets of reagents were used, to eliminate constant errors.

In Table I are given the median transmittances from about 1200 calibration observations made approximately 30 minutes after color development. Mathematical treatment of the data as a whole showed that the best agreement with the medians in Table I for all phosphate concentrations studied was obtained with systems containing 5 ml. of 9 *N* perchloric or 3 ml. of 15 *N* nitric acid per 100 ml. Delayed precipitation took place in all standards and blanks which contained only 2 ml. of 9 *N* perchloric acid per 100 ml., in the standards containing 10 ml. of 9 *N* perchloric acid and 5 mg. of phosphorus per 100 ml., and in some corresponding solutions containing equivalent amounts of nitric acid, but not in systems with intermediate acid concentrations.

In such systems transmittance readings were sufficiently constant after 10 to 30 minutes for precise measurements: transmittances were almost invariably lower after 12 to 24 hours, but rarely more than 1.0 unit lower. The minimum drop in transmittance occurred in systems containing 5 ml. of 9 *N* perchloric acid per 100 ml. On two counts, therefore, this is the optimum acid concentration. However, neither time nor acid concentration is extremely critical: transmittance readings taken from 10 minutes to many hours after making up the test solutions to 100 ml. with 2 ml. to 10 ml. of 9 *N* perchloric acid or equivalent amounts of nitric acid or nitric-perchloric acid mixtures give approximately correct results, even when precipitation of molybdic acid or the yellow complex is eventually to occur.

No significant differences due to interchange of reagents were observed during the calibrations. The order of addition of vanadate and molybdate should not be reversed.

Readings taken with water as the reference liquid were a little more reproducible than those made with blanks, but water should be used as the reference liquid only when all the developing reagents are free from phosphate and the solutions to be analyzed are colorless.

TABLE I. CONCENTRATION-TRANSMITTANCE DATA FOR PHOSPHORUS DETERMINATIONS

Phosphorus	Transmittance at Various Wave Lengths: Blank Reference 430 mμ	Blank Reference 450 mμ	Blank Reference 470 mμ	Water Reference 430 mμ	Water Reference 450 mμ	Water Reference 470 mμ
Mg./100 ml.	%	%	%	%	%	%
0.200	77.1	84.0	88.6	72.9	81.6	87.6
0.500	53.0	66.0	76.0	50.4	64.3	75.2
1.000	28.6	44.2	58.7	27.1	43.0	57.8
1.800	10.7	23.5	38.8	10.1	22.8	38.2
2.50	[5.1]	13.5	27.2	[4.8]	13.1	26.6
3.50	...	[6.6]	16.4	...	[6.3]	16.1
5.00	...	...	[8.2]	...	...	[8.1]

Graphical representation of the data in Table I shows that Beer's law is followed very closely except for the bracketed values, which were not used in the final reduction of results. The 4 per cent deviation of these values is probably due to an instrumental error; it is undesirable to use transmittances lower than 10 per cent in precise analyses.

Analytical Procedure

The following procedure is designed for the determination of phosphorus in aliquot portions of the solution obtained by ashing a 10-gram sample of material by successive treatment with 60 ml. (and, if necessary, additional 30-ml. portions) of 15 *N* nitric acid and 24 ml. of 9 *N* perchloric acid, according to the method of Gerritz (*1*).

PROCEDURE. When ashing is complete, evaporate the solution to about 50 ml., filter through a Munroe or Pyrex filtering crucible to remove silica, dilute, boil for at least 30 minutes, cool, and make up to exactly 250 ml. Most of this solution may be reserved for the determination of calcium and iron.

To analyze a colorless solution for phosphate pipet a 10.00-ml. or other suitable aliquot portion into a 100-ml. volumetric flask, add 5 ml. of 9 *N* perchloric acid or 3 ml. of 15 *N* nitric acid, and bring the volume to 70 ml. Add 10.0 ml. of ammonium vanadate solution, gradually mix in 10.0 ml. of ammonium molybdate solution, and dilute to exactly 100 ml. After 30 minutes make duplicate transmittance readings with two portions of the solutions, at the optimum wave length. If the reagents are free from phosphate use water as a reference liquid; otherwise use a blank containing the reagents. Calculate the result of the analysis from the median transmittance by appropriate substitution in Equation 1 below, or by use of a graph derived from this equation.

In analyzing colored solutions—e. g., the light yellow or yellow-green solutions derived from many foods—use as a reference liquid a system containing the same aliquot portion of colored liquid as the test solution, with all the reagents except the ammonium molybdate, which in this case must be free from phosphate.

For convenience in calculating results with maximum precision the data in Table I have been reduced by the method of least squares to equations of the form:

$$\text{Mg. of P per 100 ml. of test solution} = \frac{a \log_{10} T + b}{l} \qquad (1)$$

in which T is the percentage transmittance relative to the reference liquid, and l is the thickness of the solution in centimeters. Values of the constants a and b for the various conditions studied are given in Table II. In the present calibrations l was 1.308 cm., and the data in Table II apply to cases in which matched square cuvettes of about this size are used (*4*). The actual value of l should in any case be determined with a micrometer and calipers. Under the conditions specified, Equation 1 yields results correct to between 0.4 and 2 per cent over the range from 0.2 to 4.5 mg. of phosphorus per 100 ml. of final test solution.

Applications and Tests of Method

The procedure described above has been used in the analysis of some 80 different materials, including vegetables, meats, flour, milk, eggs, fruits, grasses, berries, vinegar, yeast, gelatin, agar, condiments, urine, feces, baking powders, wine, beer, fertilizers, oils, pharmaceuticals, chemical reagents, and about 40 food concentrates. In view of the small mineral content of most of these substances, few interferences were expected; the fact that results obtained at all three wave lengths were generally in good agreement and independent of aliquot size may be taken as evidence of the absence of serious interferences. Of course, one wave length usually provides a result of maximum precision, depending on the size and phosphate content of the aliquot. The optimum conditions may be selected by the analyst, who may use the lowest wave length to obtain sensitivity, the highest to obtain range, or more than one for confirmatory purposes.

TABLE II. CONSTANTS FOR CONCENTRATION-TRANSMITTANCE EQUATIONS

	Wave Length *Mμ*	*a*	*b*
Blank reference	430	−2.442	4.867
	450	−3.789	7.55
	470	−5.89	11.72
Water reference	430	−2.437	4.802
	450	−3.783	7.49
	470	−5.85	11.62

In addition to the various checks on the procedure provided by the use of two primary standard materials in the calibrations, an additional check was made by comparison of the method with A. O. A. C. official gravimetric methods, for representative materials. The results in Table III are typical, and further confirm the soundness of the method. The precision and accuracy of the spectrophotometric analyses are satisfactory, considering that they require only a fraction of the time needed for the gravimetric determinations.

TABLE III. SUMMARY OF TYPICAL RESULTS

Material[a]	Spectrophotometric Method 430 mμ	450 mμ	470 mμ	Official Method	
	Mg. P per 10 grams of material				
Apple vinegar	0.430	0.436	0.432	...	
Dried grass 1	7.3	...	...	7.0	(volumetric)
Dried grass 2	18.2	18.2	17.6	17.9	
Kelp concentrate	25.7	26.1	26.3	27.8	
Tomato concentrate	27.7	28.1	28.5	28.3	
Milk powder	...	70.3	70.0	70.2	
Spinach concentrate	...	72.5	70.8	70.6	
Liver concentrate	189	195	193	193	
Baking powder 1	303	309	310	303	
Baking powder 2	324	320	318	314	
Baking powder 3	841	839	834	828	
Fertilizer	560	550	544	520	(not official)
$FePO_4.2H_2O$ (1 g. per liter)	164$_6$	165$_0$	165$_2$	[1658]	(theoretical)

[a] All samples were dried before analysis by 12 to 24 hours of vacuum desiccation, except vinegar and ferric phosphate, which also were not ashed.

The authors are indebted to the Division of Chemistry of the Texas Agricultural Experiment Station for analyzed grass samples, to California Vegetable Concentrates, Inc., for the food concentrates, and to W. C. Craig for many gravimetric analyses.

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Estimation of Bromides in the Presence of Other Halides

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ESTIMATION of bromides in the presence of other halides is a problem of importance and a number of papers on the subject have been published.

Bromides in Presence of Chlorides

Most of the published methods can be classified under two general heads: (1) those depending upon the choice of certain oxidizing agents by which bromine alone is liberated and distilled off, the chloride remaining in the residue as chloride, after which bromine collected is determined iodometrically, gravimetrically as silver bromide, or colorimetrically by means of fluorescein solution or fuchsin–sulfuric acid reagent; and (2) those depending upon the quantitative oxidation of bromides to bromates either by chlorine water in the presence of sodium bicarbonate (*11*) or by the addition of the theoretical amount of hypochlorite in the presence of borate buffer and sodium chloride to half saturation (*5*, *8*). If chloride be present a small amount of chlorate is invariably formed and this must be allowed for by using controls subjected to the same procedure from the start; if iodide be present, it is quantitatively oxidized to iodate and then the apparent bromide figure includes the iodide.

TABLE I. DETERMINATION OF BROMIDES

KBr Solution Taken	$Na_2S_2O_3$ Required, $I_2=2Br$	Strength of $Na_2S_2O_3$	Weight of KBr in 100 Ml.	
			Found	Present
Ml.	*Ml.*		*Gram*	*Gram*
2.0	12.45	0.001602	0.996	1.0000
1.0	16.95	0.0005902	1.000	1.0000
5.0	42.35	0.0005902	0.4997	0.5000
1.0	16.65	0.0001200	0.1990	0.2000
1.0	8.25	0.0001200	0.0990	0.1000

To effect oxidation Baubigny and Rivals (*1*) used a mixture of copper sulfate and potassium permanganate, Clausmann (*3*) used a mixture of chromic and sulfuric acids, Baughman and Skinner (*2*) used chromic acid in concentrated solutions, Meloche and Willard (*7*) used potassium permanganate and hydrochloric acid, and Edwards, Nanji, and Parkes (*6*) used potassium permanganate and dilute phosphoric acid. These methods require distilling the bromide mixture with the oxidizing agent and collecting the liberated bromine in a vessel containing potassium iodide solutions, when an equivalent amount of iodine is liberated which can be estimated volumetrically by titrating against standard thiosulfate solution. There are other methods where bromine produced by oxidizing agents is extracted with carbon tetrachloride (*9*, *10*), which is separated and shaken with potassium iodide. Iodine thus liberated is determined volumetrically. Denoël (*4*) has recently described an extraction method for the estimation of bromides in the presence of chlorides, using potassium bromate as the oxidizing agent.

TABLE II. BLANK EXPERIMENTS WITH SODIUM CHLORIDE ALONE

Sodium Chloride Present	Sodium Thiosulfate Used
Mg.	*Ml.*
25	Nil
50	Nil
75	Nil
100	0.03
150	0.18

In the present investigation the authors used mixtures of nitric and chromic acids as oxidizing agents and removed the liberated bromine by shaking the mixtures with carbon tetrachloride.

TABLE III. DETERMINATION OF BROMIDES IN PRESENCE OF CHLORIDE

KBr Solution Taken	NaCl Added	$Na_2S_2O_3$ Required, $I_2=2Br$	KBr in 100 Ml. of Solution	
			Found	Present
Ml.	*Gram*	*Ml.*	*Grams*	*Grams*
2.0	0.025	11.12	1.0008	1.0004
2.0	0.050	11.12	1.0008	1.0004
2.0	0.075	11.12	1.0008	1.0004
2.0	0.100	11.15	1.0035	1.0004
2.0	0.125	11.20	1.0080	1.0004
2.0	0.150	11.30	1.0170	1.0004

Strength of $Na_2S_2O_3$ used, 1 ml. = 0.0018 gram of KBr

REAGENTS. A 1 per cent stock solution of potassium bromide was made from analytical reagent potassium bromide. Different volumes of this solution were taken to estimate the bromide content.

Carbon tetrachloride available in the stores was purified according to the standard methods (*12*).

Nitric acid and chromic acid were extra pure Merck quality.

PROCEDURE. A known volume of potassium bromide solution was mixed with an excess of 10 per cent chromic acid and an equal volume of concentrated nitric acid in a 100-ml. separating funnel. The mixture was shaken with 10 ml. of carbon tetrachloride. The lower layer of carbon tetrachloride containing the liberated bromine was separated. To ensure complete removal of bromine, the solution was further shaken a number of times with successive 5-ml. portions of carbon tetrachloride and the latter was separated. It was found that chromic acid dissolves to a small extent in carbon tetrachloride and interferes later on with the estimations. Therefore, carbon tetrachloride extractions were shaken with water to remove any chromic acid present. To safeguard against any loss of bromine, the water layer was again shaken with a fresh 5-ml. portion of carbon tetrachloride and this extraction added to the rest of the extractions. To the carbon tetrachloride extractions in a glass-stoppered Erlenmeyer flask potassium iodide was added and the equivalent amount of iodine liberated was titrated against standard sodium thiosulfate. The end point is indicated by the disappearance of violet color from the carbon tetrachloride.

TABLE IV. EFFECT OF IODIDES

Potassium Iodide Present	Sodium Thiosulfate Used
Mg.	
25	Nil
30	Nil
40	Nil
75	Nil

In Table I are given the results obtained on a specimen of pure potassium bromide, showing experimental results in very good agreement with the calculated ones. In all these cases 10 ml. of 10 per cent chromic acid and 10 ml. of nitric acid were used for oxidation. Carbon tetrachloride extraction was done four times.

As may be seen from Table I, very small amounts of bromides, like 0.001 gram in 1.0 ml. of 0.1 per cent solution, can be easily and accurately estimated.

A preliminary series of blank determinations was carried out to ascertain the effect of chromic acid–nitric acid mixture on varying concentrations of chloride. The figures in Table II show that no iodine is liberated from carbon tetrachloride extractions on the addition of potassium iodide when the concentration of chloride is not very high.

In Table III are recorded results of determination of potassium bromide in the presence of varying quantities of sodium chloride.

It is clear that if the amount of chloride present be not large, the amount of bromide found is almost theoretical. High results in the presence of much chloride have been noted by other investigators (*9*).

Bromides in Presence of Iodides

The interference of iodides in the oxidation method of determining bromides is overcome by oxidizing the iodide to iodate (*7*). The authors have observed that the method described above, when slightly modified, overcomes the interference of iodides, as they are completely oxidized to iodates by the excess of nitric acid–chromic acid mixture.

TABLE V. DETERMINATION OF BROMIDES IN PRESENCE OF IODIDE

KBr Solution Taken	KI Added	$Na_2S_2O_3$ Required, $I_2=2Br$	KBr in 100 Ml. of Solution	
			Found	Present
Ml.	*Gram*	*Ml.*	*Grams*	*Grams*
2.0	0.0150	15.20	1.00016	1.0002
2.0	0.0300	15.20	1.00016	1.0002
2.0	0.0450	15.20	1.00016	1.0002
2.0	0.0600	15.20	1.00016	1.0002
2.0	0.0750	15.20	1.00016	1.0002

Strength of $Na_2S_2O_3$ used, 1 ml. = 0.001316 gram of KBr

PROCEDURE. To the mixture of halides 25 per cent instead of 10 per cent chromic acid was added with nitric acid, thus ensuring complete conversion of iodides to iodate. The rest of the procedure was as described above.

Preliminary experiments were carried out with potassium iodide alone and it was found (Table IV) that no iodine is detected in the carbon tetrachloride extractions.

In Table V are given the results of determination of potassium bromide in the presence of varying concentrations of potassium iodide. The results are self-explanatory.

It is evident that the method described can be used safely with great accuracy for the determination of bromides in the presence of iodides and chlorides, provided the chlorides are not present in large amount as in saline waters, "potash salts", etc.

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An Adjustable Safety Shield

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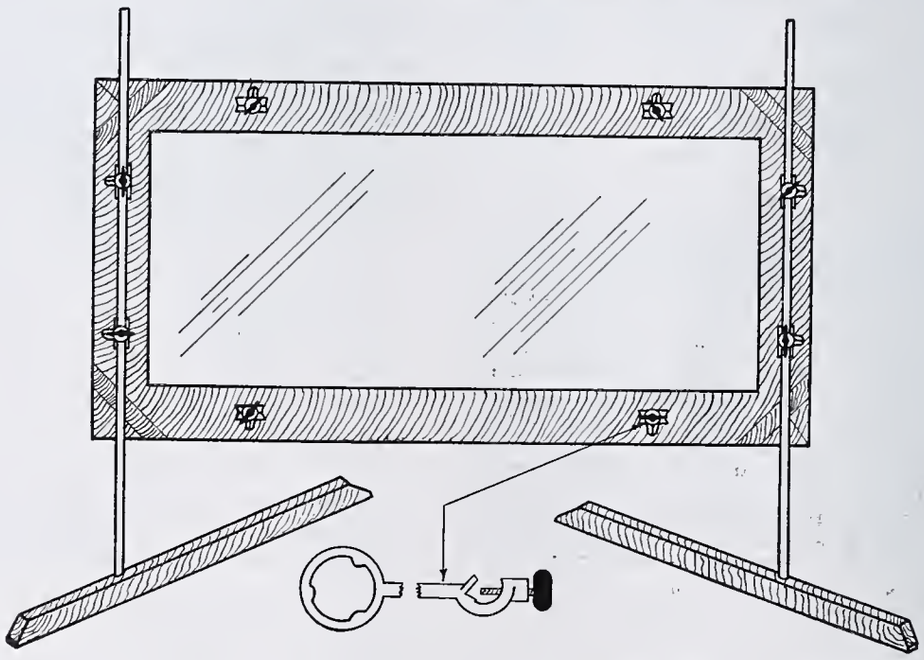

THE adjustable safety shield here described was developed by the writer to overcome the limitations to the arrangement of apparatus when a conventional safety shield is used.

The conventional shield consists of either safety glass or wire-encased glass set close to the base legs and rigidly attached to them. Protection is afforded at the very bottom where there is no real need, while at the same time the shield protects only a restricted area. The height of the shield is the limiting height of the apparatus. If the arrangement must be modified, the dimensions of the safety shield are a hampering factor. Unless an assortment of shield sizes is kept, only a limited number and types of arrangements can be used.

To give more leeway to the assembling of apparatus and still maintain complete protection, an adjustable, portable safety shield was constructed, using the safety glass from an old automobile wind wing. It was tested by shattering a liter beaker against it from a distance of 4 feet. The glass was enframed in 2 by 4 inch wood, and cushioned by strips of cloth. Into two holes bored in each side were forced the shaft ends of some broken rings. These served to clamp the shield to the ring stands which had been removed from the original iron stands and fastened to large wooden blocks.

This shield can easily be made higher or lower, and used either vertically or horizontally. Ultimate freedom and flexibility in setting up apparatus are achieved.

A Modified Noyons Thermic Diaferometer for Respiratory Gas Analysis

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A modification of the thermic diaferometer of Noyons is described, the general principle of which is the analysis of the carbon dioxide content and oxygen deficit of respiratory chamber gases based on measurements of the deflections of a sensitive galvanometer, which indicates the changes in resistance of platinum wires caused by a difference in the cooling powers of the gases surrounding the wires. The apparatus has been standardized by analyses with diluted respiratory air in comparison with atmospheric air, and constants have been established for the equivalent percentages of carbon dioxide and oxygen deficit in samples of respiratory chamber air per millimeter deflection of the galvanometer. The accuracy of functioning of the apparatus has been demonstrated by alcohol control tests. Forty-six consecutive tests on five days gave an average respiratory quotient of 0.662 with a standard deviation of ±0.0084. The standard deviation of the quotients from the theoretical alcohol quotient of 0.667 was ±0.0097.

IN 1937 Noyons (*1*) published the description of an apparatus for gas analysis, the principle of which was dependence upon measurement of the changes in resistance of platinum wires in two arms of a Wheatstone bridge caused by differences in the cooling powers of the gases surrounding the wires. In August, 1938, one of the authors (T. M. C.) saw the apparatus demonstrated at the Sixteenth International Physiological Congress in Zurich, Switzerland, and was able to obtain measurements and details with regard to its construction.

A schematic diagram indicating the general principle of the apparatus is shown in Figure 1, and photographs of the assembled apparatus are reproduced in Figures 2 and 3. The apparatus consists of two parallel pathways for two continuous streams of air, which are driven either through absorbents for both carbon dioxide and water vapor or through an absorbent for water vapor alone. After passing through the absorbents, portions of the two streams of air are aspirated by means of a constant-level hydrostatic pump through chambers containing resistance wires which form two arms of a Wheatstone bridge system. The changes in cooling power of the gas passing through the system are measured by the deflections of a delicate galvanometer.

The streams of air enter the apparatus through *A*1 or *A*2 (Figure 1), and the direction of either air current can be determined by stopcocks *B*1 and *B*2. The air, aspirated from the outside, is driven through the absorption system by pumps *P*1 and *P*2, and the rate of flow is controlled by needle valves *N*1 and *N*2. When it is desired to pass the air along the system without removing its carbon dioxide content, stopcocks *D*1 and *D*2 are closed and stopcocks *C*1 and *C*2 are turned in such a direction that the air passes directly to the absorbing vessels, *E*1 and *E*2, where the water vapor is removed. To remove carbon dioxide as well as water vapor, stopcocks *C*1 and *C*2 are closed and *D*1 and *D*2 are opened, which permits the air to pass through two parallel sets of tubes *F*1 and *F*2, containing the carbon dioxide absorbent. After leaving the carbon dioxide absorbing system, the gas in the original Noyons apparatus

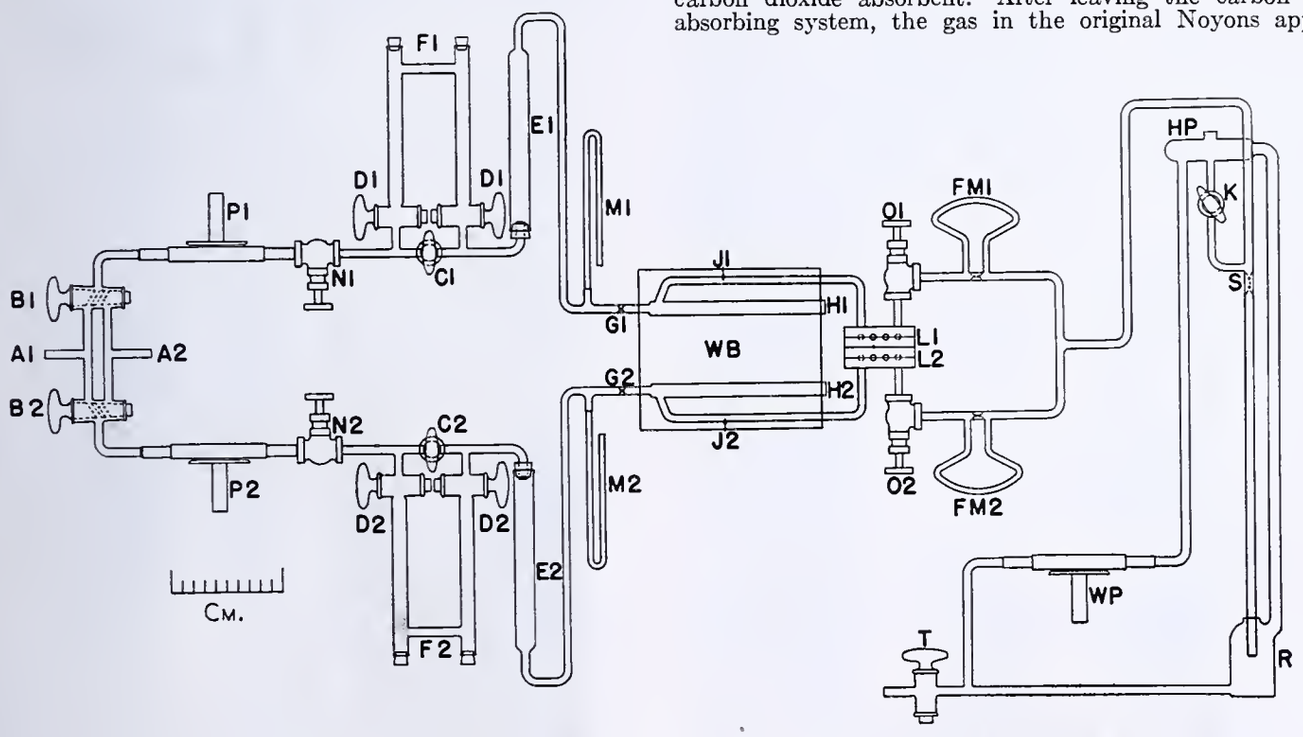

FIGURE 1. SCHEMATIC OUTLINE OF GENERAL PRINCIPLE OF MODIFIED NOYONS DIAFEROMETER

*A*1 and *A*2, openings for entrance of air. *B*1 and *B*2, stopcocks to direct air current through apparatus. *P*1 and *P*2, pumps to aspirate air. *C*1, *C*2, *D*1, and *D*2, stopcocks to direct air through *E*1 and *E*2 alone or through *F*1 and *F*2 as well, for removal of water vapor and carbon dioxide, respectively. *FM*1 and *FM*2, flowmeters. *HP*, hydrostatic pump. *M*1 and *M*2, oil manometers. *G*1 and *G*2, points of constriction in tubing. *H*1 and *H*2, tubes through which air passes out into room. *J*1 and *J*2, tubes through which air samples pass into diaferometer blocks *L*1 and *L*2. *N*1, *N*2, *O*1, and *O*2, needle valves to regulate rate of air flow. *R*, water reservoir. *K* and *T*, stopcocks. *S*, capillary suction pump. *WB*, water bath. *WP*, water pump.

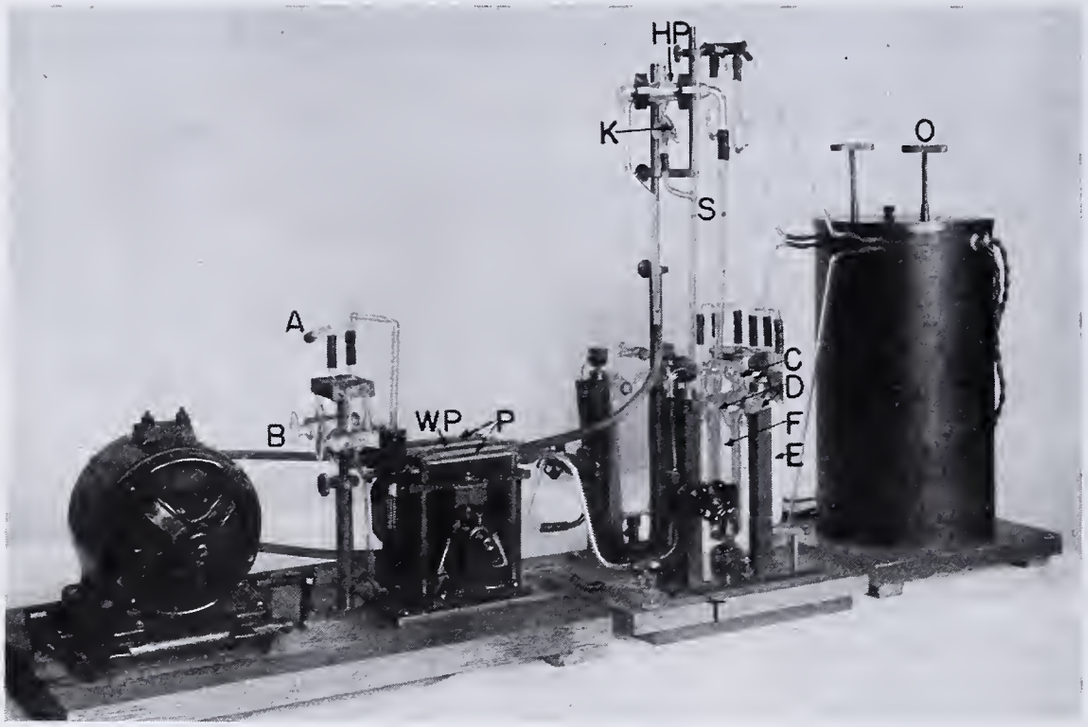

FIGURE 2. GENERAL VIEW OF MODIFIED NOYONS DIAFEROMETER

passed through a saturator, but the authors found it more satisfactory to remove the water vapor completely.

Containers $E1$ and $E2$ hold 12 grams of Dehydrite each, and the two parallel sets of tubes, $F1$ and $F2$, hold 15 grams of Ascarite each. These amounts serve for thirty analyses.

The rate of flow of the air through the continuing circuit is regulated by oil manometers $M1$ and $M2$. To have pressure at this point, a constriction is made in the tubing leading to the outside air at points $G1$ and $G2$. After leaving these points, the excess air passes out freely through the parallel tubes, $H1$ and $H2$, to the outside air. These two tubes are immersed in a cylindrical glass water bath, WB.

From these two main air streams samples are drawn for analysis through tubes $J1$ and $J2$ by means of a hydrostatic pump, HP, which aspirates the air through the two sides of the Wheatstone bridge enclosed in the diaferometer blocks, $L1$ and $L2$. The rate of flow of air through these two small side arms, $J1$ and $J2$, is regulated by two needle valves, $O1$ and $O2$. Two flowmeters, $FM1$ and $FM2$, have been installed in this model so that the rate of flow through the two sides of the Wheatstone bridge can be regulated independently. In the original apparatus of Noyons the flow was regulated by only one flowmeter, and this regulation of flow was governed by the reading of a galvanometer, which should show no change when the circuits are put into operation with the air flowing through them. The authors have found this arrangement unsatisfactory and have substituted the two flowmeters for the one.

The level of water in the hydrostatic pump, HP, is kept constant by pump WP, which continually draws water from a reservoir, R, which in turn is continually supplied with water from the overflow at the constant level at HP. The rate of suction of the air by the water flow is determined by the position of stopcock K. A capillary suction pump is located at S. When stopcock T is turned in the proper direction, the water can be drained from the hydrostatic pump.

The rate of flow through each side of the main circuit is from 4 to 5 liters per hour. The rate of flow through each arm of the Wheatstone bridge system is 3 liters per hour.

The electrical parts of the apparatus are somewhat different from other systems of gas analysis by measurement of changes in resistance in wires.

The resistance wires are enclosed in a copper box instead of in nonconducting material. A cross section of the diaferometer itself is shown in Figure 4, and general views are illustrated in Figure 5. Two copper blocks, 22 cm. long, 6.4 cm. wide, and 1 cm. thick, form a case containing the platinum wires. In each block are semicylindrical grooves (A and B, Figure 4), 20 cm. long and 2.4 mm. deep. Holes C and D, 4.4 mm. in diameter, are made through the block to connect one end of each groove with the air current. Two other grooves, F and G, 2.4 mm. deep, run the entire length of the block and carry the platinum resistance wires, which are fastened into terminals K and L at one end of the block and terminals M and N at the other end. Springs of No. 40 Manganin wire, $S1$ and $S2$, keep the platinum wires at the right tension so that they will not sag. The outside connections are insulated from the blocks. The electric current passing through the two platinum wires in each set of blocks is 56 milliamperes.

Two such blocks, when placed with the grooved sides facing one another, form one container for one arm of the Wheatstone bridge system. To permit the stream of air to pass from opening D and groove B across the wires in grooves G and F to groove A and out through opening C, the two copper blocks are separated at all points around the outside edge by a layer of aluminum foil, 0.4 mm. thick. The blocks and foil are made air-tight by shellac and held together by machine screws at various points. An end cross section of the blocks, separated, is shown in the lower part of

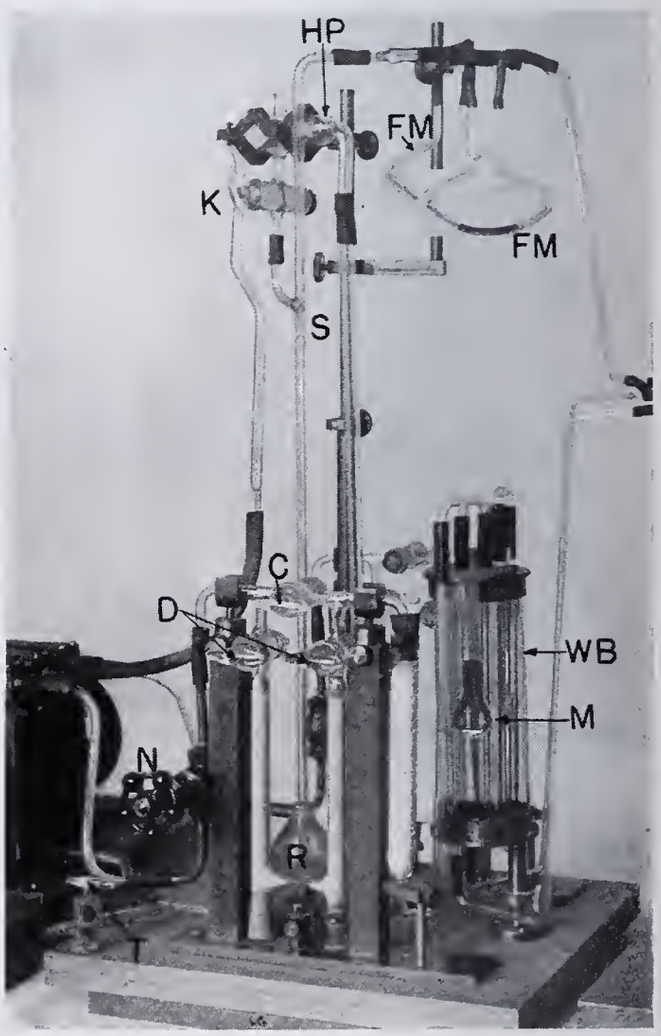

FIGURE 3. ABSORPTION SYSTEM AND HYDROSTATIC PUMP

Figure 4. Two sets of such blocks are placed together, back to back, and form the two arms of the Wheatstone bridge. This is placed in a thermostat, a copper box of double walls forming a case of about 1400 cc., which is filled with water. As the thermostat can be maintained at constant temperature, the Wheatstone bridge is protected against external thermic influences.

A diagram of the electric circuits for the Wheatstone bridge system is shown in Figure 6.

The two arms of the bridge, *A* and *B* (the resistance of one side of which is changed by the gas of unknown composition), contain the platinum wires, 19.5 cm. in length and 0.02 mm. in diameter, and each arm has a resistance of 120 ohms. The other two arms, *C* and *D*, are fixed resistances, each of 200 ohms. Final adjustment of the bridge is made by a small (0.1-ohm) slide-wire rheostat, *E*. At *F* there are three 6-volt storage batteries connected in series, at *H* there is a resistance box for regulating the amperage, and at *J* a Weston standard, portable milliammeter, Model 1, having a range of 1500 milliamperes and 150 scale divisions.

The galvanometer, *G*, is a Moll type made by Kipp, sensitive to 1.22×10^{-6} volt, and it gives a deflection of 1 mm. (equivalent to 5.7×10^{-9} ampere) at 1 meter scale distance. *LM* is a double-pole, double-throw, vacuum mercury switch which is used when the air currents through the two sets of absorbers are reversed. The full width of the galvanometer scale, which is 50 cm. in length, may thus be used with the zero setting at the same end in both sets of readings. The distance from the galvanometer mirror to the scale is 440 cm. The shunt used with the galvanometer is 40.1 ohms for oxygen and 3.9 ohms for carbon dioxide.

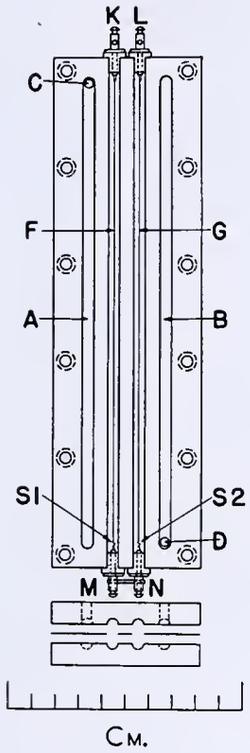

FIGURE 4. CROSS SECTION OF DIAFEROMETER

D and *C*, openings for entrance and exit of air by grooves *B* and *A* and *G* and *F*. *K*, *L*, *M*, and *N*, terminals to which platinum wires in *G* and *F* are fastened. *S1* and *S2*, springs to prevent wires from sagging.

A drawing of the pump, three of which are in the arrangement, is shown in Figure 7. This pump operates on the eccentric principle, driving a plunger which regularly closes a soft piece of rubber tubing.

The rubber tubing, *RT*, is shown in the upper part of the drawing connected to the ingoing valve, *V1*, and the outgoing valve, *V2*. These valves alternately close and open as the piston plunger, *P*, presses up against the rubber tubing. The valves are simple cylinders with lightly movable flaps placed on the ends, which are cut off at an angle. In the lower part of Figure 7 the pump is shown without the rubber tubing. The pump is driven by a 0.125-horsepower motor, with a 9-cm. pulley attached to the shaft of the pump and a 4-cm. pulley attached to the motor. The speed of the motor is cut down by resistance to about 600 revolutions per minute.

Standardization of the Apparatus

After the apparatus was constructed, the first problem was its standardization, to determine the deflection of the galvanometer per definite unit of carbon dioxide and of oxygen deficit in the gas passing through the system.

To determine the deflection for carbon dioxide, expired air diluted with atmospheric air was passed through one side of the system without absorption of the carbon dioxide, and on the other side the carbon dioxide was removed from it. This caused a deflection of the galvanometer, and from the number of millimeters' deflection and the known percentage of carbon dioxide in the air sample (as determined by the Carpenter gas-analysis apparatus), the percentage of carbon dioxide per millimeter deflection of the galvanometer could be calculated. The apparatus is so sensitive (a 500-mm. deflection on the galvanometer scale corresponds to a change in resistance of 0.012 ohm) that its sensitivity had to be cut down by shunting the galvanometer, in order to have a readable length of deflection of the galvanometer for the amount of carbon dioxide ordinarily occurring in respiration chamber air.

The standardization for the oxygen value was made by passing atmospheric air through potassium pyrogallate to remove a portion of its oxygen content and then collecting it in a gasometer. Through one side of the apparatus atmospheric air, freed from carbon dioxide, was passed and through the other side the air from the gasometer, freed from carbon dioxide. This gave a difference in the composition of the air in the two sides of the apparatus with respect to the oxygen content, and from the deflection of the galvanometer it was possible to standardize the apparatus for the oxygen measurement. In all these standardizations a comparison of the composition of the gases was made by the Carpenter gas-analysis apparatus.

A large number of standardizations were made, particularly of the value for oxygen per millimeter deflection of the galvanometer. It was necessary, first, to determine the regularity of these values, for it was evident that if the value changed with time, the apparatus would not be serviceable. In the course of these standardizations the authors tried on two occasions a mixture of carbon dioxide, oxygen, and nitrogen made from compressed gases in cylinders. However, this was found unsatisfactory, because apparently in making the mixture of these gases some one of the rare gases, which have a high cooling power, was increased in amount above that which would occur in normal atmospheric air.

Alcohol Control Tests

After the apparatus was once standardized for carbon dioxide and oxygen with diluted expired air, the next logical step, according to the experience in the Nutrition Laboratory, was to determine whether the values for carbon dioxide con-

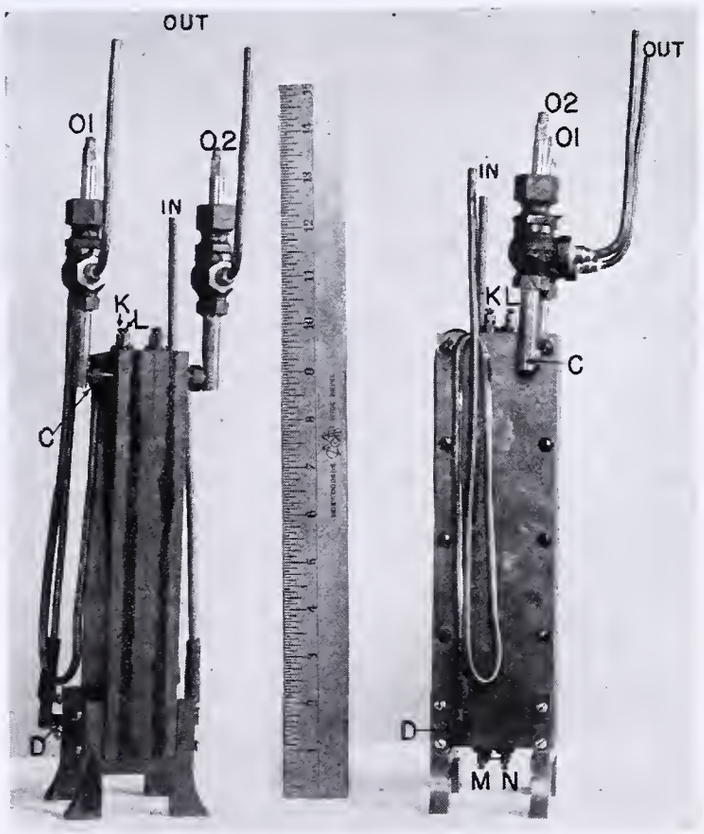

FIGURE 5. GENERAL VIEW OF DIAFEROMETER

Two sets of blocks containing platinum wires, ingoing and outgoing air tubes, openings *D* and *C* for entrance and exit of air, terminals *K*, *L*, *M*, and *N*, to which platinum wires in one block are attached, and needle valves *O1* and *O2*.

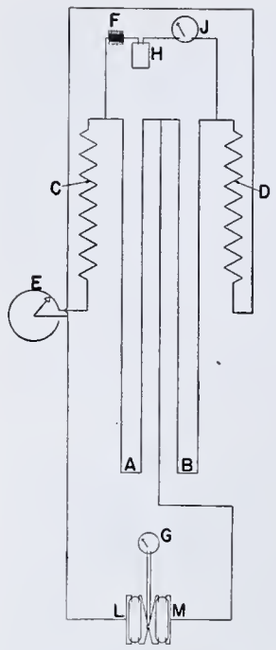

FIGURE 6. DIAGRAM OF ELECTRIC CIRCUIT FOR WHEATSTONE BRIDGE SYSTEM

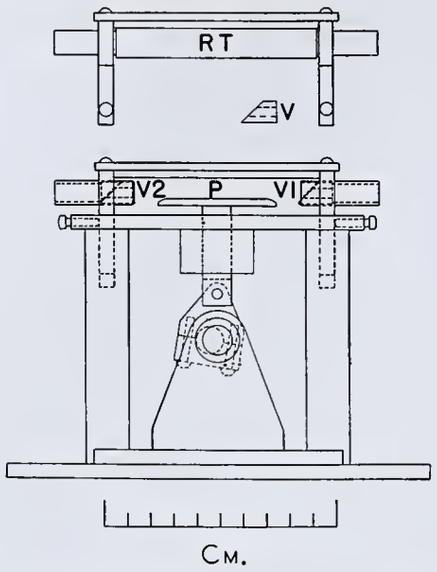

FIGURE 7. PUMP FOR ASPIRATION OF AIR THROUGH APPARATUS

tent and oxygen deficit per millimeter deflection of the galvanometer would be the same if ethyl alcohol were burned in a stream of atmospheric air. For this purpose the usual arrangement of apparatus was used (Figure 8), in which air passed over an alcohol lamp containing a small flame. At first, comparisons were made of the Carpenter gas-analysis apparatus and the modified Noyons apparatus to make sure that there was not present any gas, either unburned or foreign, which would contaminate the mixture so that it could not be read with the modified Noyons apparatus. In these tests the percentage values for carbon dioxide content and oxygen deficit per millimeter deflection of the galvanometer were no different from those obtained when atmospheric air was used to which carbon dioxide had been added or from which oxygen had been removed. Table I gives the results of eight such determinations, in which the average value per millimeter deflection of the galvanometer is 0.00238 per cent for carbon dioxide and 0.00336 per cent for oxygen deficit. The average of a large number of previous determinations with diluted expired air is 0.00238 per cent for carbon dioxide and 0.00335 per cent for oxygen deficit.

The modified Noyons apparatus was then used as a gas-analysis apparatus for analysis of air in alcohol control tests on five days (Table II). In this series of 46 consecutive tests, the carbon dioxide content ranged from 0.591 to 0.848 per cent and the oxygen deficit from 0.843 to 1.299 per cent. The average respiratory quotient was 0.662 and the standard deviation was ±0.0084. The standard deviation from the theoretical quotient of 0.667 was ±0.0097.

Procedure

For the determination of the carbon dioxide in a sample of respiratory chamber air, the first step is to have outdoor air flow through both sides of the apparatus, passing through the Dehydrite for removal of its moisture content. When the galvanometer becomes steady, which usually requires about 30 minutes, a sample of respiratory chamber air is aspirated through both sides of the apparatus, and its moisture is also removed. The position of the galvanometer is read after the sample has been flowing through for 4 minutes (reading 1, Table III). During the next 3 minutes carbon dioxide as well as water vapor is removed from one of the two streams of air and water vapor alone is removed from the other stream. At the end of these 3 minutes a second reading is made, to show the magnitude of the galvanometer deflection. Immediately after this second reading, water vapor alone is removed from both streams of air. At the end of 1.5 minutes under these conditions the largest deflection of the galvanometer will occur and should be recorded (reading 3). The deflection of the galvanometer will then move back toward the initial position and in 2.5 minutes will stop. The position of the galvanometer should then be read again (No. 4). The difference between the third and the fourth reading indicates, in terms of millimeters' deflection of the galvanometer, the content of carbon dioxide in the air sample.

TABLE I. CONSTANTS OF MODIFIED NOYONS APPARATUS

(Obtained by comparison of volumetric gas analysis with diaferometer readings in alcohol control tests)

	Galvanometer Reading		Volumetric Gas Analysis		Value per Mm. Deflection	
Date, 1941	CO_2	O_2 deficit	CO_2	O_2 deficit	CO_2	O_2 deficit
	Mm.	*Mm.*	%	%	%	%
June 10	375	311	0.891	1.041	0.00238	0.00335
June 10	378	312	0.899	1.044	0.00238	0.00335
June 11	378	315	0.902	1.055	0.00239	0.00335
June 11	386	309	0.918	1.035	0.00238	0.00335
June 16	354	290	0.840	0.971	0.00237	0.00335
June 18	343	284	0.803	0.950	0.00234	0.00334
June 19	376	309	0.893	1.040	0.00238	0.00337
June 19	355	288	0.853	0.981	0.00240	0.00341
				Av.	0.00238	0.00336

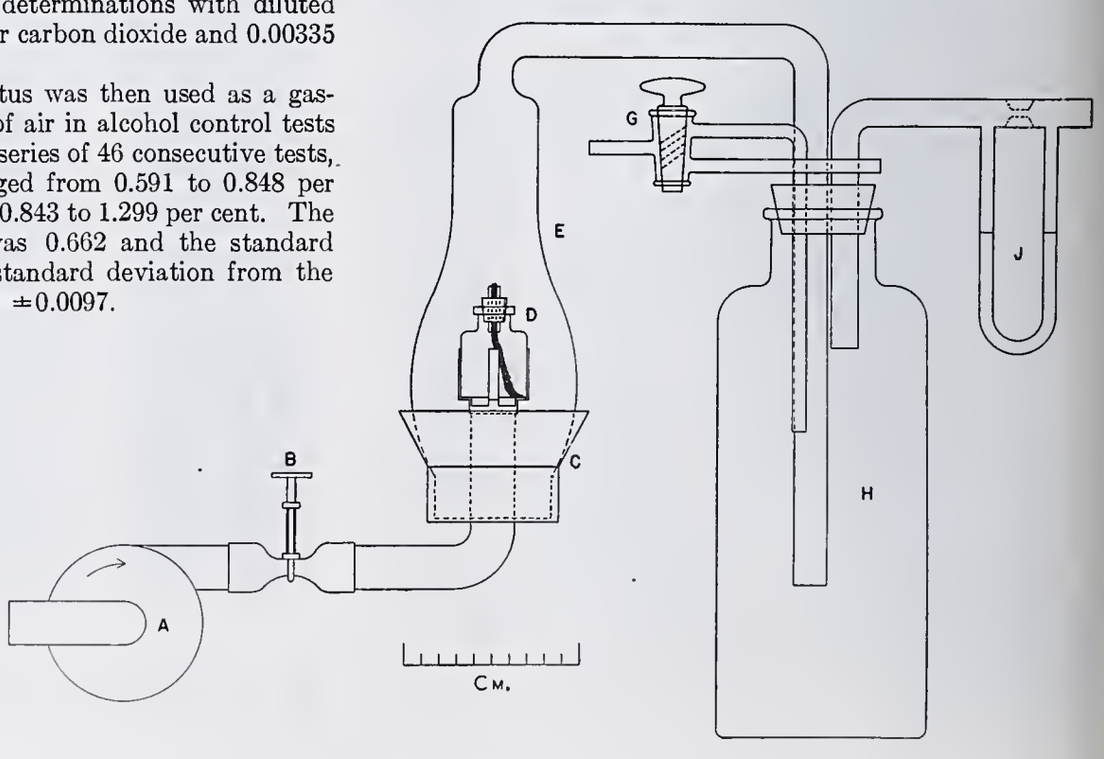

FIGURE 8. ARRANGEMENT FOR ALCOHOL CONTROL TESTS

A, rotary blower. *B*, pinchcock for regulating rate of flow of air. *C*, water seal. *D*, alcohol lamp. *E*, Pyrex lamp chimney. *G*, stopcock for drawing sample. *H*, mixing chamber. *J*, flowmeter.

Table II. Respiratory Quotients (CO_2/O_2) of Burning Ethyl Alcohol Obtained with Modified Noyons Diaferometer

(June–July, 1941)

June 25	June 26	June 30	July 1	July 2
0.638	0.654	0.649	0.677	0.659
0.657	0.667	0.653	0.664	0.663
0.649	0.664	0.657	0.677	0.661
0.657	0.668	0.657	0.657	0.668
0.662	0.663	0.660	0.683	0.657
0.666	0.661	0.662	0.661	0.664
0.673	0.646	0.659	0.670	0.668
0.672	0.668	...	0.657	0.655
...	0.655	...	0.675	...
...	0.664	...	0.668	...
...	0.664	...	0.668	...
...	...	...	0.667	...
Av. 0.659	0.661	0.657	0.669	0.662

For the determination of the oxygen deficit in a sample of respiratory chamber air, outdoor air is also allowed to flow through both sides of the apparatus, but in this case both the carbon dioxide and the water vapor are absorbed. When the galvanometer becomes steady (usually in about 30 minutes), the position of the galvanometer is read. Then for 4 minutes the sample of respiratory chamber air is passed through one side of the system, while outdoor air passes through the other side. At the end of the 4 minutes, the deflection of the galvanometer is read (reading 2). Immediately thereafter outdoor air is again allowed to flow through both sides of the apparatus, passing through both absorption systems. At the end of 2 minutes the largest deflection of the galvanometer will occur and should be recorded (reading 3). The deflection of the galvanometer will then move back towards the initial position and in 4 minutes will stop. The position of the galvanometer should then again be read (reading 4). The difference between the third and fourth readings represents the difference between the content of oxygen in the sample of respiratory chamber air (carbon dioxide–free) and the content of oxygen in carbon dioxide–free outdoor air, this difference being expressed in terms of millimeters' deflection of the galvanometer.

The initial reading of the galvanometer, both for the carbon dioxide and for the oxygen determination, is made only to see that the galvanometer is steady. The preliminary 30-minute period at the start, when the apparatus is brought into equilibrium, is necessary only at the beginning of a series of analyses on any one day and need not be repeated before each analysis in the series.

Theoretically, the third reading of the galvanometer deflection (in the carbon dioxide and oxygen determinations) should be made under the same conditions as the second reading as regards the composition of the air streams passing through the two sides of the diaferometer. The interval of time between these two readings is so short, however, that actually the respective compositions of the air streams in *L1* and *L2* (Figure 1) at the time of the third reading are still the same as at the time of the second reading. The total time required for an analysis can, therefore, be shortened by making the described changes in the air streams at the end of the second reading instead of waiting until the end of the third reading. The second reading is not absolutely necessary but is desirable as a check on the third.

Usually there is a difference of about 2 to 3 mm. between the initial reading and the fourth reading. The total time required for the analysis of an air sample for both carbon dioxide and oxygen is approximately 17 minutes, and the total volume of sample required for the complete analysis is 1 liter.

Table III. Analysis of a Prepared Sample of Diluted Expired Air Made with Diaferometer, June 17, 1941

Diaferometer Determinations

Reading No.	Carbon Dioxide Galvanometer Deflection Total	Carbon Dioxide Galvanometer Deflection Difference	Oxygen Galvanometer Deflection Total	Oxygen Galvanometer Deflection Difference
	Min. *Mm.*	*Mm.*	*Min.* *Mm.*	*Mm.*
Left side				
1	0 440		0 420	
2	3 205		4 311	
3	4.5 204		6 300	
4	7 439	235	10 424	124
Right side				
1	0 388		0 378	
2	3 158		4 280	
3	4 155		6 246	
4	7 388	233	10 370	124
		Av. 234		124

Standardization by Comparison with Volumetric Gas Analysis, Gas Analysis of Air Sample

	Reading	Difference	H_2O	True Reading
Vol.	99.925	....	0.204	99.721
CO_2	99.370	0.555	...	....
O_2	79.012	20.358	...	....
H_2O	78.808	0.204	...	....

CO_2 in 100 volumes of air sample (0.555 ÷ 99.721)	0.557
O_2 in CO_2-free outdoor air [20.940 ÷ (100 − 0.03)]	20.946
O_2 in CO_2-free air sample [20.358 ÷ (99.721 − 0.555)]	20.529
O_2 deficit (20.946 − 20.529)	0.417
CO_2 per 1-mm. galvanometer deflection (0.557 ÷ 234), %	0.00238
O_2 deficit per 1-mm. galvanometer deflection (0.417 ÷ 124), %	0.00336

Precautions in Use of Apparatus

The strength of the electric current passing through the diaferometer should be checked frequently, to make sure that it has remained unchanged.

The manometers showing the rates of flow of the sampling streams that pass through the diaferometer should be checked frequently, as otherwise incorrect readings will be obtained.

It is necessary to have clean water in the hydrostatic pump, in order for the aspiration of air to be uniform.

A check on the completeness of absorption of either carbon dioxide or water vapor or both can be obtained by reversing the currents of air and at the same time reversing the galvanometer. The same reading should be obtained for carbon dioxide and similarly for oxygen, irrespective of whether the right or the left side of the apparatus is used (Table III).

After the apparatus has been thoroughly standardized by comparison with volumetric gas analysis, it is no longer necessary to standardize it in this way, but alcohol control tests made regularly will demonstrate the accuracy of functioning of the apparatus, provided the ratio of carbon dioxide increase to oxygen deficit in the burning of alcohol is within 0.01 of the theoretical ratio of 0.667. If the apparatus gives this ratio, neither the value for carbon dioxide nor that for oxygen deficit has been changed.

The apparatus is designed primarily for analysis of respiratory chamber air. It could be adapted for analysis of expired air, if the shunts in the galvanometer system were changed to decrease the sensitivity or if the reading scale were brought closer to the galvanometer. It cannot be used for the analysis of gases from any respiratory system in which foreign gases are present that do not exist in atmospheric air, such as methane, hydrogen, and carbon monoxide. When such respiratory systems are used, these foreign gases must be removed from the air samples before analysis with the modified Noyons apparatus.

Literature Cited

(1) Noyons, A. K. M., *Ann. physiol.*, 13, 909 (1937).

Presented before the Divisions of Agricultural and Food, Biological, and Medicinal Chemistry, Symposium on New Analytical Tools for Biological and Food Research, at the 102nd Meeting of the American Chemical Society, Atlantic City, N. J.

Pressure Regulating and Indicating Apparatus for Vacuum Systems

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FOR many laboratory vacuum systems the automatically controlled air leak is the simplest and most satisfactory method of pressure control. Figure 1 illustrates such an apparatus which has been evolved to eliminate many of the objections to earlier designs of this type of control. This device has proved to be inexpensive, easy to operate and maintain, and capable of an accuracy of ±0.2 mm. for pressures of 5 to 760 mm.

With the apparatus set up as shown, stopcock *C* is opened, switch *M* is closed, and mercury is admitted through valve *D* (by means of a leveling bottle) until the mercury just makes contact at *E*, closing the electrical circuit. *D* is then closed and *M* is opened. The system is evacuated to the desired pressure at which point *C* and *M* are closed. Bulb *A* maintains the desired pressure and as the vacuum source tends to decrease the system pressure the mercury will rise in the right-hand arm of the U-tube, making contact at *E* and activating electromagnet *G*, thus opening capillary leak *H*.

The capacity of bulb *B* and the resistance offered by capillary *N* cushion the effect of this leak on the mercury, and capillary *I* prevents setting up a harmonic surging of the mercury. When sufficient air has entered *H* to increase the system pressure to that of *A*, the contact at *E* is broken and the spring flap on *G* closes *H*.

A needle valve in the line to the vacuum source may be used to adjust the frequency of this cycle; six to twelve cycles per minute are satisfactory. When increasing system pressure, or when bringing it to atmospheric pressure after use, it is customary to open stopcock *C*. If this is neglected, however, the mercury is forced into bulb *A* as the pressure is equalized, but it then falls back into position in the tube without further adjustment.

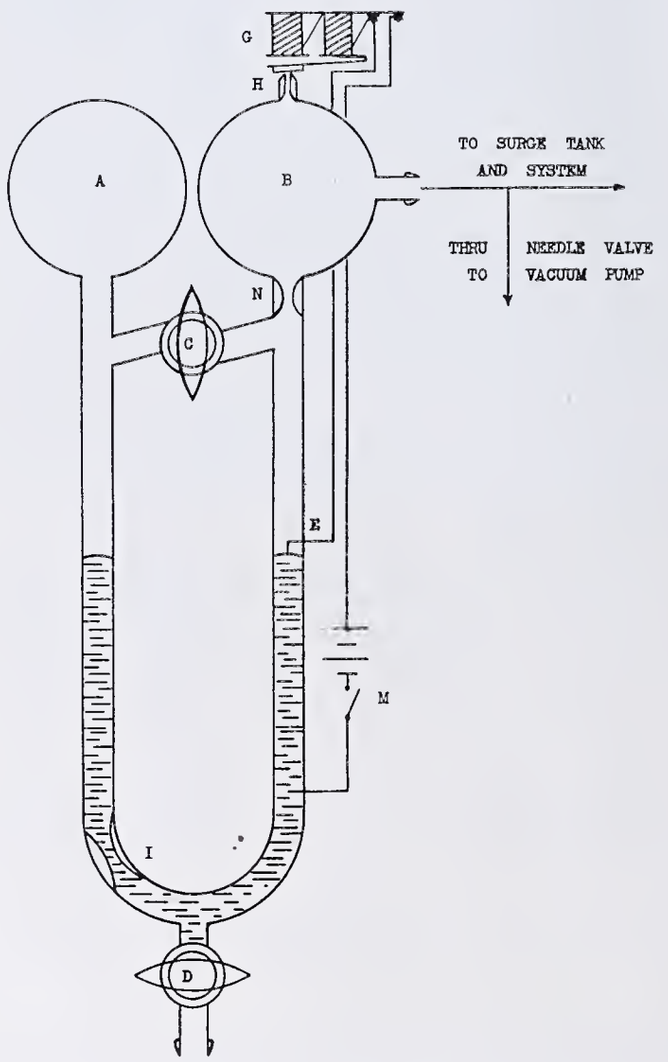

FIGURE 1. DIAGRAM OF APPARATUS

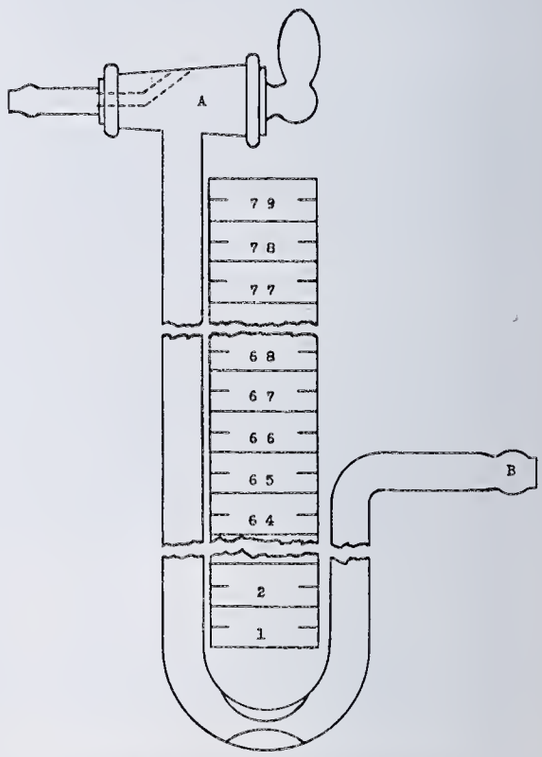

FIGURE 2. CLOSED-END MANOMETER

Oxidation of the mercury by the sparking at *E* may be decreased by floating a small quantity of oil on the mercury. White Russian mineral oil may be used after shaking with mercury to remove sulfides and filtering. The U-tube may be cleaned readily by draining the mercury from stopcock *D* and rinsing, by use of a leveling bottle, with dilute nitric acid, water, and acetone successively.

The glassware is furnished to Ugite Sales Corporation specification by Ace Glass, Inc., Vineland, N. J. The electromagnet is an ordinary twin-coil buzzer, obtainable at any electrical supply shop. It is then wired directly to the coils instead of through the make-and-break buzzer as furnished. Dry cells or a direct current source is used. The device may be mounted on a board for convenience in handling.

A closed-end manometer has several advantages over the open-end type for use in vacuum systems. It is independent of atmospheric pressure and obviates the necessity for continual barometric readings and corrections. It is read more easily and accurately, since under increasing vacuum its legs approach a common height, while the reverse is true of the open-end type.

The disadvantages of the closed-end manometer are mainly the difficulties encountered in filling the manometer properly, and in removing the mercury and cleaning the manometer.

Figure 2 illustrates a design for a closed-end manometer which overcomes these troubles. Stopcock *A* may be opened and after

sufficient mercury has been admitted through *B*, *A* is attached to a source of vacuum, exhausting the air above the mercury in the left-hand column. The closed arm of the manometer may be heated with a Bunsen burner to drive off air adsorbed to the surface of the glass and dispersed in the mercury. Now a slight air pressure is applied at *B*, forcing the mercury up into stopcock *A*. When the left-hand column is completely filled with mercury, *A* is closed. The manometer may be checked against the barometer to determine the success of the filling operation. It should agree with the barometer within 1 mm. at 760 mm., which amounts to a much smaller error at reduced pressure—(e. g., 0.05 mm. at 40 mm.).

For cleaning, the entire plug of stopcock *A* is removed, and dilute nitric acid, water, and acetone, consecutively, are circulated through the tube.

A constriction at the bottom of the tube cushions pressure changes. A sliding wooden scale may be used to simplify reading the differential heights of the two columns. This glassware is made to Ugite Sales Corporation specifications by Ace Glass, Inc., Vineland, N. J.

Continuous Washing Apparatus for Solutions in Organic Solvents

A. L. LEROSEN, California Institute of Technology, Pasadena, Calif.

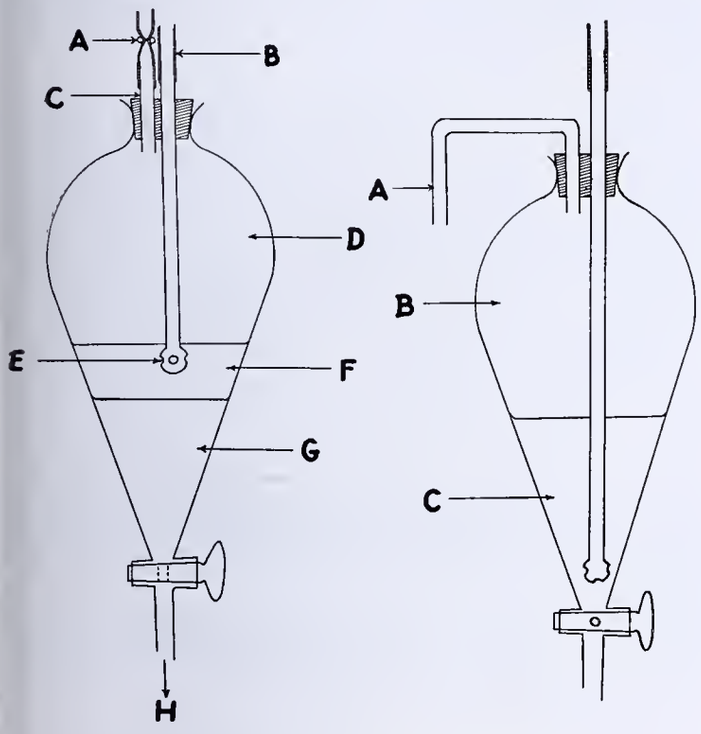

FIGURE 1 (*Left*). WASHING APPARATUS
A pinchclamp, *B* water inlet, *C* air adjustment tube, *D* constant air space, *E* washing tube outlet, *F* solution, *G* water, *H* outlet to drain

FIGURE 2 (*Right*). MODIFIED APPARATUS
A water outlet, *B* water, *C* solution

OFTEN it is necessary to wash petroleum ether, benzene, chloroform, etc., solutions free from a water-soluble solvent, alkali, or acid. If many solutions must be washed the operations are tedious and time-consuming, but these difficulties may be eliminated by the device shown in Figure 1. A constant air space is maintained in a separatory funnel while a stream of water is passed through the solvent.

To operate, water is added to the solvent mixture. After this first wash water is drained off as usual, the washing tube is inserted with pinchclamp open. The water is then turned on at a suitable rate and when the upper layer has reached the washing tube outlet, the pinchclamp is closed and the stopcock is opened. The closed air space maintains a constant level, so that the quantities of water entering and leaving are always equal. This washing is continued as long as necessary—e. g., 5 to 10 minutes for ligroin plus alcohol in a 500-cc. funnel. Then the bottom stopcock is closed, the pinchclamp is opened, the washing tube is rinsed with some pure solvent, and the water stream is stopped. This procedure has given satisfactory results with volumes of solvent from a few cubic centimeters up to 8 liters. The setup used in this laboratory, a series of 8 units, is shown in Figure 3.

For liquids heavier than water the washing tube is slightly modified (Figure 2) and reaches near the bottom of the separatory funnel. The water flows out at the top through a glass tube. The stopcock remains closed until the washed solvent is drawn off. No air pocket is present in this case.

GATES and Crellin Laboratories of Chemistry, California Institute of Technology, Contribution 849.

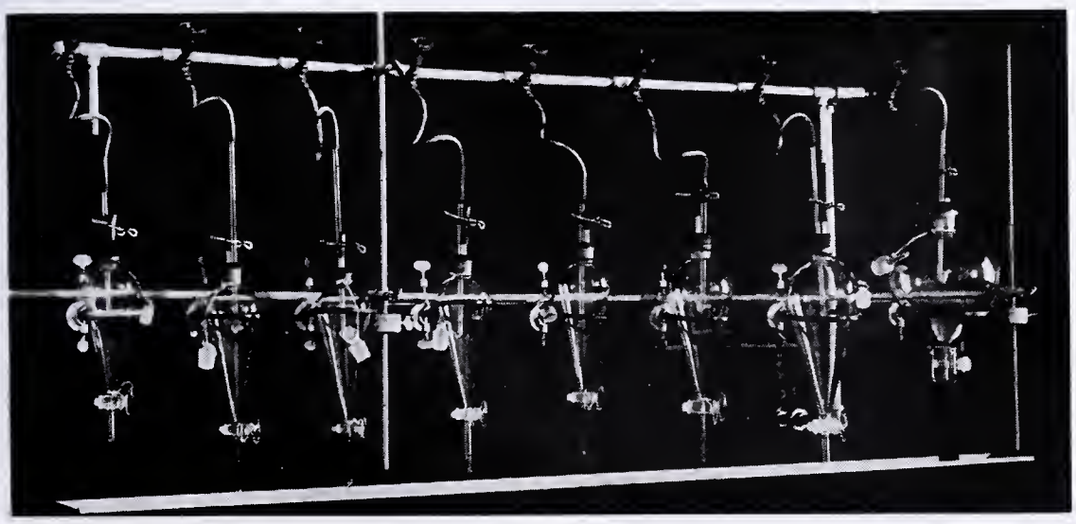

FIGURE 3

Determination of Solubilities of Gases at High Temperatures and High Pressures by the Rotating Bomb

V. N. IPATIEFF AND G. S. MONROE, Universal Oil Products Co., Riverside, Ill.

VERY little information can be found in the literature concerning the solubility of gases in liquids at high temperatures and high pressures.

The first work in this direction was carried out by V. V. Ipatieff, Jr., in 1930 when he employed the rotating bomb for this purpose (*1*, *2*). (This preliminary investigation was made in June, 1930, in the Institute of High Pressures, Leningrad. It was mentioned by V. N. Ipatieff at the meeting of the Scientific Technical Department before his departure from Russia.)

Since the literature contains data about the solubility of methane in some hydrocarbons at temperatures not higher than about 100° C., the authors decided to make experiments to determine the solubility of the gas in benzene over a temperature range approaching the critical.

For this purpose it was necessary to modify the rotating bomb, adding sampling lines by means of which either liquid-phase or vapor-phase samples could be taken. This change made it possible not only to determine solubilities but also to establish phase conditions within the bomb in the determination of the critical temperatures of individual substances and two-component systems to be described in a later paper.

The apparatus can be improved for the more accurate measurement of temperature and pressure. The authors recommend this type of apparatus not only for the determination of solubilities at high temperatures and high pressures but also for the study of reactions involving heterogeneous systems, the arrangement permitting the removal of samples of the different phases for investigation.

Apparatus and Procedure

The apparatus used for the solubility determinations consisted of an inclined (28° from the horizontal) modified Ipatieff rotating bomb of 3420-cc. capacity, heated by an external electric furnace, with a special head provided with two needle valves attached to the vapor- and liquid-phase sampling lines, respectively. An inner thermocouple pocket extended from the center of the bomb head equidistant from the sides of the bomb to about 1.25 cm. (0.5 inch) from the bottom. The vapor-phase sampling line extended 10 cm. (4 inches) below the head, and was supported by clamping to the thermocouple pocket. The 28° inclination of the bomb from the horizontal was considered ample to raise the vapor-phase sampling line above the liquid charge. The liquid-phase sampling line extended through the center of the bomb to the end of the thermocouple pocket and was bent at the end almost to touch the side of the bomb. When the bomb was mounted in the rotation rack, this arrangement made it possible to draw off liquid-phase sample from the lowest part of the bomb. The sampling lines inside the bomb were made of 3-mm. (0.125-inch) copper tubing to reduce the capacity to the minimum, that of the longer liquid-phase line, including the valve to the seat, being only 1.7 cc.

On stopping rotation of the bomb in the position shown in Figure 1 and connecting the sampling apparatus (Figure 2), liquid-phase and vapor-phase samples could be taken at any temperature and corresponding pressure.

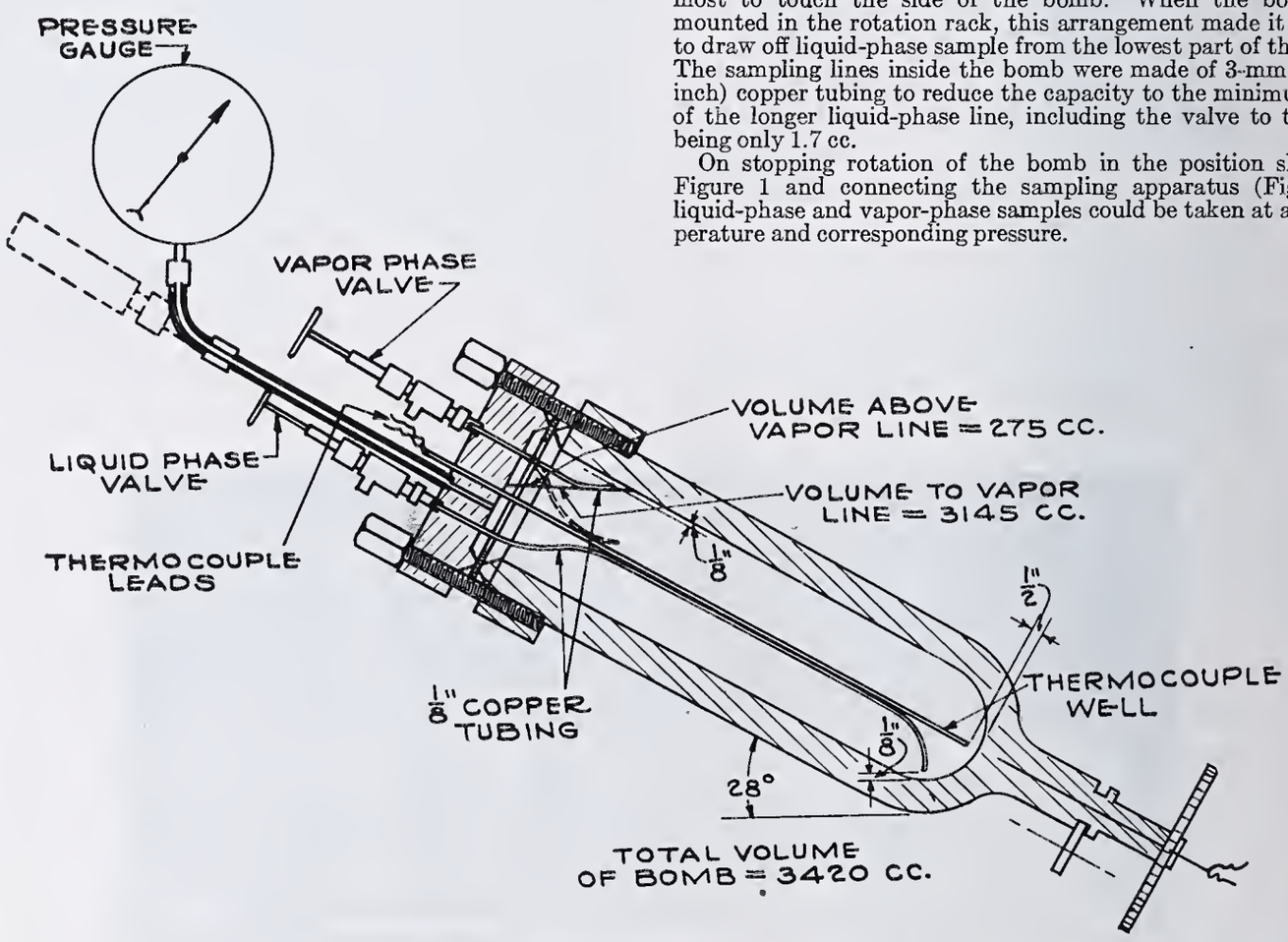

FIGURE 1. ROTATING BOMB FOR DETERMINING SOLUBILITIES AND CRITICAL TEMPERATURES

Broken lines show locations of gage and vapor-phase sample line for solubility determinations, solid lines show positions for critical temperature determinations.

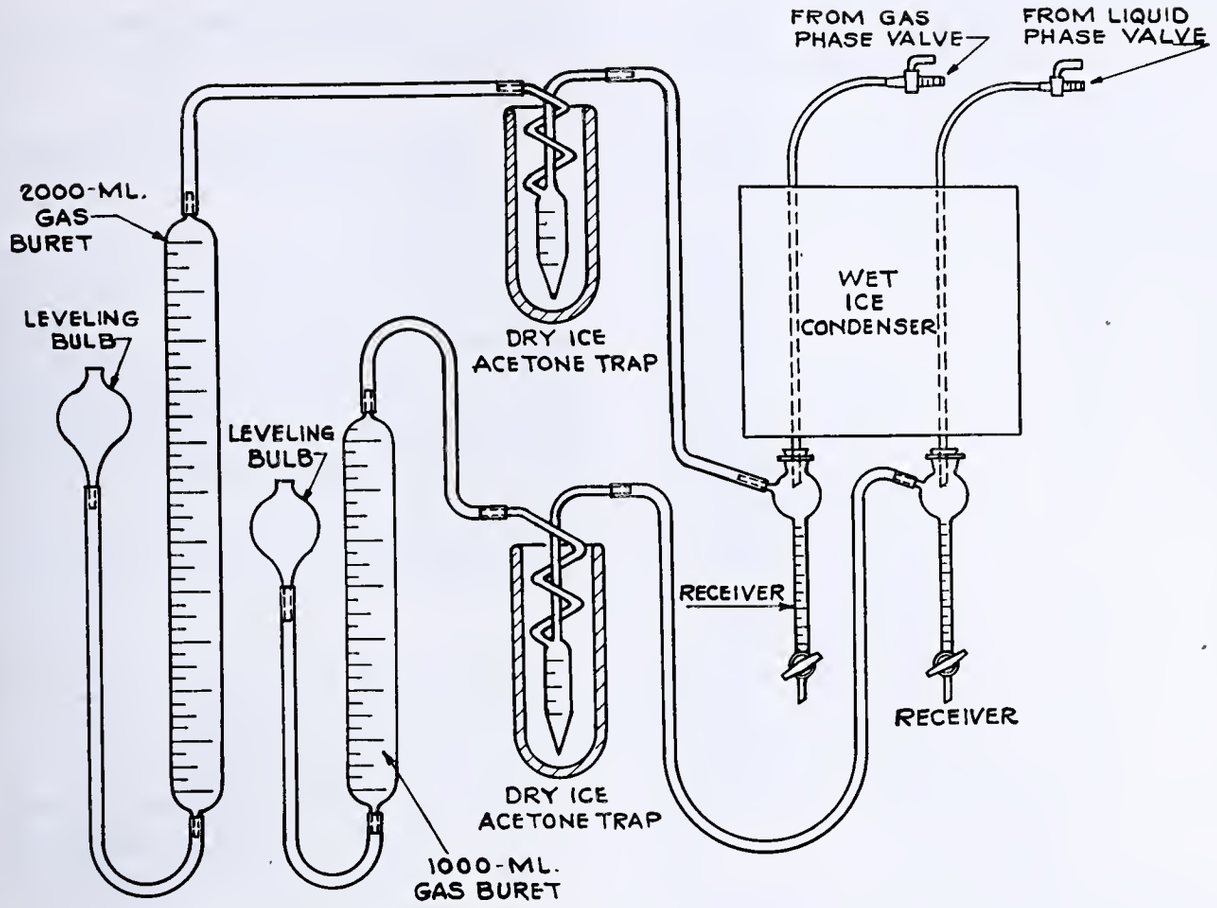

FIGURE 2. SAMPLING APPARATUS FOR DETERMINING SOLUBILITIES

The sampling device used for both liquid-phase and vapor-phase samples consisted of a wet ice condenser with a calibrated receiver (10-cc. capacity) which condensed most of the benzene from the methane. The outlet of the receiver led to a graduated dry ice trap (10-cc. capacity) to condense any benzene escaping previous condensation. The methane thus stripped free from benzene was collected over salt water in gas burets of 1000-cc. and 2000-cc. capacity for the liquid-phase and vapor-phase samples, respectively. After sampling, the cooling baths were removed from the traps and the contents of the same, together with the gas in the gas burets, allowed to reach room temperature equilibrium by standing 1.5 to 2 hours before reading the volumes.

The purges were made with a similar but smaller apparatus consisting of a wet ice trap connecting to a gas buret of about 500-cc. capacity.

PROCEDURE FOR SOLUBILITY DETERMINATIONS. The solubility bomb was evacuated to high vacuum and the benzene (1200 to 1500 cc.) was charged from a graduated cylinder by suction. Methane was then charged into the bomb to the desired pressure (25, 50, 75, and 80 atmospheres, initial in the experiments described). The bomb was placed in the rotation rack and heated to the desired temperature level. After rotation for 0.5 hour, which was considered sufficient to establish equilibrium, the sampling lines were purged, about 50 cc. of gas being taken from the vapor-phase line and a sufficient amount of sample taken from the liquid-phase line to give about 1.7 cc. of condensate, which was the capacity of the liquid-phase sampling line. The bomb was again rotated for a few minutes to ascertain whether the temperature of the bomb had changed during the purging operation.

The liquid-phase and vapor-phase samples required for the solubility determinations were then taken, about 7 to 9 cc. of benzene condensate for the liquid-phase sample and about 700 to 1000 cc. of gas for the vapor-phase sample. From the data thus obtained the composition of the vapor and liquid phases could be calculated and the solubility of methane in benzene determined from the composition of the liquid phase. The solubility of the methane in the benzene separating in the traps was ignored, since according to the Bunsen absorption coefficient the amount dissolved would be less than experimental error. It was necessary to determine the composition of the sample from the vapor-phase sample cock as well as that from the liquid-phase cock in order to establish the existence of a one-phase or two-phase condition within the bomb.

In making the solubility determinations, because of the relatively large amount of benzene in the bomb, usually 1200 to 1500 cc., compared with the small amount drawn from the bomb during the sampling operation, it was not necessary to charge the bomb for each determination. The bomb was discharged, cleaned, and recharged only when a change in the initial pressure was made.

Solubility determinations were made for each pressure level (25, 50, 75, and 80 atmospheres initial) at various temperatures, and the data thus obtained reduced by means of curves to isobars and isotherms as indicated in the tables.

Solubility of Methane in Benzene

The benzene used in these experiments was Baker's C. P., thiophene-free, $n_D^{20} - 1.5012$.

The methane used was obtained from the Carbide and Carbon Chemicals Corporation, Charleston, W. Va., and had the following Goeckel analysis:

Carbon dioxide	0.0
Olefins	0.0
Oxygen	0.0
Carbon monoxide	0.0
Hydrogen	0.0
Nitrogen	4.0
Paraffins	96.0
Index	1.01

In determining the effect of pressure on the solubility of gas in liquid (methane in benzene) the ideal procedure would be to hold the temperature constant when the pressure was varied for isotherm values, or to hold the pressure constant and vary the temperature for isobar determinations.

It is evident that such a procedure cannot be followed when the rotating bomb is used, since pressure and temperature change simultaneously. This condition necessitates recourse to the type of curves in which temperature and the

TABLE I. EFFECT OF PRESSURE ON SOLUBILITY OF METHANE IN BENZENE AT DIFFERENT TEMPERATURE LEVELS

(See Figure 5, upper)

Temperature Level ° C.	Pressure Atmospheres (gage)	Solubility G. CH_4/100 g. C_6H_6	Values Derived from Figures 3 and 4
100	30	1.3	3, upper
100	70	3.4	3, lower
100	98	4.8	4, upper
100	102	5.1	4, lower
125	33	1.35	3, upper
125	72	3.4	3, lower
125	102	5.0	4, upper
125	112	6.0	4, lower
150	38	1.4	3, upper
150	79	3.65	3, lower
150	108	5.7	4, upper
150	121	7.0	4, lower
175	42	1.55	3, upper
175	86	4.0	3, lower
175	118	6.7	4, upper
175	131	8.2	4, lower
200	49	1.75	3, upper
200	95	4.6	3, lower
200	133	8.5	4, upper
200	141	9.6	4, lower
225	57	2.1	3, upper
225	103	5.55	3, lower
225	152?	13.2?	4, upper
225	152	11.9	4, lower
250	70	2.7	3, upper
250	116	7.9	3, lower

corresponding pressures are plotted against solubility. The effect of pressure change on solubility at various temperature levels or the effect of temperature change on solubility at different pressures can readily be determined from special curves (Figures 3 and 4). The values given in Tables I and II and Figure 5 were obtained in this way.

Sage, Webster, and Lacey (*3*) have plotted the bubble point pressure *vs.* mass per cent methane for 100° to 220° F. isotherms for the methane-benzene system. If we compare these results with the bomb results we see that the agreement for the two methods is good.

TABLE II. EFFECT OF TEMPERATURE ON SOLUBILITY OF METHANE IN BENZENE AT DIFFERENT PRESSURE LEVELS

(See Figure 5, lower)

Pressure Level Atmospheres (gage)	Temperature ° C.	Solubility G. CH_4/100 g. C_6H_6	Values Derived from Figures 3 and 4
90	...	...	3
90	185	4.2	4
90	...	...	5
90	65	4.0	6
100	...	...	3
100	215	5.1	4
100	120	5.0	5
100	120	4.9	6
110	...	...	3
110	240	6.5	4
110	155	5.9	5
110	122	5.9	6
120	...	...	3
120	252	9.0	4
120	177	6.9	5
120	147	6.9	6
130	...	...	3
130	...	...	4
130	195	8.1	5
130	172	8.1	6
140	...	...	3
140	...	...	4
140	208	9.6	5
140	198	9.5	6

Unfortunately, no further comparisons can be made, as 220° F. was the highest temperature investigated by Sage, Webster, and Lacey.

Solubility of Propane in Benzene

The propane used was C. P. grade obtained from the Phillips Petroleum Company.

The general procedure was the same as described for methane with the exception that salt ice traps (−20°) were substituted for the dry ice traps, this arrangement permitting the measurement in the gaseous state of the major portion of the propane in the samples drawn from the bomb. The relatively small amount of propane remaining dissolved in the condensed benzene after the major portion had collected in the gas buret was calculated from solubility data in the International Critical Tables and from data supplied by G. M. Webb (*4*).

The calculation of the solubility of propane in benzene from the results of the bomb experiments followed the same plan

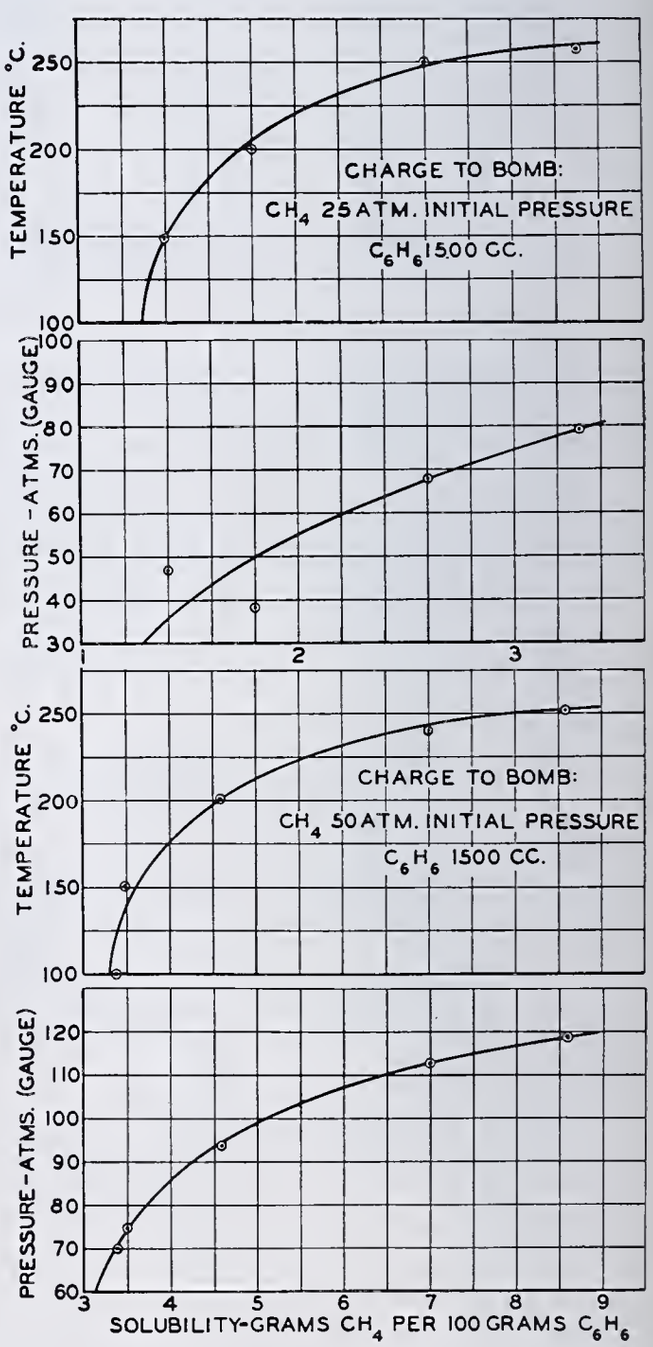

FIGURE 3. EFFECT OF PRESSURE AND TEMPERATURE ON SOLUBILITY OF METHANE IN BENZENE

Table III. Comparative Values

Bomb Method			Sage, Webster, and Lacey		
Total pressure absolute	Temp.	CH_4 in liquid phase	Bubble point pressure absolute	Temp.	CH_4
Lb./sq. in.	*° F.*	*Wt. %*	*Lb./sq. in.*	*° F.*	*Wt. %*
1065	216	3.3	1065	220	3.2
1455	217	4.6	1455	220	4.5
1605	216	5.2	1605	220	5.0

as outlined for methane in benzene. In the case, however, of propane only two concentrations of propane in benzene (7.7 and 19.9 weight per cent) were used (Figure 6). This gave only two points for plotting the solubility curves. In Figure 7 the corresponding two points on the solubility curves are joined by straight broken lines. While the degree of curvature is not shown, the general direction of the curves is indicated.

As no solubilities of propane in benzene under high pressures and high temperatures could be found in the literature, it was impossible to check the results herein described with those obtained by other methods.

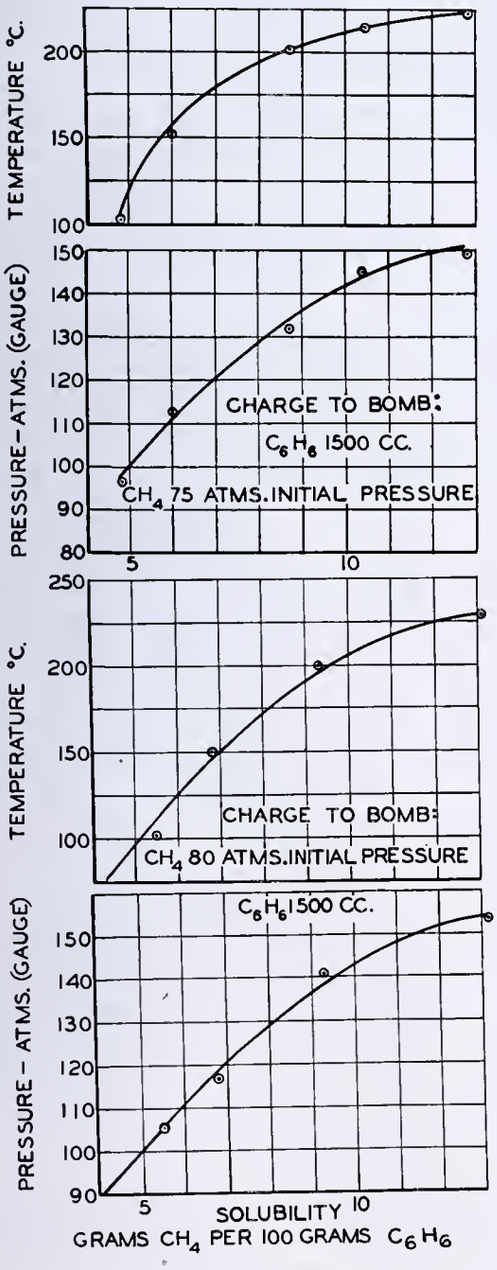

Figure 4. Effect of Pressure and Temperature on Solubility of Methane in Benzene

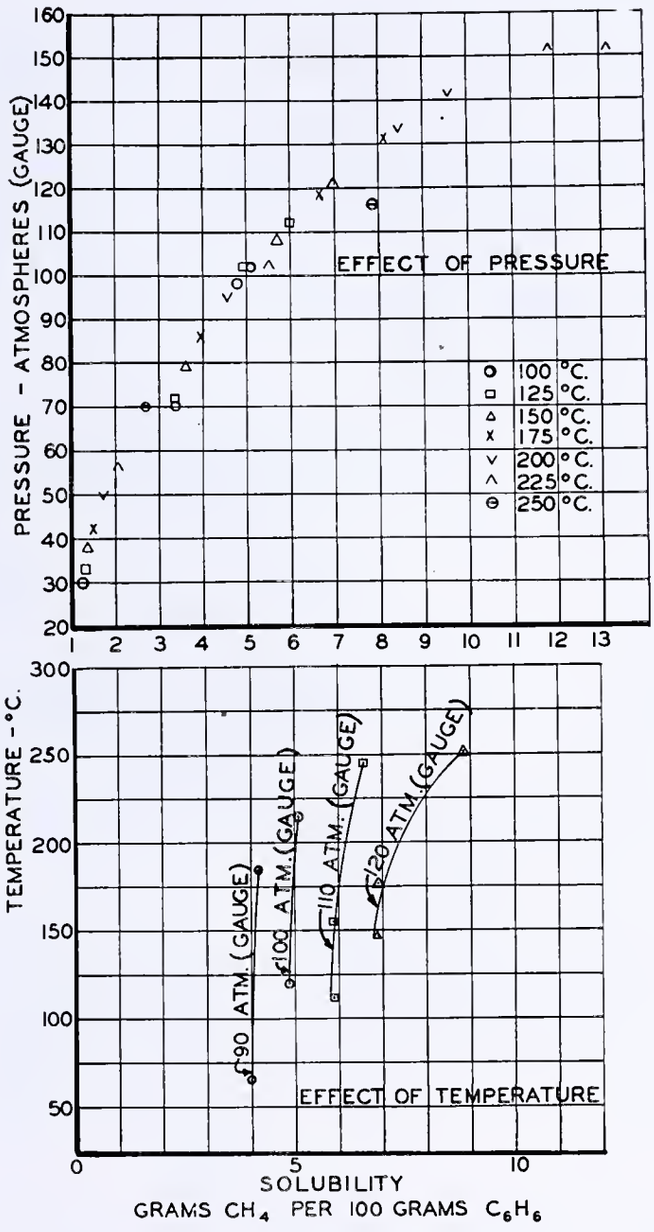

Figure 5. Effect of Pressure and Temperature on Solubility of Methane in Benzene

Solubility of Sulfur Dioxide in Benzene

The procedure for determining the solubility of sulfur dioxide in benzene at 26° C. under pressures from 1.2 to 2.7 atmospheres (absolute) was essentially the same as previously described, the determinations being made with the bomb at

Table IV. Effect of Pressure on Solubility of Propane in Benzene at Different Temperature Levels

Temperature Level	Pressure	Solubility	Values Derived from Figure 7
° C.	*Atmospheres (gage)*	*G. C_3H_8/100 g. C_6H_6*	
150	10.0	7.41	Upper
150	20.0	22.25	Lower
160	12.0	7.54	Upper
160	23.5	22.36	Lower
170	14.5	7.66	Upper
170	27.5	22.50	Lower
180	17.0	7.77	Upper
180	32.0	22.70	Lower
190	20.0	7.87	Upper
190	37.5	22.95	Lower
200	23.0	7.97	Upper
200	42.5	23.24	Lower
210	27.0	8.07	Upper
210	48.0	23.56	Lower

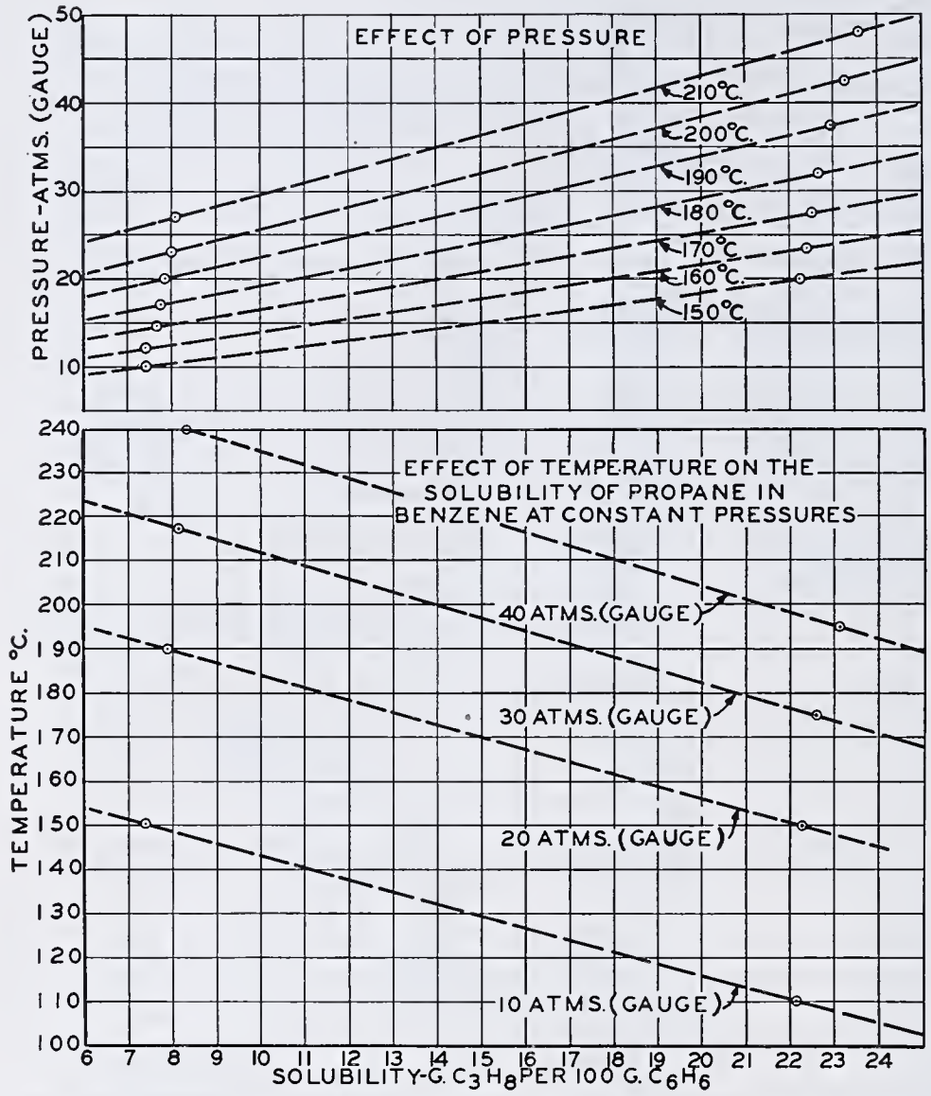

FIGURE 6. EFFECT OF PRESSURE AND TEMPERATURE ON SOLUBILITY OF PROPANE IN BENZENE

TABLE V. EFFECT OF TEMPERATURE ON SOLUBILITY OF PROPANE IN BENZENE AT DIFFERENT PRESSURE LEVELS

Pressure Level *Atmospheres (gage)*	Temperature ° C.	Solubility *G. C_3H_8/100 g. C_6H_6*	Values Derived from Figure 7
10	150	7.41	Upper
10	110	22.12	Lower
20	190	7.88	Upper
20	150	22.26	Lower
30	217	8.13	Upper
30	175	22.60	Lower
40	242	8.32	Upper
40	195	23.08	Lower

room temperature and the sulfur dioxide being added to the bomb until the desired pressure was reached after the benzene had been charged.

The liquid-phase samples were collected in a dry ice–acetone trap provided with a ground-glass joint for attachment to a modified Podbielniak fractionating column. The sulfur dioxide was removed from the sample by fractionation, collecting the evolved sulfur dioxide in a gas buret over mercury. When the vapor had reached 80° C., the distillation was stopped and the apparatus after reaching room temperature was purged with a stream of air to remove any sulfur dioxide remaining in the apparatus. The purge was added to gas previously obtained during the distillation and the amount of sulfur dioxide determined by absorption in potassium hydroxide solution. The residue remaining in the trap after distillation was weighed as benzene.

The results obtained with benzene and sulfur dioxide are given in Table VI.

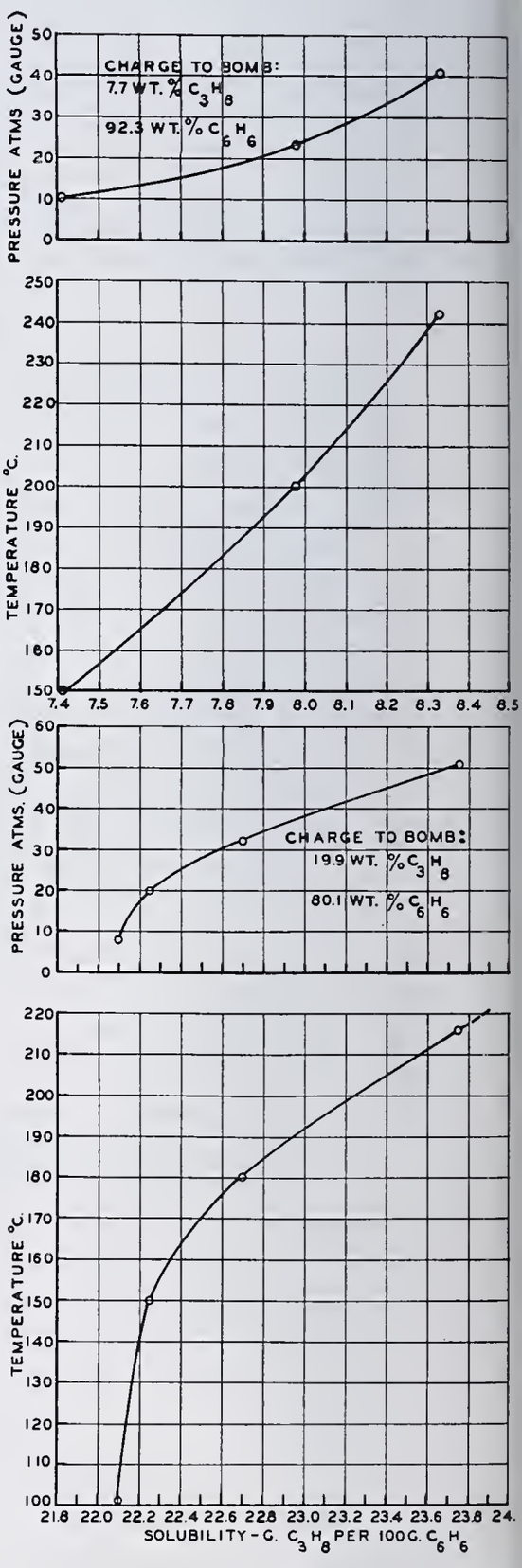

FIGURE 7. EFFECT OF PRESSURE AND TEMPERATURE ON SOLUBILITY OF PROPANE IN BENZENE

Table VI. Solubility of Sulfur Dioxide in Benzene

Temperature of Bomb ° C.	Total Pressure *Atmospheres (abs.)*	Soluhility *G. SO_2 dioxide/100 g. C_6H_6*
26	1.20	24.7
26	1.75	37.6
27	2.40	84.8
25	2.65	125.5

Conclusions

The results obtained show that the modified rotating bomb can be used to determine the solubility of gases at high pressures and high temperatures approaching the critical.

The method applied to the determination of the solubility of methane in benzene indicates that pressure is the principal factor affecting the solubility. The effect of temperature is minor, becoming more noticeable at higher pressures.

The solubility determinations of propane in benzene indicate that both temperature and pressure have a marked influence on the solubility.

Acknowledgment

The authors wish to express their appreciation to Charles Rohlfing and Lee Fischer for assistance rendered on these experiments.

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Presented before the Division of Petroleum Chemistry at the 102nd Meeting of the American Chemical Society Atlantic City, N. J.

Determination of Critical Temperatures by the Rotating Bomb

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THE methods usually employed in determining critical temperatures involve the determination of the pressure-volume relation along various isotherms or the appearance or disappearance of the meniscus at the phase boundary at the critical temperature. These procedures, though capable of giving a high degree of accuracy, require special apparatus and skillful technique. In cases where an accuracy of only ±2° C. is required, a method involving less elaborate apparatus would be desirable. It was with this in mind that the authors developed a method for determining critical temperatures by the rotating bomb.

A study was made of the pressure-temperature curves of various pure substances to ascertain if any change occurred in the pressure-temperature relation in the region of the critical temperature that could be employed in determining critical temperatures. The investigation was also extended to two-component systems.

The pressure-temperature curves were determined in the presence and absence of hydrogen for propane, cyclohexane, hexane "practical", and benzene.

The pressure-temperature relationship was also studied for the following two-component systems over the temperature ranges indicated:

Methane, 27 mole %
Benzene, 73 mole %
Temperature range, 62° to 261° C.

Benzene, 70 mole %
Propane, 30 mole %
Temperature range, 94° to 256° C.

Hexane "practical", 32 weight %
Benzene, 68 weight %
Temperature range, 150° to 288° C.

Table I. Comparison of Points of Discontinuity on Pressure-Temperature Curves of Pure Substances

(Determined by the rotating bomb with critical temperatures obtained from literature)

Substance	Propane	Propane + H_2	Hexane "Practical"	Hexane "Practical" + H_2	Cyclohexane	Cyclohexane + H_2	Benzene			Benzene + H_2		
Amount charged, cc.	2000	2200	1400	1400	1800	1800	700	1300	1500	700	1300	1500
Temperature of break, ° C.	96–98	96–98	226–229	234–239	276–281	277–280	283–290[a]	286–289	285–288.5	288–290.5	288.5–289.5	286–288
Critical temp. (literature), ° C.	96.9	96.9	234.8[b]	234.8[b]	281	281	288.6	288.6	288.6	288.6	288.6	288.6
Test samples:												
Above break temp., ° C.	...	101	...	235	...	283	...	...	...	...	292	294
Liquid-phase sampling cock, cc. liquid/100 cc. gas	...	7.7	...	5.4	...	8.6	...	...	...	...	5.2	6.2
Vapor - phase sampling cock, cc. liquid/100 cc. gas	...	7.8	...	5.5	...	8.7	...	...	...	...	5.1	5.9
Below break temp., ° C.	...	SS	...	228	...	270	...	...	...	...	288	282
Liquid - phase sampling cock, cc. liquid/100 cc. gas	...	10.5	...	S.9	...	9.1[c]	...	...	...	...	5.1	8.7
Vapor - phase sampling cock, cc. liquid/100 cc. gas	...	2.2	...	0.5	...	9.2[c]	...	...	...	...	1.4	0.4
Curve	1-A	1-B	2-A	2-B	3-A	3-B	4-A	4-B	4-C	5-A	5-B	5-C

[a] Determination of additional points on pressure-temperature curve between 283° and 290° would reduce range of discontinuity.
[b] *n*-Hexane.
[c] Apparently with amount of cyclohexane charged (1800 cc.), liquid level was still above vapor-phase sample line.

Apparatus and Procedure

The Ipatieff rotating bomb used for the determination of the solubility of methane in benzene was also used in this work, modified as follows:

The vapor-phase sampling line was bent outward to within 0.31 cm. (0.125 inch) of the bomb wall. This gave the bomb a capacity of 3145 cc. up to the vapor-sampling line or 92 per cent of the total capacity of the bomb (3420 cc.). This change made possible the study of two-phase systems over a greater range of temperature than by the previous arrangement by which solubilities were determined (*1*).

The gage was mounted at approximately 120° to the axis of rotation of the bomb. This made it possible to stop the rotation of the bomb momentarily and read the gage in a vertical position, thus giving greater accuracy in the pressure readings.

In studying the pressure-temperature relationship with pure substances the procedure was essentially as follows:

The bomb was evacuated and the charge drawn into the bomb by the vacuum from a graduated cylinder until the desired amount of liquid was charged. In case of propane the bomb was evacuated and the propane charged from a graduated pressure charger.

When hydrogen was used, the bomb was charged with the substance to be investigated according to the procedure outlined above and enough hydrogen added to increase the gage pressure about 10 atmospheres. With hydrogen present, it was possible to determine when a two-phase or one-phase condition existed in the bomb by comparing the ratio of the liquid to gas from the samples taken from the vapor-phase and liquid-phase sample lines.

In studying the two-component systems the procedure for charging was as follows:

In case of benzene-methane, the benzene and methane were charged as described above when hydrogen was used with pure substances.

When the propane-benzene system was studied, the benzene was charged to the bomb after evacuation, and propane was charged from a pressure charger in the desired amount.

The hexane-benzene was mixed in the desired proportions outside the bomb, and charged to the bomb after evacuation.

The heat input to the heating furnace was controlled to raise the temperature approximately 1° C. per minute.

The procedure for drawing samples when the bomb charge consisted of two-component systems has been previously outlined (*1*).

In studying the compositions of samples from the hexane-benzene system, the weight per cents of the two constituents were determined by refractive index curves, the compositions of sam-

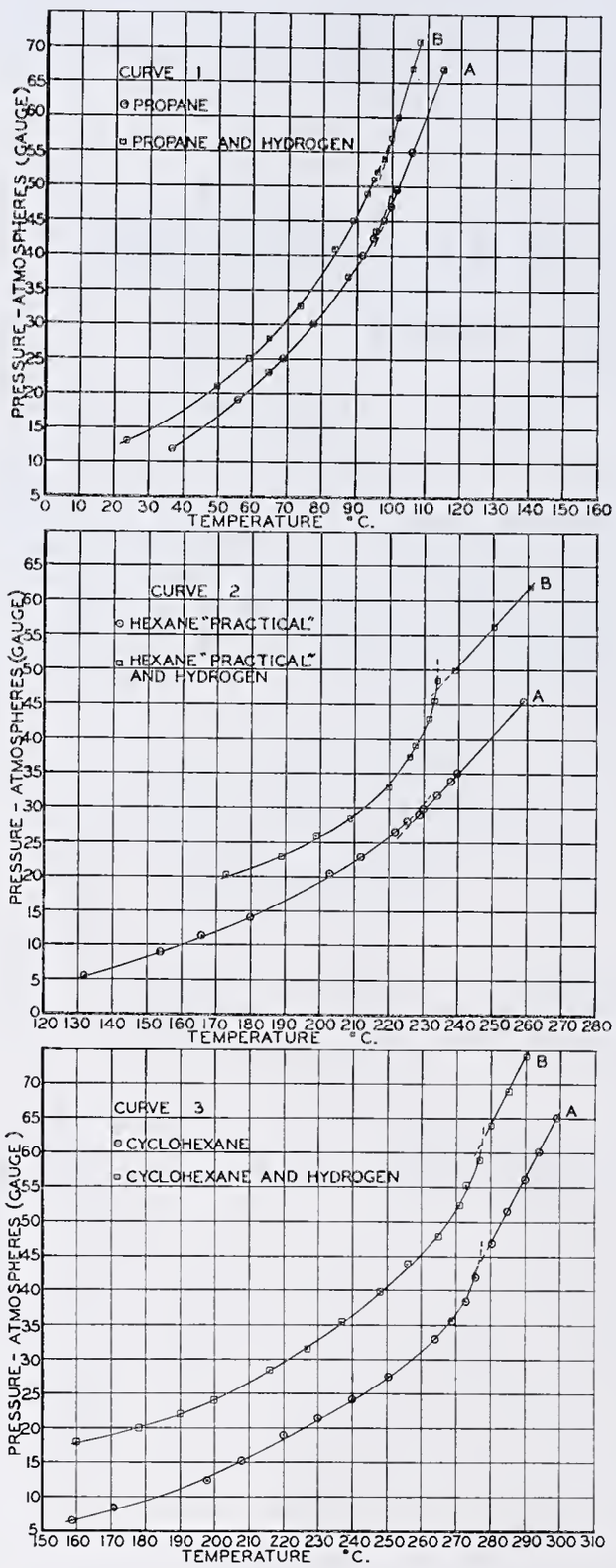

FIGURE 1

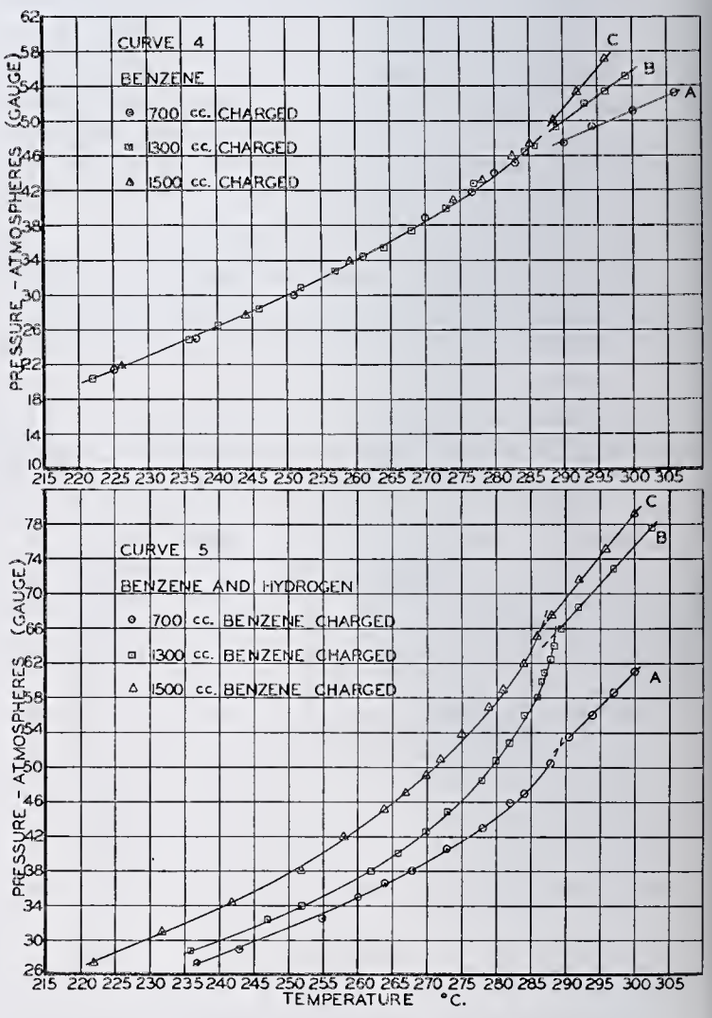

FIGURE 2

ples from the other systems investigated being determined by the ratio of liquid to gas.

Pressure-Temperature Relation of Pure Substances. Table I and Figures 1 and 2 summarize the results obtained with pure substances alone and in the presence of a small amount of hydrogen (10 atmospheres, initial). In all experiments the pressure-temperature curves became discontinuous in the region of the critical temperature, the part below the critical temperature being curved and the section above the critical temperature being practically straight. Variations over a relatively wide range in the amount of material charged did not noticeably affect the temperature of break on the curve.

In case of propane (curves 1-A and 1-B), the break in both curves occurred over a range of 96° to 98° C., the hydrogen having no noticeable effect on the temperature of the break in the curve. Bomb samples drawn from the vapor-phase and liquid-phase sampling lines when hydrogen was used showed that at a temperature of 100–101° C. the bomb charge was a single phase, the hydrogen content of the two samples being the same. When samples were taken at 88° C., the hydrogen content indicated the existence of two phases in the bomb.

Curves 2-A and 2-B, using hexane "practical" from petroleum, showed the characteristic breaks in the pressure-temperature curves at 226–229° and 234–239° C., respectively. The hydrogen content of samples drawn (curve 2-B) indicated the presence of a single-phase region at 235° C. and a two-phase region below 228° C. The critical temperature for *n*-hexane is 234.8° C. Closer agreement with the critical temperature of *n*-hexane cannot be expected, as the sample used probably had hexane isomers.

The experiments with cyclohexane (curve 3-A) and with hydrogen (curve 3-B) gave points of discontinuity on the pressure-temperature curves at 276–281° C. The critical temperature for cyclohexane according to the literature is 281° C. The presence of hydrogen did not noticeably displace the break on the curves. In this case samples drawn from the bomb when runs were made with hydrogen did not indicate a two-phase region below the critical temperature. This may be due to the possibility that the level of the liquid phase was above the vapor-phase draw-off line and samples of liquid phase were obtained from both sampling cocks. This suggests the difficulty of obtaining satisfactory samples when working in the region of the critical if the liquid level of the charge in the bomb is high. However, the presence of hydrogen was beneficial in that the temperature of the break on the curve was more clearly defined.

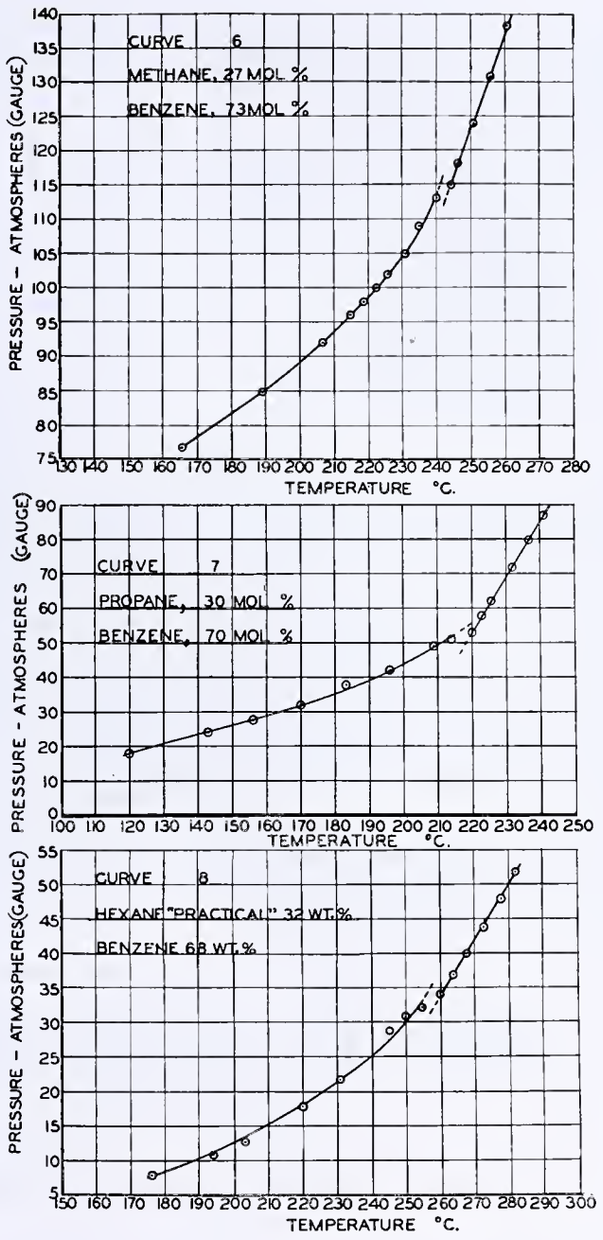

Figure 3

Table II. Summary of Determination of Critical Temperatures of Two-Component Systems by the Rotating Bomb

System			
Benzene, mole %	73	70	68 wt. %
Methane, mole %	27	..	..
Propane, mole %	..	30	..
Hexane "practical", weight %	..	..	32
Amount charged, liquid, cc.			
Benzene	1600	1415	..
Benzene + hexane "practical"	..	..	1500
Methane, atmospheres (initial)	55	..	..
Propane	..	600	..
Temperature of break, ° C.	240–244	214–220	255–260
Test samples:			
Above break temperature at, ° C.	246	222	266
Liquid-phase sample cock, cc. liquid/100 cc. gas	1.0	0.9	68.5 wt. % C_6H_6 31.5 wt. % C_6H_{14}
Vapor-phase sample cock, cc. liquid/100 cc. gas	1.0	0.9	68.5 wt. % C_6H_6 31.5 wt. % C_6H_{14}
Below break temperature at, ° C.	238	216	256
Liquid-phase sample cock, cc. liquid/100 cc. gas	1.1	1.0	69.5 wt. % C_6H_6 30.5 wt. % C_6H_{14}
Vapor-phase sample cock, cc. liquid/100 cc. gas	0.1	0.4	66.0 wt. % C_6H_6 34.0 wt. % C_6H_{14}
Curve	6	7	8

The results of determinations of the critical temperature of benzene in the absence of hydrogen (curves 4-A, B, and C) and in the presence of hydrogen (curves 5-A, B, and C) were on the whole satisfactory. The hydrogen did not noticeably displace the break on the pressure-temperature curve. The critical as determined by the break on the pressure-temperature curve (in the presence and absence of hydrogen) ranged from 286° to 291° C. (critical temperature of benzene according to the literature is 288.6° C.). In the case of benzene, samples drawn from the bomb, when runs were made with hydrogen, indicated a two-phase region below the break on the temperature-pressure curve and a one-phase condition above the break.

In the case of benzene the presence of hydrogen again gave a sharper break on the pressure-temperature curve at the critical than did runs made in the absence of hydrogen.

In the runs with benzene, variations in the amount charged (700 to 1500 cc.) produced no noticeable effect in the temperature of break on the curve.

Pressure-Temperature Relation of Two-Component Systems. The pressure-temperature relationship of the following two-component systems was studied over the temperature ranges indicated:

Benzene, 1600 cc.	(73 mole %)
Methane, 55 atmosphere initial (gage)	(27 mole %)
Temperature range, 62° to 261° C.	
Benzene, 1415 cc.	(70 mole %)
Propane, 610 cc. (liquid)	(30 mole %)
Temperature range, 100° to 250° C.	
Benzene, 1440 cc.	(70 mole %)
Propane, 605 cc. (liquid)	(30 mole %)
Temperature range, 94° to 256° C.	
Hexane "practical", 32 weight %	
Benzene, 68 weight %	
Temperature range, 150° to 288° C.	

The results obtained are given in Table II and Figure 3. As in the case of pure substances, the pressure-temperature curves exhibited well-defined regions or points of discontinuity, the section of the curve below the break being curved and the section above the break being practically straight. Samples drawn from the liquid-phase and vapor-phase sampling cocks at temperatures above the break had approximately the same composition, indicating a one-phase condition, while samples drawn from the liquid-phase and vapor-phase cocks below the temperature of break on the curve had different compositions, indicating a two-phase region.

The points of discontinuity evidently correspond to the critical temperatures of the two-component systems studied, since analyses of samples taken above and below the temperature of discontinuity showed the existence of one-phase and two-phase regions, respectively.

Conclusions

The results obtained with pure substances in either the presence or absence of hydrogen indicate that the critical temperatures may be determined with a fair degree of accuracy and by determining a sufficient number of points in the range of discontinuity the accuracy should be ±2° C.

The amount of material charged to the bomb may vary from 20 to 50 per cent of the total capacity of the bomb without any appreciable shift in the temperature of discontinuity.

It is probable that a bomb of smaller size (200 to 300-cc. capacity) might serve equally well if no samples are removed. The smaller bomb could be easily rotated in a liquid heating bath and the temperature determined with greater accuracy than in case of the larger bomb used in these experiments.

The pressure-temperature curves of two-component systems exhibited points of discontinuity similar to those shown by individual substances, and the critical temperatures of the systems probably lie within the break on the curves. Up to the present time, however, check determinations have not been made on two-component systems of known critical temperatures.

Acknowledgment

The authors are indebted to Lee Fischer and Charles Rohlfing for valuable assistance rendered during the course of the experiments.

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Presented before the Division of Petroleum Chemistry at the 102nd Meeting of the American Chemical Society, Atlantic City, N. J.

Glass Electrode Measurements of the pH of Chromic Acid Solutions

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The effect of temperature, purity of acid, and water on the pH of aqueous chromic acid solutions has been measured and shown to be negligible, except for a slight variation with temperature in dilute solutions.

A considerable variation in the readings obtained at pH below 2 with different types of measuring instruments exists. It increases as the pH of the solution decreases, and may amount to 0.2 pH at pH 0. A large portion of this discrepancy results from various liquid junction potentials, while a residual portion cannot at present be explained.

Calculations show that the correct pH value is probably not far from that obtained with certain of the instruments used. All instruments are self-consistent and may be used for control purposes, if standardized against known solutions.

THE pH of chromic acid solutions has been measured by means of the glass electrode by Thompson (*17*), Buzzard and Wilson (*2*), and Neuss and Rieman (*15*), and by means of the hydrogen electrode in fairly dilute solution by Britton (*1*). (The term "chromic acid" is used in this paper to apply to both solid chromium trioxide and its aqueous solutions.) It is only under unusual circumstances that the latter method is satisfactory, and with the development of modern equipment for glass electrode measurements, the glass electrode is practically universally used for such determinations, and has become of industrial importance in the control of baths for the anodic oxidation of aluminum (*2*). It is the purpose of this paper to show the effect of varying apparatus and conditions on the pH readings obtained with chromic acid solutions, and to present certain calculations which corroborate data obtained with one type of apparatus.

Apparatus

The equipment used for these measurements consisted of six commercial-type instruments of three different makes with various types of electrode assemblies, as shown in Table I. All instruments were provided with a temperature compensator and used a saturated calomel electrode as reference half-cell, except Nos. 2A and 5A, in which a silver–silver chloride reference electrode was substituted.

No difference was discernible between setups 1 and 2 and these were used unless otherwise specified.

Materials

The material used in most of the work was technical flake chromic acid, analyzing 99.8 per cent chromium trioxide, 0.03 per cent sulfate, and 0.017 per cent chromic oxide. Chromium trioxide was determined by electrometric titration according to the method of Kelley (*9*), sulfate gravimetrically as barium sulfate after reduction of the hexavalent chromium by alcohol and hydrochloric acid, and chromic oxide by cerimetry according to

Table I. Types of Electrode Assemblies Used

No.	Instrument	Glass Electrode	Liquid Junction
1	A-1	Quinhydrone-HCl[a]	Small ground-glass, with pinhole
2	A-1	Sealed[a]	Small ground-glass, with pinhole
2A	A-1	Sealed	Small ground-glass, with pinhole
3	A-1	Sealed[a]	Fiber diaphragm
4	A-1	Sealed	Capillary junction
5	A-1	Sealed	Broad junction
5A	A-1	Sealed	Broad junction
6	A-2	Sealed	Small ground-glass, as above
7	A-2	Sealed	Fiber diaphragm
8	A-2	Sealed	Broad junction
9	A-2	Sealed	Flowing junction
10	B-1	Sealed	Broad junction
11	B-2	Sealed	Broad junction
12	B-3	Sealed	Fiber junction
13	B-3	Sealed	Large ground-glass
14	C	Quinhydrone-HCl, 30 meg.	Capillary junction
15	C	Quinhydrone-HCl, 30 meg.	Large ground-glass
16	C	Quinhydrone-HCl, 3 meg.	Capillary junction
17	C	Quinhydrone-HCl, 3 meg.	Large ground-glass

[a] Several glass electrodes used.

a modification of the method of Willard and Young (*20*). For some experiments a special grade of C. P. chromium trioxide, prepared by washing specially selected C. P. material with distilled water, was used. It tested 99.9 per cent chromium trioxide, 0.0005 per cent sulfate, and 0.008 per cent chromic oxide. As reference standard for the pH measurements, Bureau of Standards potassium acid phthalate, 0.0500 *M*, was used. This solution had a pH of 4.00 at 25° C., and varies therefrom by about ±0.008 pH at 19° or 31° C., according to the data supplied with the sample (*14*). Secondary pH standards were sodium acetate–acetic acid buffers, 0.1 *N* hydrochloric acid solutions, and hydrochloric acid–potassium chloride buffers. The meter was checked at least once each day. Determinations were frequently performed on separate days on identical samples, and results were reproducible within 0.02 pH.

Effect of Varied Conditions

Temperature. Solutions were tested at 19°, 25°, and 31° C. (Table II). It will be noted that the effect of raising temperature is to cause a slight decrease in pH, this effect is, however, nearly negligible except at low concentrations of chromium trioxide.

Water. Measurements of solutions of equal concentration in Baltimore city water and in laboratory distilled water showed no discernible difference. Slight corrections should be made in the case of waters of high alkali or calcium bicarbonate content.

Purity of Chromium Trioxide. The technical grade and C. P. material described above were tested with the following results:

Mole of CrO_3/Liter	pH, Technical Grade, 27° C.	pH, C. P. Grade, 27° C.
0.9695	0.10	0.10
0.1939	0.80	0.80
0.0485	1.39	1.39

The small amount of impurities present in technical chromium trioxide has no effect on the pH of solutions.

Effect of Variation in Measuring Equipment

The effect of various pH cells and instruments for effecting the measurements was next studied, with the results shown in Table III.

The wide variations observed obviously require explanation. Several sources of inaccuracy are inherent in commercial glass-electrode instruments, and these will be discussed.

Errors Due to High Acidity and Concentration of Solutions. MacInnes and Belcher (*13*) and Dole (*5*) have observed erroneous results from glass electrodes in highly acid solutions, especially at pH below 0, and in solutions of high electrolyte content. The generally accepted explanation is that the effect is due to the fact that the hydrated proton, $(H_3O)^+$, is transferred through the glass membrane, and that the low activity of the water in these solutions is responsible for the discrepancy. While no definite figures are available for the activity of the water in the chromic acid solutions under consideration, the concentration and pH are such that only slight indication of the effect would be expected. Approximate calculations indicate that the correction at 1.0 *M* chromium trioxide should be of the order of 0.012 pH, which is less than the experimental error. Hubbard, Hamilton, and Finn (*8*) have indicated that the "acid error" may vary with the composition of the electrode; however, commercial instruments use Corning 015 glass for the electrode and any errors existing in the measurement would be common to all cells. This has been confirmed by the fact that measurements with assemblies identical except for the glass electrodes (Table III, 1, 2, 3; 14, 16; 15, 17) gave results identical within experimental error. The electrodes used gave normal readings in 0.1 *M* hydrochloric acid, and did not show the error reported by Hubbard, Hamilton, and Finn (*8*).

The same considerations which render pH measurements on chromic acid solutions feasible up to 1 *M* make it necessary to state that any values which may be obtained in more concentrated solutions—e. g., plating baths—are of value for comparison only.

Errors Due to Action of Chromic Acid on Glass Electrode. Watson (*19*) and Kerridge (*10*) have observed that glass electrodes cleaned in a mixture of chromic and sulfuric acids are not suitable for immediate use in the measurement of pH, and Dole (*4*) has observed increase in weight of glass cleaned in this manner, caused by adsorption of chromic acid. Laboratory cleaning solutions normally contain large quantities of sulfuric acid and might be expected to be dehydrating in action, with resultant change in the properties of the glass. No such action is to be expected from the relatively dilute chromic acid solutions under consideration, and this is borne out by the fact that electrodes with which several samples of chromic acid have been measured give accurate readings immediately when checked against a buffer. Tubbs (*18*) has reported high readings in the field

Table II. pH Readings of Chromic Acid Solutions with Equipment 1 and 2

CrO_3 Mole/l.	pH 19° C.	pH 25° C.	pH 31° C.
1.00	0.11	0.10	0.08
0.80	0.21	..	..
0.60	0.31	..	..
0.50	..	0.40	0.39
0.40	0.51	..	..
0.20	0.77	0.79	0.78
0.10	..	1.09	1.07
0.05	1.40	1.39	1.37
0.04	1.51	..	..
0.03	1.61	..	..
0.02	1.82	1.80	1.77
0.01	..	2.08	2.06

Table III. Effect of Variations in Measuring Equipment

Apparatus No. (from Table I)	Mole of CrO_3 per Liter: 1.00	0.50	0.20	0.10
	pH at 25° C.			
1	0.10	0.40	0.79	1.09
2	0.10	0.40	0.79	1.09
2A	0.12	..	..	..
3	0.11	0.41	0.79	1.09
4	0.13	..	..	..
5	0.20	..	..	..
5A	0.18	..	..	..
6	0.14	..	..	..
7	0.14	..	..	..
8	0.20	..	..	..
9	0.20	..	..	..
10	0.27	..	..	..
11	0.30	..	..	..
12	0.21	..	..	..
13	0.25	0.53	0.89	1.18
14	0.20	0.47	0.84	1.13
15	0.26	..	..	..
16	0.24	..	..	..
17	0.25	..	..	..

with an electrode kept immersed in chromic acid instead of distilled water between readings, but such high readings may be due to diffusion in the liquid junction rather than any action on the glass.

Slide-Wire Inaccuracies. Dole (*3*) has reported work by Blair indicating that errors exist in the slide-wire of a commercial pH electrometer, although another electrometer studied by Lanford and Kiehl (*11*) showed little difference when checked against the Type K potentiometer. Repeated checks with standard solutions of hydrochloric acid at a pH of 1.08, where a variation in the observed pH of chromic acid of 0.04 to 0.09 pH (see Table III, column 5) was noted with different electrode assemblies, indicate that a definite effect exists under conditions where both acid errors and slide-wire inaccuracies are completely balanced out. While it is possible that the variation of 0.2 pH noted at pH 0.10 might be due to slide-wire inaccuracies, it seems unlikely that slide-wire errors would amount to as much as 10 per cent over the range pH 0 to 1, or that the error would be nearly identical for all instruments of the same make.

Liquid Junction. Examination of the data in Table III indicates that the type of liquid junction is an important factor in determining the value obtained for the pH of chromic acid solutions. Algebraic analysis of the results indicates that approximately half the discrepancy is due to variations in the method of forming the liquid junction, while half is due to some factor at present unknown. The data show further that those liquid junctions possessing the broadest surface of contact give the highest readings. The increase in pH appears to be connected with diffusion in the broad junction, since calomel electrodes of the sleeve type in which diffusion has accidentally taken place invariably give readings of higher pH.

The excellent check results obtained with all instruments on other solutions of low pH indicate that the variations observed are peculiar to the liquid junction between chromic acid and saturated potassium chloride. Extensive work on junction potentials (*6*, *7*, *16*) has shown that the ground-glass sleeve or fiber-type junction is normally the least reproducible of liquid junctions; the author has found in the data given above that all junctions studied were reproducible within experimental error, but that diffusion effects appear to introduce a drift toward high pH in the broad types of junction. In this particular case, it would seem that the broad types of junction are more subject to error. This is confirmed in some degree by the calculations given below.

Theoretical

Neuss and Rieman (*15*), in connection with their measurements of the ionization of chromic acid, determined values for the ionization constants in a cell designed to eliminate liquid junction errors, and calculated the variation of the constants with the ionic strength, according to the Debye-Hückel theory. From their data, at 25° C.:

$$K_1' = \frac{[H^+][HCrO_4^-]}{[H_2CrO_4]} = 0.18 \text{ at } \mu = 0.16$$

where

$$pK_1' = 1.05 - \frac{\sqrt{\mu}}{1 + 0.7\sqrt{\mu}}$$

and

$$\frac{(HCrO_4^-)^2}{(Cr_2O_7)^{--}} = 0.023, \quad \frac{[HCrO_4^-]^2}{[Cr_2O_7]^{--}} = K'$$

where

$$pK' = 1.64 + \frac{\sqrt{\mu}}{1 + 0.7\sqrt{\mu}}$$

(Concentrations are here denoted by brackets, activities by parentheses.)

Finally, $pH = -\log (H^+) = -(\log[H^+] + \log \gamma)$

where $\log \gamma$ is derived from values given by Scatchard (*16*) or MacInnes (*12*). If the chromic acid molality $= M$, and $M - [H^+] = y$, since $[H^+] = [HCrO_4^-] + 2[Cr_2O_7]^{--}$, on elimination of $[HCrO_4^-]$ and $[Cr_2O_7]^{--}$:

$$y = \frac{K'[H^+]}{4K_1'}\left(\sqrt{1 + \frac{8[H^+]}{K'}} - 1\right)$$

whence:

$[H^+]$	μ	K_1'	K'	γH^+	y	M	(H^+)	pH
0.01	0.0118	0.116	0.0178	0.921	0.000514	0.01051	0.00921	2.04
0.02	0.0248	0.123	0.0166	0.888	0.00153	0.02153	0.01776	1.75
0.04	0.0518	0.140	0.0145	0.870	0.00395	0.04395	0.0348	1.46
0.08	0.107	0.164	0.0123	0.848	0.00942	0.08942	0.0678	1.17
0.10	0.136	0.175	0.0117	0.842	0.0122	0.1122	0.0842	1.07
0.20	0.279	0.216	0.0093	0.838	0.0261	0.2261	0.1676	0.78
0.40	0.579	0.277	0.0074	0.863	0.0528	0.4528	0.3452	0.49

Plotting M against pH gives a curve which is in best agreement (± 0.04 pH) with the data determined with instrument A with small junctions up to 0.2 M, which represents the highest concentration at which the equations are valid. However, any of the assemblies employed in this work give self-consistent results, and no false e. m. f.'s are developed by the action of the chromic acid up to 1 M on the glass electrode in any case, as shown by the reproducibility of readings with standard buffer at all times. Because of the acid error, the possible action of chromic acid on the glass electrode, the increase in liquid junction errors, the lack of any theoretical check on the measurements, and the fact that many commercial meters do not read below pH 0 or -0.5, it did not seem feasible to obtain pH values for higher concentrations of chromic acid.

Acknowledgments

The author wishes to express his thanks to the following who have made pH measurements on chromic acid solutions: A. O. Beckman, National Technical Laboratories, Pasadena, Calif.; F. R. McCrumb, W. A. Taylor & Co., Baltimore, Md.; and Mutual Chemical Co. of America, Jersey City, N. J. He is much indebted to William Rieman III of Rutgers University for his assistance in the calculations of pH.

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Molecular Still Heads

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MANY devices for molecular distillation have been developed and made available to chemists in general through publication. Morton (*3*) reviewed a number of the types most useful in the laboratory, Detwiler and Markley (*2*) cited over a hundred publications, and others are readily found in the literature.

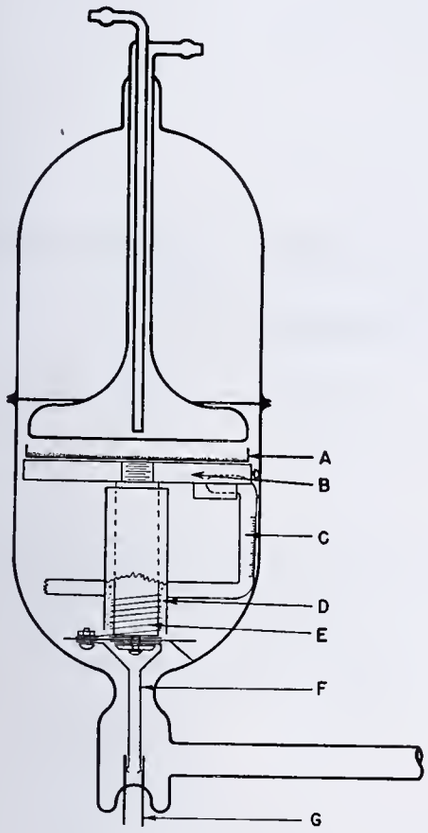

FIGURE 1. MOLECULAR STILL

A, lead sample pan; *B*, brass hot plate; *C*, thermometer; *D*, glass wool insulation; *E*, No. 30 Nichrome wire (20 ohms) insulated above and below by glass wool and wrapped on a brass bar; *F*, spring bronze contacts insulated by mica washers; *G*, tungsten wires sealed in

Experience in this laboratory has shown that the use of standard glass parts contributes greatly to the economy of construction, rigidity, and mechanical strength of the still, and interchangeability and general serviceability of the device. Several designs are of sufficient value to justify more general use.

Figure 1 shows one type of molecular still made from two standard Pyrex microbell jars. The parts are inexpensive, and the simple and rugged construction contributes to rapid and safe manipulation. The design shown includes improvements indicated by many months of continuous operation, and is free from the usual vagaries such as temperamental heater, thermal control, and measuring devices, and erratic and undependable vacuum seals.

A feature of early designs which caused endless trouble was the material which insulated the heating element. Materials such as mica and glazed or unglazed porcelain were unsatisfactory because of the large quantities of gas given off when heated. Glass wool taken from a bat in the general laboratory supply and wrapped around the heater proved admirably effective as insulation. The various glass tapes, cloths, and sleeves supplied by the Owens-Corning Fiberglas Corporation, Toledo, Ohio, permit easy application. An input to the heater of about 20 watts sufficed for temperatures up to 300° C. The most satisfactory wax for completing the vacuum seal between the two bell jars was picein; it was equally effective whether built up as a dam on the outside of the joint or allowed to form a layer between the opposite joint faces, although it was usually easier to make a successful seal at the first attempt by the latter method. The condenser effectively cooled the wax seal so that the vapor pressure of the wax did not interfere. The details of construction are apparent in Figure 1.

While several types of vacuum systems were employed, best results were obtained by using a simple, short-coupled vacuum line. The still was coupled to a freezing trap which was attached to the mercury pump. A McLeod gage was also connected directly to the mercury pump. Between the mercury pump and the mechanical forepump a three-way stopcock was inserted. The third outlet led to the high-vacuum side of the mercury pump, thus making it possible to by-pass the mercury pump completely to prevent contaminating the mercury. On the low-vacuum side of the mercury pump, a liter flask was connected to serve as a reservoir. It was then possible to reduce the running time of the mechanical pump to two or three brief periods in 24 hours. Using the molecular still and vacuum system as described, a two-stage Kurth mercury pump and Cenco Hyvac fore-pump reduced the reading of the McLeod gage to 10^{-6} mm. of mercury in about an hour if the heater was not energized, and to the same level in about 2 hours with the heater and sample at 200° C. The combination of McLeod gage and freezing trap records the pressure of permanent gases only and these not in the distillation gap. The total pressure of temporarily uncondensable molecules in the gap during distillation was probably higher than 10^{-6}. The effectiveness of this still was demonstrated by successfully distilling lignin for the first time (*1*) and it has proved to be dependable, sturdy, and simple to operate.

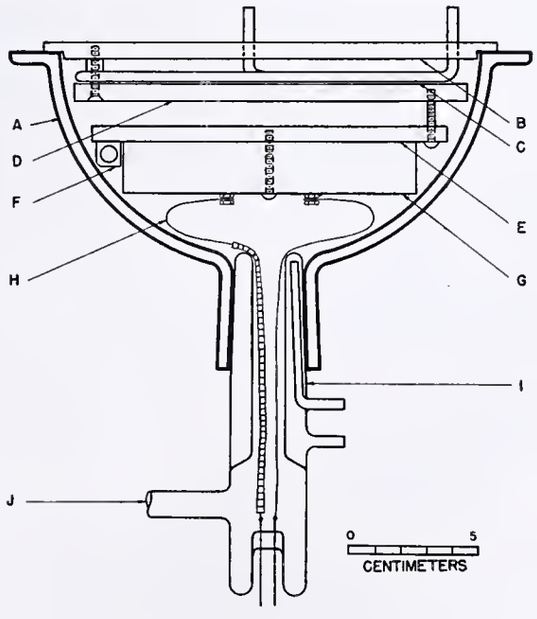

FIGURE 2. LARGE MOLECULAR STILL

A, vacuum distilling dome; *B*, iron cover; *C*, cooling coil; *D*, condenser plate; *E*, stove plate; *F*, thermometer well; *G*, heater; *H*, electrical lead; *I*, water jacket for tapered joint; *J*, vacuum line

Large Molecular Still

For larger capacity, a still of somewhat similar design was developed (Figure 2).

The central unit of construction was a standard Pyrex vacuum distilling dome (Catalog No. 3480) which contained the heater, hot plate, condenser, and thermometer. The heater was an inexpensive commercial hot plate 11.25 cm. (4.5 inches) in diameter and was fastened to a circular brass plate 13.75 cm. (5.5 inches) in diameter and 0.625 cm. (0.25 inch) thick: these two constituted the stove. It was suspended by three machine screws from the condenser, and the length of the screws permitted varying the stove-condenser distance. The condenser consisted of a circular brass plate 15 cm. (6 inches) in diameter and 0.625 cm. (0.25 inch) thick with a copper cooling coil soldered to the upper surface, and fastened rigidly to the iron cover. The electrical connections were made by wires, one threaded through magnesium oxide beads, soldered to a nickel bead fused to the tungsten wires sealed through the glass. An internal condenser was attached to the male ground joint to maintain the vapor pressure of the wax on the joint at a safe value. Picein wax was used to seal both the cover joint and the tapered joint. The thermometers for both stills were made from the broken stems of regular thermometers, with bulbs of proper size blown, and stems curved to fit the walls of the stills. Glass plates were used to cover both stove and condenser when direct contact with metal was undesirable. This still had a heating surface of over 150 sq. cm. (23 square inches) and a condensing surface of over 180 sq. cm. (28 square inches).

The rapidity of pumping systems is increased by large, short tubing, and elimination of constrictions caused by stopcocks, the latter also being a prolific source of leaks in high vacua. Satisfactory service can best be achieved by an all-glass, sealed system; all joints should be regarded with suspicion.

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A Special Slide Rule for Calcium Carbonate Equilibrium Problems

In Corrosion Control of Water Supplies

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PROTECTION of distribution systems against corrosion by means of a calcium carbonate film was first proposed by Tillmans (*4*), but a comprehensive theoretical formulation of conditions for deposition in the system CO_2—HCO_3^-—CO_3^{--}—Ca^{++} was evolved recently by Langelier (*7*). His complete equation involves pH, titratable alkalinity as calcium carbonate, calcium concentration, ionic strength, and temperature:

$$pH_s = (pK_2' - pK_s') + pCa + p\left[Alk + (H^+) - \frac{K_w}{(H^+)}\right] + \log\left[1 + \frac{2K_2'}{H_s^+}\right] \quad (1)$$

where pH_s = the hypothetical pH a water must have, without other changes in composition, for calcium carbonate stability
pK_2' = negative logarithm of the second dissociation constant of carbonic acid
pK_s' = negative logarithm of the solubility product constant of calcium carbonate
pCa = negative logarithm of the molal calcium concentration
Alk = equivalent concentration of alkalinity by titration
K_w = dissociation constant for water

The influence of ionic strength and temperature is shown in the term $(pK_2' - pK_s')$. From the actual pH value of the sample is obtained:

$$\text{Langelier saturation index} = pH - pH_s \quad (2)$$

a negative value of which indicates undersaturation and possible corrosion; a zero value denotes balance, and a positive value evinces supersaturation and possible scaling, the actual effects being dependent on numerical magnitude. DeMartini (*2*) concluded from a survey of California waters that a saturation index more positive than −0.5 generally implied freedom from serious corrosion.

By showing, under conditions, that the effect of (H^+) and (OH^-) on the alkalinity term and the influence of the final log parenthesis may be omittably small, Langelier simplified Equation 1 with negligible error for ordinary waters whose pH_s lies within the limits 6.5 and 9.5 to

$$pH_s = (pK_2' - pK_s') + pCa + pAlk \quad (3)$$

At the ordinary temperature of 20° C. $(pK_2' - pK')$ approximates a value of 2.3, which substituted in Equation 3 gives a short form:

$$pH_s = 2.3 + pCa + pAlk \quad (4)$$

A further simplification of the Langelier formula is ultimately possible in certain restricted cases where, in the absence of a calcium determination, it may be assumed that the calcium and alkalinity values are proportional. If calcium and alkalinity are taken as equivalent, Equation 4 becomes

$$pH_s = 2.6 + 2pAlk \quad (5)$$

which resembles in form the empirical equation of Baylis' experimentally established calcium carbonate equilibrium curve (*1*) and is equivalent to Strohecker's short formula (*9*).

To facilitate further the calculation of pH_s Hoover and Riehl (*6*) prepared a nomogram to solve Equation 3 graphically. Their drawing provides for temperature and salinity influences but omits corrections for the final log term of Equation 1 and the effect of (H^+) and (OH^-) ions.

The slide rule described below was designed primarily to provide immediate and easy solution to the full Langelier formulation with suitable provisions for all corrections; its application in solving the simplifications, Equations 3 and 4, is especially rapid and effortless.

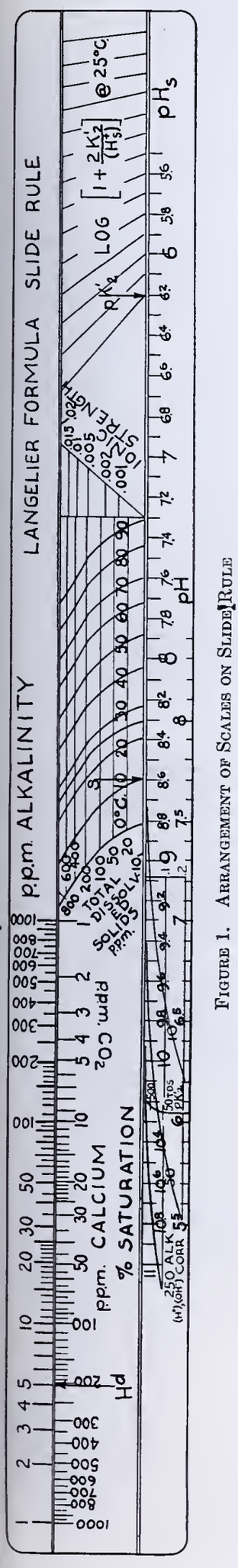

FIGURE 1. ARRANGEMENT OF SCALES ON SLIDE RULE

This special slide rule enables mechanical calculation of (1) the full Langelier equation and its contractions; (2) degree of calcium carbonate saturation; and (3) dissolved carbon dioxide from pH and alkalinity values, or vice versa. Incidentally, ordinary multiplication and division may be performed by using the logarithmically divided calcium and alkalinity scales.

Description

Arrangement of the scales is shown in Figure 1. On the upper stock is ruled the alkalinity scale, which gives the p*Alk* value for the alkalinity in p. p. m. as calcium carbonate figures marked. Although total dissolved solid correction factors extend only to 800 p. p. m., the scales for alkalinity and for calcium are ruled to an even 1000 to fill out the log cycle and enable their use as regular logarithmic scales.

The slide carries pCa values for calcium content. The semilog graph labeled "total dissolved solids", with temperature parameters, gives the $(pK'_2 - pK'_s)$ term. Data for its construction were taken from the Hoover-Riehl nomograph; hence proper provision is included for a corrected temperature effect (*6*). An accompanying scale of ionic strength is provided for use in cases where this fundamental information is known. The log $\left[1 + \frac{2K'_2}{(H^+_s)}\right]$ correction is supplied by gage lines on the right-hand end of the slide.

The top edge of the crowded bottom stock is ruled arithmetically to give pH_s; the lower edge is divided likewise but enumerated in the opposite direction to allow calculation of carbon dioxide from actual pH. In the region of $pH_s = 10.2$ is located an arc for use in conjunction with the log term correction gage lines; this short curve shows the variation of pK'_2 with total dissolved solids at 25° C. Correction for (H^+) and (OH^-) ions is provided by the graph plotted for alkalinity levels of 10, 50, and 250 p. p. m.

Applications

LANGELIER FORMULA CALCULATIONS. *Simple Form, Equation 4.* Method. Set the calcium figure on the slide opposite the alkalinity value on the top scale. The position of arrow *S*, located within the total solids plot, at a $(pK'_2 - pK'_s)$ value of 2.3, points directly to the pH_s value on the inside edge of the bottom stock.

Computation may be best illustrated by a specific example, using the following analysis:

	P. p. m.		*P. p. m.*
Na	18	Mg	6
Cl	16	Ca	15
Alkalinity as $CaCO_3$	33	SO_4	44
Total dissolved solids (by analysis), p. p. m.		132	
pH		10.0	

To find pH_s set 15 on the calcium scale opposite 33 on the alkalinity scale, and read at the point of arrow *S* that $pH_s = 8.9$. Since the actual pH is 10.0, the saturation index is $+1.1$, indicating a tendency to scale.

Simple Form with Temperature and Total Dissolved Solids. Correction, Equation 3. Method. The alkalinity and calcium settings remain as before, but the position of $(pK'_2 - pK'_s)$ is now obtained from the total dissolved solids–temperature graph. Total dissolved solids is the quantity obtained by summation of the ions and not the result of evaporation; this quantity may also be obtained from electrical conductivity, using the formula of Gustafson and Behrman (*3*). In the graph 40 p. p. m. total dissolved solids is taken equivalent to 0.001 ionic strength, as suggested by Langelier, who showed that many natural waters contain equal equivalent concentrations of monovalent and divalent ions. The graph may also be employed in terms of ionic strength; this is preferable for treated waters, especially zeolite effluent using the sodium cycle, wherein the relation between equivalent concentrations of the two valence types is altered considerably. A special stoichiometric slide rule (*5*) facilitates the calculation of ionic strength from the analytical statement.

This type of calculation will suffice to establish pH_s for most waters. The plot lucidly demonstrates that temperature influence on pH_s is dominant compared to the total dissolved solids effect; a wide variation from 50 to 400 p. p. m. dissolved solids is needed to produce practically the same shift of pH_s as a drop of only 10° C. below usual room temperature.

Solution with Correction for Log $\left[1 + \frac{2K'_2}{(H^+_s)}\right]$. The gage lines to the right of the slide are designed to follow Langelier's tabulated values of $\log \left[1 + \frac{2K'_2}{(H^+_s)}\right]$ in terms of the difference $(pK'_2 - pK'_s)$. pK'_2 in turn depends on temperature and solids content, but for simplicity its values are given only at 25° C. as its variation with ordinary temperatures is negligible. The short vertical curve near $pH_s = 10.2$ gives the value of pK'_2 from 1 to 800 p. p. m. dissolved solids, within which range its variation is limited.

Method. The pK'_2 arrow, located in the gage line area, is set opposite the proper pK'_2 value on the short vertical arc and the gage line opposite the preliminary pH_s, corrected for temperature and dissolved solids, is followed to its top edge and then projected back vertically to the pH_s scale to give a value corrected for $\log \left[1 + \frac{2K'_2}{(H^+_s)}\right]$. Below $pH_s = 8.9$ this correction becomes negligible, as noted from the nearly vertical slopes of the gage lines for large differences between pK'_2 and preliminary pH_s. On the other hand, above $pH'_s = 8.9$, consideration of this term becomes increasingly important.

Solution with Correction for (H^+) and (OH^-) Ions. Previously the term $p\left[Alk + (H^+) - \frac{K_w}{(H^+)}\right]$, which for equilibrium conditions reached through theoretical (H) adjustment only should properly be written $p\left[Alk + (H^+_s) - \frac{K_w}{(H^+_s)}\right]$, has been assumed to have the same value as p*Alk*. However, a plot of the difference

$$p\left[Alk + (H^+) - \frac{K_w}{(H^+)}\right] - pAlk$$

in Figure 2 shows a correction, ΔpH_s, exceeding 0.05, applicable within the shaded zone. This correction is significant for waters of low alkalinity and mineralization, especially in calcium. On

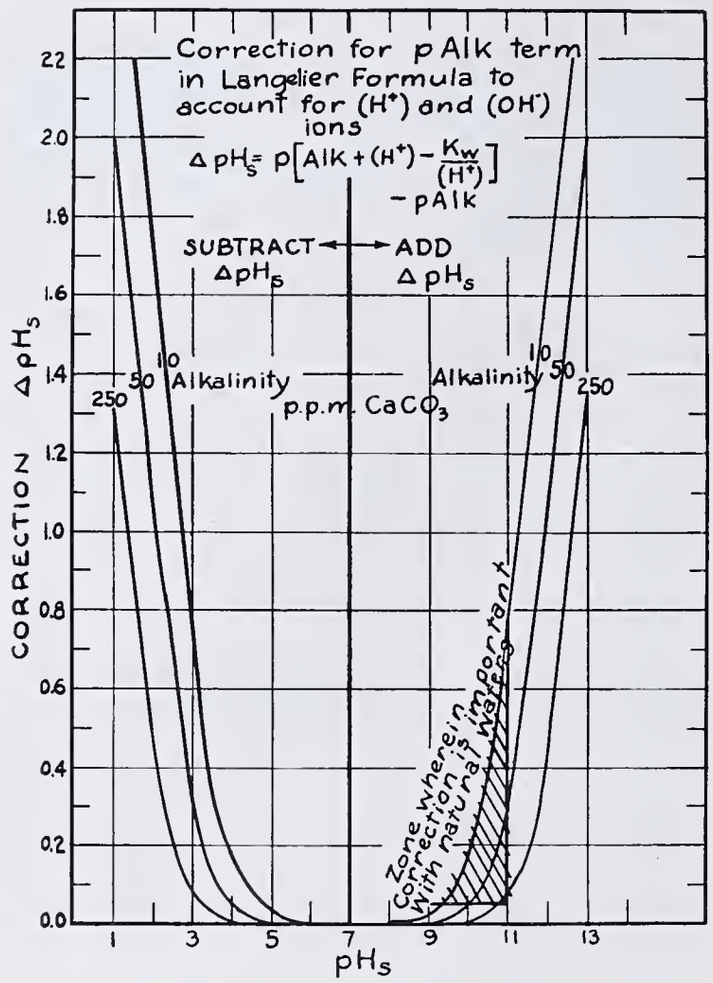
Correction for pAlk term in Langelier Formula to account for (H+) and (OH-) ions
Δ pHs = p[Alk + (H+) − Kw/(H+)] − pAlk
SUBTRACT Δ pHs
ADD Δ pHs
Alkalinity p.p.m. CaCO3
10
50
250
Zone wherein correction is important with natural waters
CORRECTION Δ pHs
pHs

FIGURE 2

the acid side this correction is without practical importance but is drawn in Figure 2 merely to show symmetry. These three curves are reproduced on the bottom of the slide rule to enable this final correction for those cases above about $pH_s = 9.5$ where needed.

Method. The ordinate from the edge of the stock at the uncorrected pH_s value vertically downward to the alkalinity parameter, interpolated if necessary, is noted and the correction read from the auxiliary scale of ordinates near $pH_s = 9.1$. This increment is added to the preliminary value of pH_s preferably before making the preceding log term correction, as the rate of variation of the latter with respect to initial pH_s is the larger. Adjustment of corrections by iteration may be disregarded.

Problem. Using the previous lime-softened water analysis, find pH_s at a temperature of 10° C. with all corrections included. Set 15 on the calcium scale opposite 33 on the alkalinity scale as before. Move the glass index to a point in the total dissolved solids–temperature plot corresponding to 132 p. p. m. total solids and 10° C. temperature. Under the hairline, the preliminary value of pH_s corrected for temperature and solids is 9.09. To correct for the log term, set the pK_2' arrow opposite the 132 p. p. m. solids point on the pK_2' arc. Project $pH_s = 9.09$ along the gage line direction to the upper edge of the slide, then drop this point vertically to the pH_s scale to read $pH_s = 9.14$. No final correction for (H^+) and (OH^-) is necessary, as the intersection of the hairline at $pH_s = 9.14$ with the interpolated location of the 33 p. p. m. alkalinity parameter is at zero.

DEGREE OF CALCIUM CARBONATE SATURATION. It is evident from Equation 3 that a corrosive water may be balanced if the calcium and/or the alkalinity is increased so that the log of their product is augmented to the extent of the saturation index. The saturation index represents the deficit in the product of calcium by alkalinity ions. Rewriting Equation 3 using logs

$$pH_s = (pK_2' - pK_s') - (\log Ca + \log Alk) \quad (6)$$

Since balance is attained when $pH - pH_s = 0$, this condition will be established by making

$$\log Ca_{sat.} + \log Alk_{sat.} = \log Ca + \log Alk + \text{saturation index} \quad (7)$$

$$\text{whence} \quad \text{Saturation index} = \log\left[\frac{Ca_{sat.} \times Alk_{sat.}}{Ca \times Alk}\right] \quad (8)$$

$$\text{or} \quad \frac{Ca \times Alk}{Ca_{sat.} \times Alk_{sat.}} = \frac{1}{\log^{-1} \text{saturation index}} \quad (9)$$

This procedure presupposes no changes in pH nor in factors affecting the term $(pK_2' - pK_s')$, thus properly neglecting any slight elevation in salinity.

Method. It is sufficient to find the reciprocal of the antilog of the saturation index. This is done by setting 100 on the per cent saturation scale opposite the previously calculated pH_s value and noting the reading on the per cent saturation scale by the actual pH, also along the pH_s scale. Enumeration of the per cent saturation scale above and below 100 permits this calculation for both scaling and corrosive waters. A solution is also possible using the slide in reverse.

Problem. Find per cent saturation when pH = 10.0 and $pH_s = 9.14$. Set 100 on the per cent saturation scale opposite 9.14 on the pH_s scale and opposite 10.0 on the same scale read the hairline at 700 per cent saturation.

DISSOLVED CARBON DIOXIDE CALCULATION FROM pH AND ALKALINITY VALUES. Carbon dioxide is more accurately calculated from the alkalinity titration and glass electrode readings, both of which are very reliable, than determined by direct titration with phenolphthalein according to standard methods. This simple mass law calculation of the hydrogen-ion concentration of a mixture of a weak acid and its completely dissociated salt was applied by Henderson and Hasselback (*8*) in 1908 to blood chemistry equilibria.

$$pH = pK + pCO_2 - pAlk \quad (10)$$

since pK = 6.3 in distilled water

$$pCO_2 = pH + pAlk - 6.3 \quad (11)$$

The extent to which carbon dioxide removal by simple aeration raises the pH value of a water sample may be conveniently calculated by this method.

Method. The slide is reversed and set so that the pH arrow (located to allow for the constant) points to its value on the very bottom scale. The hairline is set at the given value on the alkalinity scale and p. p. m. carbon dioxide is read directly underneath on the scale normally devoted to calcium.

Problem. Given alkalinity = 95 p. p. m. as calcium carbonate and pH = 6.5, find the carbon dioxide content. Reverse the slide, set the pH arrow opposite 6.5 on the bottom pH scale, and across from 95 on the alkalinity scale read 70 p. p. m. carbon dioxide on the carbon dioxide scale. To raise the pH value to 7.0, carbon dioxide must be reduced to 26 p. p. m., as can be found by shifting the slide toward the right to place the pH arrow over 7.0 on the bottom scale and reading the carbon dioxide scale without shifting the hairline.

Summary

This special slide rule expedites Langelier formula calculations and enables all corrections to be included with a minimum of effort. It also permits easy mechanical calculation of the degree of calcium carbonate saturation and the carbon dioxide content from the actual pH value and alkalinity.

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An Improved Methoxyl Apparatus

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COMMON methods of methoxyl assay employ a cumbersome and complex apparatus. The standard method of the Technical Association of the Pulp and Paper Industry (*3*) uses three separate pieces of apparatus for maintaining temperature in the assay system, while that of Peniston and Hibbert (*2*) employs four separate units; other methods require generally similar equipment. The proposed apparatus represents a simplification in apparatus with greatly improved operating characteristics.

Apparatus

A diagram of the apparatus is shown in Figure 1. The apparatus was patterned, in part, after the standard T. A. P. P. I. apparatus (*3*), but embodies an internal electrical heater rather than an external heating unit, and the absorption system is simplified. Greatly simplified control is one of the major advantages.

OPERATION. Best results were obtained by heating the glycerol bath (*I*, Figure 1) on a small adjustable, thermostatically controlled hot plate (Central Scientific Co., Catalog No. 16,627), which maintained the bath at 130° C. with almost no change for hours on end. The heater immersed in the jacket was connected to the alternating current line through a fixed resistance of such size as to maintain the jacket temperature at 55°. With the trap and absorption tubes filled, the sample was introduced, and the apparatus was closed and lowered into the glycerol bath. Introduction of a slow stream of carbon dioxide completed the necessary adjustments. This apparatus was in full-day operation for several weeks at a time; once adjusted, virtually no further adjustments were required and supervision was necessary only at hourly intervals to change absorption tubes and scrubbing liquid and to introduce a new sample and, if necessary, new hydriodic acid and phenol.

It was found desirable to add samples in a small glass boat and to use an antibump tube as shown. Three or four samples were hydrolyzed with 15 ml. of hydriodic acid (specific gravity 1.7) before the flask was replaced with a new flask and fresh reagent. The addition of phenol was not necessary, but contributed to a shorter time and smoother reaction.

As Clark (*1*) and Peniston and Hibbert (*2*) noted, the apparatus and reagents must be heated before use to free the system completely of methoxyl. All grades of phenol used in this laboratory have given positive tests for methoxyl. A convenient method of testing for completion of removal of methoxyl is to use an absorption tube containing alcoholic silver nitrate. In addition to the causes of error recorded by Peniston and Hibbert necessitating a blank determination, a prolific source of blank correction was found to be the instability of potassium iodide solution. Even short standing caused a faintly yellow solution (formation of free iodine) and consequent large blank correction.

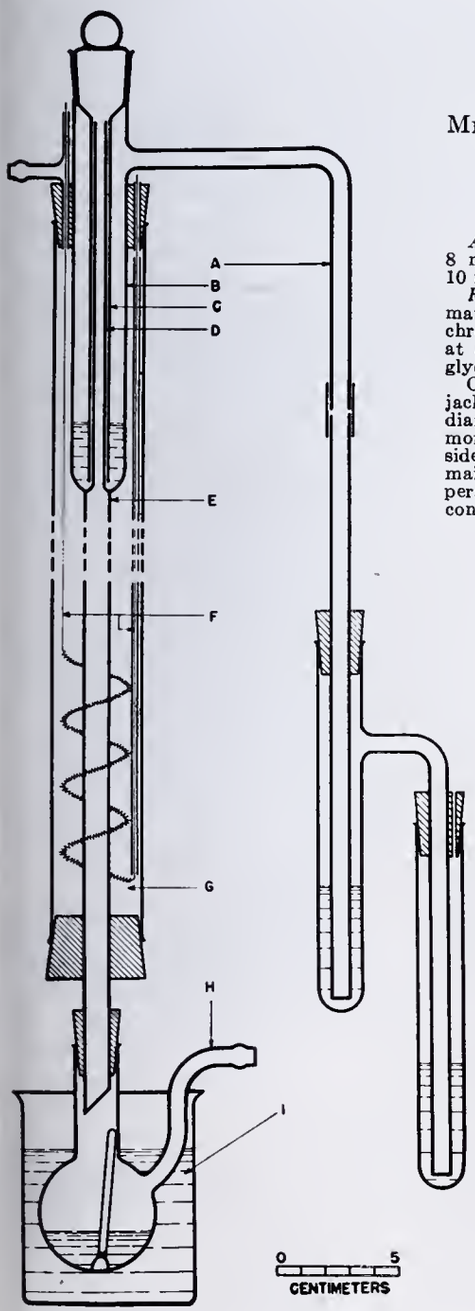

FIGURE 1
METHOXYL APPARATUS
Drawn to scale

A, 7 mm.; *B*, 22 mm.; *C*, 8 mm.; *D*, 5 mm.; and *E*, 10 mm. in outside diameter; *F*, electrical leads, approximately 4 feet of No. 26 Nichrome wire; *G*, water jacket at 55° C.; *H*, CO_2 inlet; *I*, glycerol bath at 130°.

Over-all length of water jacket was 60 cm., outside diameter 38 mm.; a thermometer was suspended inside. Glycerol bath was maintained at constant temperature by thermostatically controlled heater.

Variations in Method

This apparatus was used with equal success in the Zeisel (*5*), Vieböck and Schwappach (*4*), and Peniston and Hibbert (*2*) methods and gave an accuracy identical to that obtained by the older apparatus. It should give identical accuracy in Clark's (*1*) and similar methods. In the Peniston and Hibbert method the addition of phenol to the flask was not essential and carbon dioxide was used directly from a tank. A phosphorus suspension and a 5 per cent cadmium sulfate–5 per cent sodium thiosulfate solution gave identical results as a scrubbing medium. With the size of sample ordinarily used in the Zeisel method, the silver nitrate absorbing solution and subsequent gravimetric determination gave lower accuracy than the bromine–potassium acetate–glacial acetic acid absorbing solution (*2*) and subsequent volumetric determination of the iodine. The latter method possesses great advantages in simplified procedure, high inherent accuracy, freedom from interference, rapidity, and ability to use extremely small samples.

This apparatus has been in successful use in the author's laboratory for several years in the analysis of methoxy phenols and lignin preparations, and has been used by Irwin A. Pearl of this laboratory in recent months with similar success and satisfaction. The method employing the volumetric determination of iodine, substantially as described by Peniston and Hibbert but modified as above, has been found to be most accurate and convenient. It consistently gave values that varied less than 0.1 per cent from the theoretical with 3- to 7-mg. samples of vanillin; in general, accuracy was similar to that reported by Peniston and Hibbert.

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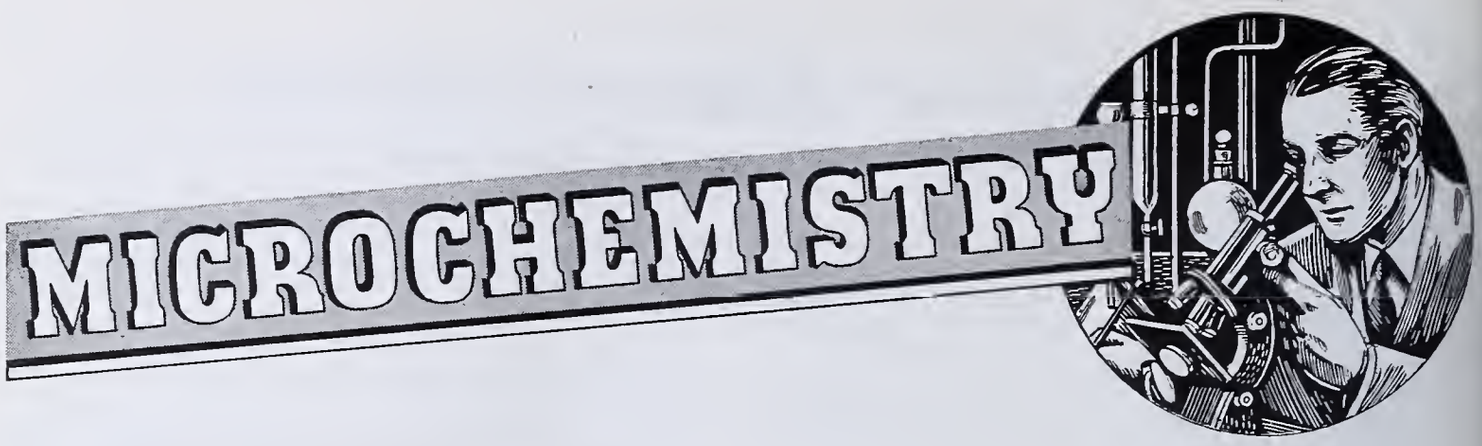
CONTRIBUTION from the Department of Chemistry and Chemical Engineering and Lignin and Cellulose Research Laboratories.



Colorimetric Determination of Phosphorus in Soils

Provision for Eliminating the Interference of Arsenic

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A colorimetric method that is both rapid and accurate has been developed for the determination of phosphorus in soils. The soil is digested with 60 per cent perchloric acid which quickly destroys the organic matter and converts the phosphorus present to the inorganic form. A final boiling temperature of 203° C. is, no doubt, responsible for the complete extraction of the soil phosphorus. Sixty per cent perchloric acid is not a hazardous reagent and has been used for over two years in hundreds of analyses without a single explosion.

An aliquot of the perchloric acid solution of the soil is treated with ammonium molybdate and 1,2,4-aminonaphtholsulfonic acid. The depth of the blue color formed is measured in a photoelectric colorimeter and the phosphorus value calculated from the calibration curve of the instrument. The effect of variations in acidity, time, and temperature upon the development of this color is discussed.

Arsenates also give a blue color with the reagents used in this method. A slight modification, however, permits the determination of phosphorus in the presence of arsenic: An excess of sodium bisulfite in the presence of perchloric acid at room temperature reduces any arsenates to arsenites which do not interfere.

The values for the determination of phosphorus in thirteen soil samples of varying iron and organic matter content are presented and are shown to be in close accord with those obtained by the conventional gravimetric method of W. O. Robinson.

The working time for six determinations is 3 hours.

THE standard procedures for the determination of phosphorus in soils are the official method (*2*), in which the sample is digested with magnesium nitrate followed by a volumetric determination of the yellow ammonium phosphate, and the Robinson method (*13*), in which the sample is digested with hydrofluoric acid followed by a gravimetric determination of the magnesium pyrophosphate. Both methods give satisfactory results and demand only the equipment found in any general laboratory, but their many steps take a good deal of time and increase the possibilities of error. There seemed to be a real need for a simple, direct, rapid, and accurate method for the determination of soil phosphorus.

The perchloric acid method that King (*7*) developed for the analysis of biological materials has been applied successfully in this laboratory to the determination of phosphorus in plant materials, in crystalline organic compounds, and in water extracts of soils that had previously been treated with organic or inorganic phosphates. Furthermore, perchloric acid gives theoretical values for the phosphorus in nucleic acid derived from yeast or from cultures of the soil bacterium, Azotobacter. The ordinary micromethod of digesting the sample with a mixture of nitric and sulfuric acids (and even with hydrogen peroxide) had shown only one fourth of the phosphorus which would be expected from the values obtained for carbon and hydrogen. Therefore it was natural, when undertaking some Neubauer tests, to try using perchloric acid in soil analysis. No reference could be found to its use in the determination of phosphorus in soils[a], although perchloric acid has had many analytical applications (*4, 6, 15, 16*).

Since the 60 per cent acid had used in this laboratory for 18 months in hundreds of analyses without a single explosion, no anxiety was felt when starting these experiments. The somewhat general distrust of perchloric acid is due, no doubt, to the hazardous properties of the anhydrous form. The 60 per cent acid has proved to be perfectly safe even when it becomes a 72 per cent constant-boiling mixture at 203° C. Nevertheless, when soils of unknown origin are first used, a screen should be employed and the initial boiling temperature lowered by the addition of nitric acid.

This paper presents the new method, certain factors that influence its accuracy, and a modification which permits the determination of phosphorus in the presence of arsenic. The analytical results thus obtained, using a photoelectric cell colorimeter, are compared with the values found by the gravimetric method of Robinson (*13*).

Experimental

The soils studied are listed in Table I. The first eight samples were very kindly furnished by M. S. Anderson of the Division of Soil Chemistry and Physics of this bureau. The other samples

[a] While this paper was in press, Shelton and Harper (*14*) published a method which uses 4 ml. of 72 per cent perchloric acid on a 2-gram soil sample and hydrazine sulfate for the reducing agent.

were described in previous reports (*5*, *11*). The soils were air-dried and rolled; the stones and stems held on a 10-mesh sieve were discarded, and the entire sample was ground to pass a 100-mesh sieve according to the directions of Robinson. Thirty-five mesh is fine enough for the perchloric acid method, but since it was to be checked by Robinson's method, his directions were followed explicitly.

Extraction of Soil Phosphorus. The soil is boiled with 60 per cent perchloric acid which quickly destroys the organic matter and converts the phosphorus present to the inorganic form. This digestion is usually complete in 40 minutes. The final boiling temperature of 203° C. is responsible, no doubt, for the complete extraction of the soil phosphorus. An aliquot of this solution can then be treated with ammonium molybdate and reduced to give a blue color that is proportional to the phosphorus present.

Conditions Affecting Color Development. King used a Duboscq colorimeter and 1.2 ml. of 60 per cent perchloric acid in a 15-ml. volume to "contain almost the same 'total acidity' as the sulfuric acid used by Martland and Robison" (*8*). With a photoelectric cell colorimeter, however, it was soon evident that variations in acidity, temperature, and time affected the development of the blue color.

If too little acid is present when the reducing agent is added, some of the excess ammonium molybdate is reduced and the results are too high. If too much acid is present, the reduction of the phosphomolybdate is inhibited and the phosphorus values will be low. Experiment showed that the use of 2 to 4 ml. of 60 per cent perchloric acid in a final volume of 25 ml. gives reliable results, and 2.5 ml. of acid were adopted as the minimum concentration to be used. This acidity range was later confirmed by Rae and Eastcott (*12*).

A series of low results coincided with a sudden cold spell (14° C. on the laboratory bench). Investigation showed that with reagents below 20° C. the readings were low; above 29° C. the results gradually became too high; and from 33° to 50° C. they were erratic. A working temperature of 25° ± 4° C. was therefore adopted as the standard procedure.

The time required for the development of the maximum depth of blue color was also studied. After the reagents are made up to volume and thoroughly mixed, the color deepens gradually for 15 minutes. Then for 5 minutes very little change can be noted in the colorimeter. A slight increase follows and upon standing overnight a fine blue precipitate settles out, leaving the color of the liquid much less intense. All readings are, therefore, made between 15 and 25 minutes after the reagents are mixed. In determining the phosphorus in a series of solutions, it is very easy to make the readings at exactly 15 minutes.

Since boiling with perchloric acid displaces nitric and hydrochloric acids and dehydrates the silica (*17*), no exhaustive study of interfering substances was made. Solutions containing hydrogen peroxide or nitric acid, however, must be boiled with perchloric to obtain the correct values even when the phosphorus is already in the inorganic form.

The presence of 5 mg. of iron in the 25-ml. final volume gives a greenish cast to the blue color but it does not affect the reliability of the readings when using a red filter. Smaller aliquots are usually taken when this greenish tinge becomes apparent to the eye. Although titanium and vanadium react with ammonium molybdate, the high acid concentration prevents any such interference. Should magnesium chloride be present in solutions digested according to the official method, it does not interfere with the color formation.

Stannous chloride was tried but was discarded in favor of 1,2,4-aminonaphtholsulfonic acid. Although smaller amounts of phosphorus (0.003 mg.) may be detected in the optimum range of the instrument when using stannous chloride, as little as 2 p. p. m. of ferric iron interferes with the reaction, whereas the phosphorus values are still accurate in the presence of 200 p. p. m. of iron when using the sulfonic acid reagent and perchloric acid. Then, too, extremely slight variations in the size of the stannous chloride drops cause decided differences in the readings of the instrument.

Reagents (phosphorus-free). Perchloric acid, 60 per cent.

Ammonium molybdate, 5 grams mixed with 100 ml. of distilled water, allowed to stand overnight, then filtered into a dark-glass bottle.

1,2,4-Aminonaphtholsulfonic acid, recrystallized according to Fiske and Subbarow (*3*, p. 388). A 0.125-gram sample of this reagent is mixed with 44 ml. of 15 per cent sodium hydrogen sulfite solution in a dark, glass-stoppered bottle. A 20 per cent solution of sodium sulfite is then added drop by drop until the solution is clear. This should require 5 to 7 ml.

The reagents and the distilled water used in making the solutions to volume are kept at 25° ± 4° C. Fresh solutions are prepared at least every two weeks.

Calibration of the Photometer. The depth of the blue color is measured in a V-type Aminco photometer, equipped with red filters, calibrated by using various dilutions of two different standard solutions of recrystallized monopotassium phosphate. The readings were checked at several points with phosphates of known purity. W. Edwards Deming of this bureau made a statistical analysis of the resulting curve which showed the optimum range of the instrument to be between 0.015 and 0.1 mg. of phosphorus in 25 ml., although 0.0025 mg. of phosphorus gives a visible blue. This consideration governed the size of the soil sample and the final dilution after the digestion with perchloric acid. Thus for a soil of low phosphorus content, such as Norfolk sandy loam, a 5-gram sample and a final volume of 100 ml. are necessary for a color intensity falling within the range of the instrument. For the Barnes loam, however, a 2-gram sample in 200 ml. is adequate. The same conditions of acid concentration, temperature of reagent solutions, and age of the blue complex which had been adopted for this method, were observed in calibrating the photometer. Frequent checks on the calibration curve have shown no change in two years.

Analytical Procedure. The prepared soil sample of 2 to 5 grams is weighed into a 300-ml. Erlenmeyer flask, mixed with 30 ml. of 60 per cent perchloric acid, and digested in a hood until the dark color due to the organic matter disappears. Heating is continued 20 minutes longer to be sure that all the phosphorus is converted to the inorganic form. At this stage heavy white fumes of perchloric acid appear and the insoluble material becomes like white sand. An additional 1 to 2 ml. of perchloric acid may be used to wash down any black particles that stick to the sides of the flask; occasionally the flask is shaken. The total time on the hot plate averages 40 minutes.

When the sample is sufficiently cool to avoid spattering, it is diluted with distilled water and filtered into an appropriate-sized volumetric flask. The residue is carefully washed to bring the volume to the mark. The equivalent of about 20 ml. of 60 per cent perchloric acid was found in this diluted volume. If the aliquot used thus contains 0.5 ml. or more of perchloric acid, due allowance therefor is made when adding acid to develop the blue color.

After thorough mixing, an aliquot of 1 to 15 ml. is pipetted into a 25-ml. volumetric flask, perchloric acid is added to total 2.5 ml., and the volume is diluted with distilled water to about 20 ml. The flask is shaken after each addition. Then 0.8 ml. of the sulfonic acid reagent is added and finally 2 ml. of ammonium molybdate. The volume is adjusted to 25 ml., mixed well, and the color measured in the photometer when the solution is 15 minutes old. There is no apparent turbidity if the sulfonic and molybdate reagents are added when the volume is near the final dilution.

The zero setting of the instrument is adjusted for the blank of the reagents just before each series of readings by following a procedure and time interval identical with those used for the unknowns. This compensates for the age of reagents and differences in time of preparations for carrying out the color reaction. The phosphorus present is then quickly calculated from the calibration chart of the instrument. The results of the phosphorus determinations by this method are shown in the last column of Table I.

For preliminary trials, Erlenmeyer flasks graduated at 200 and 250 ml. were used and the sample was diluted therein without filtering off the sandy residue. After thorough mixing on a me-

Table I. Phosphorus Contents of Soils Determined by Gravimetric and Colorimetric Methods

Sample No.	Kind and Location of Soil	Horizon	Depth	Gravimetric: P_2O_5 as $Mg_2P_2O_7$	Gravimetric: P_2O_5 in residue[a]	Gravimetric: P_2O_5 in $Mg_2P_2O_7$ + residue	P_2O_5, Colorimetric
			Inches	%	%	%	%
C-2929	Barnes loam, Labolt, S. D.	A	0– 9	0.150	0.024	0.174	0.173
				0.147	0.033	0.180	0.174
C-2930	Barnes loam, Labolt, S. D.	B_1	9–17	0.097	0.009	0.106	0.102
				0.097	0.007	0.104	0.102
C-2916	Carrington loam, Buchanan County, Iowa	A_1	0– 3	0.115	0.015	0.130	0.133
				0.126	0.006	0.132	0.133
C-2917	Carrington loam, Buchanan County, Iowa	A_2	3–13	0.112	0.008	0.120	0.119
				0.115	0.006	0.121	0.118
C-3871	Kirvin fine sandy loam, Tyler, Texas	A	0–12	0.044	0.011	0.055	0.053
				0.046	0.010	0.056	0.053
C-3872	Kirvin fine sandy loam, Tyler, Texas	B	12–24	0.079	0.005	0.084	0.077
				0.078	0.005	0.083	0.076
C-3174	Russell silt loam, Morgan County, Ind.	A	0– 2	0.087	0.005	0.092	0.085
				0.090	0.004	0.094	0.086
C-3175	Russell silt loam, Morgan County, Ind.	B	2– 8	0.047	0.005	0.052	0.053
				0.045	0.005	0.050	0.052
C-1600	Cecil clay loam, Abbeville County, S. C.	B	3– 8	0.044	0.007	0.051	0.047
				0.046	0.005	0.051	0.046
C-1671	Chester loam, Rockville, Md.	A_2	2–10	0.105	0.003	0.108	0.107
				0.103	0.005	0.108	0.106
C-1672 and 3	Chester loam, Rockville, Md.	B	10–32	0.080	0.0004	0.080	0.079
				0.081	0.0008	0.082	0.078
	Las Vegas, Clark County, Nev.	A	...	0.062	0.009	0.071	0.070
				0.066	0.010	0.076	0.071
C-1601	Norfolk sandy loam, Edgefield County, S. C.	A	0–14	0.021	0.000	0.021	0.021
				0.021	0.000	0.021	0.020

[a] Determined colorimetrically.

chanical shaker, the needed aliquot was filtered off. This saved considerable time in working out such details as the amount of acid, length of heating, and particle size necessary. Even with such differences in technique, in all the sixty-seven preliminary determinations, the maximum variation from the finally accepted value was only 0.013 per cent of phosphorus pentoxide.

In this study an accuracy of 0.001 per cent was desired and all factors, therefore, were carefully controlled. A working curve for the instrument can, however, be quickly prepared with as few as eight points if an accuracy of 0.01 per cent is all that is demanded. In this case, the reagent solutions need not be made up so often, since the zero setting of the colorimeter compensates for their cloudiness. The method can also be adapted for use in a visual colorimeter.

The same samples of soil were analyzed by Robinson's method, in which the sample is digested with hydrofluoric acid after destroying the organic matter with nitric acid and by igniting with a Bunsen burner. The phosphorus is precipitated from a nitric acid solution by ammonium molybdate, redissolved and precipitated as magnesium ammonium phosphate, ignited, and weighed on a Kuhlmann balance as magnesium pyrophosphate. The results of this method are given in the fifth column of Table I.

Details inherent in the gravimetric method would tend to make its results lower than those of a colorimetric method. However, the unusual difference between the two methods in the values for the Barnes loam A sample led to a few qualitative tests for phosphorus in the residues discarded after the hydrofluoric acid digestion. In all preliminary trials, the colorimetric tests showed phosphorus present in the residue.

Since the qualitative tests were all positive, each of the residues from the hydrofluoric acid treatment was boiled with 25 ml. of perchloric acid and the phosphorus therein was determined colorimetrically. The results are shown in column 6. It is also evident from column 7 that the sum of phosphorus found in the magnesium ammonium phosphate precipitate and that left in the residue from the nitric acid extraction is in close accord with the phosphorus values obtained colorimetrically.

The residues from several perchloric acid digestions were also tested for possible phosphorus inclusions in the silicates present in the soil.

The following samples were weighed in duplicate: 2 grams of Barnes loam A, 2 grams of Carrington loam A_1, 2.5 grams of Chester loam A, 3 grams of Cecil clay loam B, and 4 grams of Kirvin fine sandy loam B. The samples were digested in 30 ml. of perchloric acid in the usual manner and the residue was washed until acid-free. The filter paper containing the residue was then placed in a platinum dish, and Robinson's hydrofluoric acid procedure followed. After the nitric acid had been expelled by boiling with perchloric acid, the phosphorus was determined colorimetrically on the total sample. The Barnes loam residue contained 0.005 per cent of phosphorus pentoxide, but the residues from the other four soils gave negative results. Where there are strong reasons to suspect that the soil phosphorus is present in some unusual form which might be unaffected by digestion with perchloric acid, a preliminary sodium carbonate fusion is recommended.

The perchloric acid method was also tried on Bureau of Standards standard phosphate rock samples to determine the magnitude of dilution errors. A Kuhlmann balance and certified glassware were used, and the results were corrected for moisture determined by the technique previously described (*9*). The Tennessee phosphate rock No. 56a results corresponded to 99.15 per cent of the certificate value (32.90 per cent phosphorus pentoxide) and the Florida land pebble rock No. 120, to 97.84 per cent of the certificate value (35.20 per cent phosphorus pentoxide). Both samples were soluble in perchloric acid at room temperature and six determinations were made in 90 minutes.

Determination in Presence of Arsenic. Arsenic is known to react with ammonium molybdate to form an arsenomolybdate which also gives a blue complex upon reduction. Digestion of the soil with perchloric acid converts any spray residues to the pentavalent form, causing an interference that has an additive effect upon the phosphorus values. Since preliminary comparisons showed that the colorimetric method gave higher results than the gravimetric, it seemed advisable to look for possible interference from arsenic in the samples tested.

Pett (*10*) has pointed out that arsenites, in contrast to arsenates, do not give a blue color with the phosphorus reagents and he has developed a method which permits the determination of phosphorus in the presence of arsenic. Using sodium bisulfite and sulfuric acid, he found that a 60-minute reduction period at 50° C. was necessary to complete the reaction. But the effect of heat upon the intensity of the blue color had been observed since early in 1939—for example, 1 ml. of a standard monopotassium phosphate solution gives an instrument reading of 40.5 at a room temperature of about 23° C. But if the standard is diluted and warmed to 50° C. in the presence of perchloric acid prior to the color development, the instrument readings will range from 40.5 to 191.0, depending upon the amount of acid added. These observations were later corroborated by Allen (*1*) and these factors were taken into consideration in the calibration of the colorimeter and in the development of the procedure.

Preliminary tests on potassium arsenate showed that heat was unnecessary to effect the reduction of the arsenite form if perchloric acid were used and details of this substitution were worked out. Pett's findings for the necessary concentration of sodium bisulfite for this reaction were verified. One-tenth gram was ineffective, but 0.4 gram was adequate and his concentration was adopted.

The time necessary to reduce the arsenates completely is dependent upon their concentration when using sodium bisulfite and perchloric acid at room temperature. Amounts

below 0.02 mg. of arsenic are reduced in less than 30 minutes, whereas 0.1 mg. of arsenic must stand 4 to 5 hours. There is no harm in letting the solutions stand overnight if the reagent blank for setting the photometer is allowed to stand in a volumetric flask for the same length of time.

The blank for 2.5 ml. of perchloric acid in 15 ml. of water increases according to the time it stands in the flask. The presence of 0.4 gram of sodium bisulfite is inconsequential in this connection. But when the molybdate and sulfonic acid reagents are added to a dilute acid solution that has stood for two days in a volumetric flask, a milky-blue tinge appears. It is imperative, therefore, that the reagent blank be prepared and determined for the same time interval as that used for the sample.

Since the straight colorimetric readings had been made on these samples, and it was known that none of them was high enough to approach a combined value of 0.1 mg. of arsenic, a 3-hour reducing period was adopted to be sure that all the arsenic which might be present was in the arsenite form.

Procedure for Elimination of Arsenates. An aliquot of the perchloric acid extract of the soil is placed in a 25-ml. volumetric flask and enough acid is added to bring its concentration up to 2.5 ml. An appropriate-sized glass scoop is used to add 0.4 gram of solid sodium bisulfite. Since the bisulfite does not affect the blank, such a measurement is close enough. The neck of the flask is washed with water and the volume brought to 20 ml. The flask is shaken a little to dissolve the solid, and then is allowed to stand on the bench for 3 hours. A blank is prepared at the same time and in the same manner. After standing, the procedure for the development of the blue color is continued as described above. If a lower second reading is obtained, it indicates that arsenic is present in the sample and that the lower reading is the true value for the phosphorus.

The values obtained with this modification agreed with those of the straight determination and proved conclusively that no arsenic was present in the soils used in this study.

The perchloric acid method was also run on six samples of soil that were very kindly supplied by R. D. Chisholm of the Moorestown, N. J., laboratory of the Bureau of Entomology and Plant Quarantine. These soils had been treated with lead arsenate for the control of Japanese beetles and contained 0.017 to 0.059 per cent of arsenic. After the arsenates had been converted to arsenites by the procedure just described, the phosphorus was easily determined. The instrument used, however, gives only arbitrary readings not simply related to transmission values and has no spectral dispersion. It would be necessary, therefore, to measure true spectral transmissions in order to determine arsenic in the presence of phosphorus.

Acknowledgment

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Semimicropycnometer for Heavy Water

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In the course of investigating some exchange reactions, small samples (about 1 cc.) of heavy water were manipulated and purified by distillation in a high-vacuum line. A simple and convenient pycnometer was devised which took advantage of the use of the high-vacuum line for filling it. The densities determined by means of this pycnometer are reproducible to 1 to 2 parts per 10,000, without thermostatic control. The results can be calculated for any temperature between 15° and 40° C. within this limit of error, provided the difference between the temperature of the actual determination and the temperature at which the density value is desired is not greater than 5°. For the usual determination of d_4^{25} or d_4^{20} the pycnometer is then conveniently weighed at room temperature.

Table I. Typical Pycnometer Calibration

(Weight of pycnometer, 3.4616 grams)

Weight of Water W	Temperature ° C.	D/W[a]	Deviation from Mean × 10^{-4}
0.7935	20.9	1.2578	+1.5
0.7937	21.2	1.2574	−2.5
0.7935	20.7	1.2578	+1.5
0.7936	21.1	1.2576	−0.5
	Av.	1.25765	±1.5

[a] D = density of water at temperature of determination; D/W then is reciprocal of volume of pycnometer.

The method employed by Gilfillan and Polanyi (*3*) in filling their flotation micropycnometer suggested the construction of an ordinary weight pycnometer which could be filled by the same method. Their micropycnometer was evacuated and the sample allowed to draw up into the pycnometer upon re-admitting air to the system. The authors' pycnometer is illustrated in Figure 1, *a*. Sizes of from 0.5- to 1-cc. capacity made from ordinary Pyrex tubing have been constructed and used.

The pycnometer is lowered into the removable tube, Figure 1, *b*, with the ground tip resting at the bottom of the bulb. The tube is inserted in a vertical position at a suitable place in the high-vacuum line. When the sample has been suitably purified, it is frozen (carbon dioxide or liquid air), and the system, including the pycnometer arm, is evacuated to 10^{-5} mm. or better for 15 to 30 minutes. Such a high degree of evacuation is necessary to

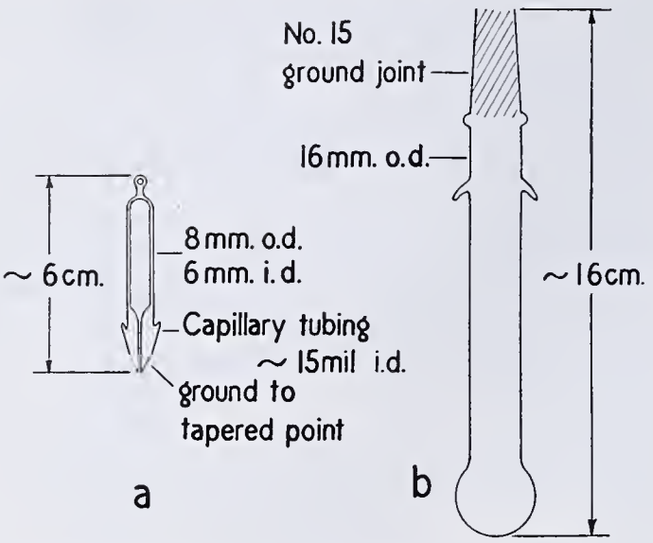

Figure 1. Diagram of Pycnometer and Vacuum Line Attachment

prevent the formation of an air bubble when the sample is introduced into the pycnometer. The sample is then thawed and distilled over into the pycnometer arm at room temperature by immersing the arm in a cooling bath (carbon dioxide or liquid air). The sample is again melted and air is carefully allowed to re-enter the evacuated system. The sample should rise into and completely fill the pycnometer with no visible air bubble at the top of the bulb. Not more than 0.05- to 0.1-cc. excess of water over the capacity of the pycnometer is required by this method. If a very small air bubble appears, it will usually dissolve if the pycnometer is allowed to stand, with tip still immersed, for an hour or more. The weight of the dissolved air is negligible. When the pycnometer is removed from the tube and suspended on the balance pan the sample should preferably be about 5° to 10° below room temperature.

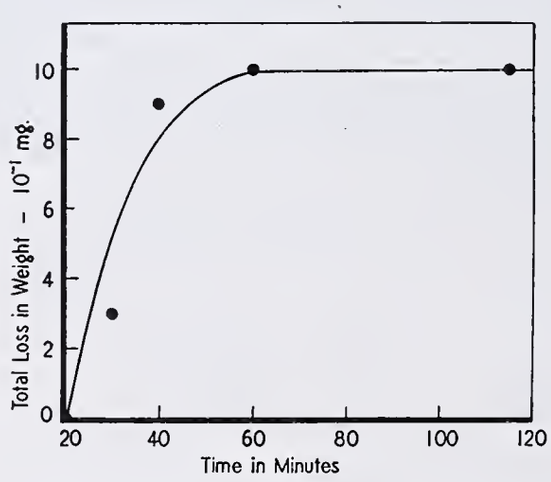

Figure 2. Typical Pycnometer Weighing

The pycnometer is carefully wiped and then suspended with ground tip up. The balance case is then closed (thermometer in balance case) and the pycnometer is allowed to stand for about one hour before weighing. As the sample warms up to room temperature the water expands through the capillary tip and evaporates at the tip as fast as it appears. Constant weight is reached in less than an hour and is maintained for a long period after. This is illustrated in Figure 2, where a typical example of a series of weighings of a filled pycnometer is given, starting 20 minutes after the sample was put into the balance case.

To empty, the pycnometer is replaced in its tube with the tip resting on a small metal coil a short distance above the bottom of the bulb. The sample usually will not pump out directly upon evacuating, but the water slowly evaporates along the capillary until a small bubble appears at the top of the capillary. On readmitting air to the system and tapping the pycnometer, the air bubble will rise to the top of the bulb; evacuating now readily expels the sample.

The pycnometer may be cleaned with chromic acid cleaning solution by alternately filling and expelling, with the use of an ordinary water aspirator.

The reproducibility of the density determinations is illustrated by the typical calibration results tabulated in Table I for a pycnometer of about 0.8-cc. capacity, with four separate samples of ordinary water. The average deviation from the mean is seen to be slightly greater than 1 part in 10,000.

In order to calculate the composition of the sample of heavy water, using the formula determined by Longsworth (*6*, *9*), the density at 25° C. is required. The density at 25° C. (or at any other temperature, t_2, between 15° and 40°) can be readily calculated from the determined density at temperature t_1. The difference in density, $d_2 - d_1$, at the respective temperatures, t_2 and t_1, is obtained for ordinary water from any suitable tables (*4*). This difference is added to the observed value of the density to give the desired density.

Table II. Variation of Difference in Density of D_2O and H_2O with Temperature[a]

t° C.	5	10	15	20	25	30	35	40
$\Delta(\Delta d_4^t) \times 10^{-4}$	..	+7.0	+4.9	+2.6	+1.2	−0.8	−2.7	−2.6

[a] The recent data of Chang and Chien (*1*, *3*) indicate that these values are very probably in error. The effect of the new data is to make $\Delta(\Delta d)$ slightly larger (approximately 1.5×10^{-4} part larger) in the intervals of 15–20° C. and 20–25° C. only. The newer data, of course, should be used to estimate corrections as suggested herein.

That this transformation can ordinarily be done with negligible error is seen from the data of Lewis and MacDonald (*5*) as recalculated by Farkas (*2*). Table II lists the differences between the difference in density of heavy water and water at various temperatures calculated from the data of Farkas (*2*). The difference in density between ordinary water and pure deuterium oxide is seen to vary by less than 4 parts per thousand from the 25° C. value over the range from 15° to 40° C.—that is, in this range the largest error possible in a density value so transformed would be 4 parts in 10,000 when interpolating from a value at 15° to one at 25° C. Ordinarily the error will be much less than this—for example, transforming densities over the 5° ranges from 20° to 25° or 25° to 30° results in an error of 1 to 2 parts in 10,000. In more dilute solutions of deuterium oxide these errors should be of the same order of magnitude or less. For greater accuracy throughout the whole range of temperatures given, the data of Farkas (*2*) may be used to estimate small corrections for the transformations. The nonlinearity of density with mole fraction (*7*) must, however, be borne in mind when making estimates of the corrections for mixtures of deuterium oxide and water.

Though especially suited to the determination of the density of heavy water, this pycnometer might be used for other pure liquids or volatile mixtures. In such cases, however, temperature control would probably be desirable.

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A Polarograph Reading Directly in Percentage

Analysis of Lead Arsenate

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When the conditions can be established so as to obtain a polarographic "wave" or "step" such that the upper and lower levels are essentially parallel to the voltage axis and to each other, a manually operated polarograph may be so manipulated as to read directly in terms of percentage. A polarograph capable of being so operated was constructed. A continuously variable shunt, with coarse and fine adjustments, is required in place of the usual stepwise shunt. The apparatus consists of three inexpensive radio-type potentiometers, a voltmeter, and a wall-type galvanometer with lamp and scale, together with battery and dropping mercury electrode.

The technique of using a manually operated polarograph so as to give direct percentage readings is described and an application to the analysis of commercial lead arsenate insecticides is given.

WHILE automatic polarographs (*1*) are convenient and even essential for many types of work, the basic principles of polarography may often be usefully applied by means of relatively simple manually operated devices. These, on the other hand, may range from the precision research instrument of Kolthoff and Lingane (*4*) to the very simple arrangements of Müller (*7*) or of Petering and Daniels (*9*).

Neither the automatic nor the manually operated polarographs are designed to read directly in terms of percentage of a given substance. Quantitative determinations are based on (1) the measurement of the wave height of the full wave, as obtained automatically or plotted from manual readings, (2) the increase in current between two voltage settings selected after an examination of the full wave, the "increment" method, or (3) the proportional increase in wave height caused by the addition of a known concentration of a particular substance to a solution containing an unknown concentration of the substance, the "internal standard" method. Since the observed wave heights depend, among other things, on the constants of the galvanometer used, each of these methods requires a calibration curve, a series of standards, or a calculation of some sort to determine the concentration from the wave height.

While Kolthoff and Lingane (*5*) describe a shunt circuit which allows the observed diffusion current to be read directly in microamperes, it is believed that a circuit designed to read directly in terms of percentage of a given substance would further simplify routine analyses.

To use such a circuit it is assumed, as in the increment method, that the diffusion current is directly proportional to the concentration and that the wave form is reasonably close to the ideal—i. e., the two levels are parallel to one another and cover an appreciable voltage interval.

The stepwise construction of the Ayrton shunt usually employed precludes exact adjustment of the galvanometer beam to a predetermined point on the scale. On the other hand, if the shunt consists of a continuously variable resistance, the galvanometer scale should be susceptible of being set to read directly in terms of percentage. The instrument described was so constructed.

The application to the analysis of lead arsenate insecticides is intended merely as an illustration of the technique of using the instrument. Normally, the lead content of such a product is determined more precisely by chemical methods. In this application the polarographic method serves as a rapid sorting-out method. The chief usefulness in the method would lie in the determination of relatively minor constituents, present in the order of 1 per cent or less, with a precision equal, in such cases, to most chemical methods.

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Apparatus

The shunt (arranged in an Ayrton connection, so that the total resistance across the galvanometer is constant) consists of two General Radio Company potentiometers arranged in tandem (Figure 1). A fixed resistance is inserted in the galvanometer circuit to bring the total resistance up to the critical damping resistance of the galvanometer employed.

A third General Radio Company potentiometer serves to deliver any desired potential from a battery, which may consist of dry cells or a 4- or 6-volt storage battery. The applied potential, as indicated by the Weston Model 301 voltmeter, V, may be adjusted to 0.02 volt, which is sufficiently close for most applications.

In more precise instruments the voltage is determined by a potentiometer balanced against a standard cell. While a low-resistance voltmeter (most cheaper types) may show some deviation from the true reading, this is immaterial in practical work, since standards and unknowns are electrolyzed at the same dial (voltage) settings.

The galvanometer was a Leeds & Northrup 2239 D type, with a period of 28 seconds, a sensitivity of 2×10^{-9} ampere per mm. per meter, and a critical damping resistance of 11,500 ohms.

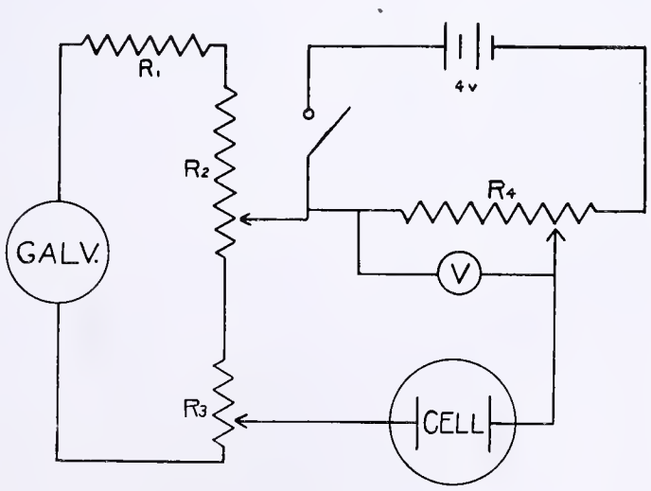

FIGURE 1. DIAGRAM OF CIRCUIT

R_1. 9000-ohm fixed resistance
R_2. 2000-ohm Model 314-A General Radio Co. potentiometer
R_3. 20-ohm Model 214-A General Radio Co. potentiometer
R_4. 50-ohm Model 214-A General Radio Co. potentiometer

Table I. Calibration of Galvanometer Scale in Terms of Percentage

Voltmeter Reading	Scale Readings					
	Blank[a]	0.01% Pb	0.01% Pb[b]	0.015% Pb	0.020% Pb	0.025% Pb
Volts						
0.4	−0.8	...	...	...	...	...
0.5	0.0	0.0	0.0	0.0	0.0	0.0
0.6[c]	0.0	0.0	0.0	0.2	0.2	0.5
1.1	2.2	86.0	...	...	...	...
1.2	2.8	93.5	...	...	...	...
1.3	3.5	98.2	102.5	148.5	198	244
1.4	4.0	100.2	104.0	153.5	201	248
1.5	4.5	101.1	105.3	155.1	205	251
1.6	6.1	103.5	106.7	157.0	208	255
1.7	12	108.0	110.2	158.8	211	259

[a] 20 cc. of foundation solution diluted to 50 cc.
[b] Solution set to read 104.0 at 1.4 volts to allow for blank.
[c] Readings between 0.6 and 1.1 volts omitted to condense table.

With a fixed resistance of 9000 ohms, the total resistance of the shunt circuit was 11,020 ohms, thus leaving the galvanometer slightly overdamped. The galvanometer was used with a Leeds & Northrup lamp and scale, No. 2100.

A small (10- to 15-cc.) beaker was used as the electrolysis cell. No attempt was made to protect the contents from air, since the sulfite present was sufficient to take care of any oxygen diffusing into the solution for a considerable period. A large calomel electrode of a simple type (*3*) served as an anode; the dropping mercury cathode consisted of a 10-cm. piece of "marine barometer" tubing (Corning) attached to a 60-cm. length of heavy-walled rubber tubing previously washed with alkali, acid, and water to remove talc and surface sulfur. With the particular capillary and head of mercury used, the drop rate in distilled water, under zero voltage, was 4.0 seconds.

An on-off single-pole snap switch serves to disconnect the battery.

Procedure

To employ the instrument so that a reading is obtained directly in terms of percentage, the following procedure is employed:

The approximate range of concentrations at which a most nearly ideal step is obtained must first be ascertained. At too low concentrations the residual current, due to traces of oxygen, impurities, "condenser current", etc. (*4*), approaches the diffusion current of the ion being determined. At too high concentrations the characteristics of the curve become such as to make it less useful for quantitative comparisons. The slope becomes flatter, so that the step occupies too great a voltage range and the upper plateau is no longer parallel to the lower.

In the present case, for example, it was found that concentrations of lead up to about 0.01 per cent were suitable. The step, beginning to rise above 0.6 volt, flattened out at 1.4 volts, and did not rise appreciably until about 1.9 volts. While even this is greater than the usual range of the polarographic step, it is satisfactory where a single constituent is being determined in the absence of interfering substances.

A detailed plot of the current voltage curve for 0.01 per cent lead showed an apparent half-wave potential of about −0.9 volt as compared with −0.755 reported by Müller and Petras (*8*) and −0.82 by Hohn (*2*). The difference is largely accounted for by the relatively large iR drop. The galvanometer deflection of 100 scale divisions, at an approximate sensitivity of $^1/_{100}$, amounts to a current of 2×10^{-5} ampere. Assuming a solution resistance of 5000 ohms (*6*) the iR drop is of the order of 0.1 volt, which is to be subtracted from the applied voltage. If half-wave potentials are desired they are determined from the voltmeter readings by making correction for iR drop, and anode potential, if necessary. In actual analysis the voltmeter readings are convenient dial settings to indicate that the same applied voltage has been used for standard and unknown.

At lead concentrations around 0.01 per cent it is necessary to remove dissolved oxygen, normally present in solution to the extent of about 8 p. p. m. This was conveniently accomplished by the use of sodium sulfite and by working in alkaline solution to prevent precipitation of lead sulfite. Alkali, sulfite, and Methocel (Dow brand of methyl cellulose, grade M-361, here used as a "maximum" suppressor) were incorporated in the foundation solution. By using the same dilution of this solution throughout, the composition was kept constant except for the concentration of the material being determined. Temperature control was not found necessary, other than to allow the solutions, previously made to volume in volumetric flasks, to stand at room temperature in the vicinity of the polarograph together with the standards. As a reading takes but a few minutes by the method described, the samples and standards may all be run within a time interval during which the room temperature is not likely to change appreciably. If a change of more than 0.5° C. is anticipated, some sort of bath for temperature control may be necessary.

Samples (0.500 gram) are dissolved in 50 cc. of 5 per cent sodium hydroxide with the aid of heat if necessary, and made to 500 cc. To a 5-cc. aliquot are added 20 cc. of foundation solution (10 cc. of 5 per cent sodium hydroxide, 2.5 cc. of 1 per cent Methocel, and 2.5 cc. of 10 per cent sodium sulfite made to 20 cc.) and the sample is made to 50 cc. The final dilution thus contains 0.01 per cent of sample, 1 per cent of sodium hydroxide, 0.05 per cent of Methocel, and 0.5 per cent of sodium sulfite. The proportions of the constituents of the foundation solution may be allowed to vary somewhat, but the volume of solution added to sample and standard must be accurately measured (pipetted), so that the concentrations are the same in samples and standards. The foundation solution is stable except for the sulfite, which should be prepared daily.

Five to 10 cc. of a standard containing 0.01 per cent of lead, prepared by addition of foundation solution to a suitable volume of a standard lead nitrate solution, are poured into the beaker serving as electrolysis cell, the galvanometer scale is set at 0 with the voltmeter reading 0.5 volt, and the coarse and fine shunts are manipulated so that the 0.01 per cent solution gives a reading of approximately 100 on the scale at a voltage setting of 1.4 volts. The standard solution is removed, the cathode and agar-salt bridge are connected to the anode washed with water, and a beaker containing foundation solution, diluted in the same manner (20 to 50 cc.) is introduced. With the shunt settings as before, the displacement of the galvanometer due to the residual current in the range 0.5 to 1.4 volts is determined. The 0.01 per cent lead standard is re-introduced, after rinsing the electrodes by immersion in a beaker containing the solution to be measured, the galvanometer is set at 0 at 0.5 volt as before, and the voltmeter is again set at 1.4 volts. The fine adjustment is then manipulated so that the reading at 1.4 volts is 100, plus the correction due to the residual current (Table I, column 4). Subsequent readings are then directly in terms of percentage, after subtracting the scale divisions equivalent to the residual current.

Table II. Lead Content of Lead Arsenate Insecticides

Sample	Pb Content by Chemical Analysis[a]	Scale Readings 0.5 volt	1.3 volts	1.4 volts	1.5 volts	Pb Content from Scale Reading[b]
23	70.1	0.0	74.2	74.8	75.1	70.8
25	59.8	0.0	64.0	64.3	64.9	60.3
33	59.6	0.0	63.5	64.0	64.2	60.0
72	59.4	0.0	62.0	63.2	63.8	59.2
93	58.5	0.0	60.9	61.3	61.7	57.3

[a] Gravimetric chromate method.
[b] From scale reading at 1.4 volts by subtracting 4.0 units to correct for residual current.

With shunt settings undisturbed, the sample solutions were measured with the results shown in Table II. The samples consisted of lead arsenate and basic lead arsenate insecticides, previously analyzed for lead content by chemical assay by W. J. Kirby, Insecticide Division, Agricultural Marketing Service. After subtracting the blank (4 scale divisions at 1.4 volts) the readings are compared in terms of lead content with the chemical analysis.

The zero setting is checked at the end of each reading to compensate for galvanometer drift. Another variation to be allowed for is the oscillation of the galvanometer beam due to the changing size of the mercury drops. With a long-period galvanometer this amounts to about one scale division under the above conditions, but the amplitude of swing is very constant and it is satisfactory to take either the maximum, the minimum, or the middle of the swing for each reading, so long as a consistent practice is followed.

It is seen (Table I) that after setting the shunt so that the reading at 1.4 volts in column 4 represents 0.01 per cent of lead, after subtracting the blank, the subsequent readings at the same voltage are directly in terms of percentage up to concentrations of about 0.025 per cent when the proportionality between wave height and concentration begins to fall off. Within this range the precision is within 2.5 per cent and somewhat better than this at the lower concentrations.

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PRESENTED before the Division of Analytical and Micro Chemistry at the 102nd Meeting of the AMERICAN CHEMICAL SOCIETY, Atlantic City, N. J. From a dissertation submitted by S. Reznek to the graduate faculty of the Polytechnic Institute of Brooklyn in partial fulfillment of the requirements for the degree of doctor of philosophy, June, 1940.

Micromethod for Identification of Volatile Liquids

Vapor Pressure, Boiling Point, and Olefin Content of Cyclobutane and *cis*-2-Butene

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A PREVIOUS paper described a micromethod for identifying hydrocarbons by their physical properties (*1*), using the vapor pressure of the material as one of the properties for identification. In the apparatus vapor pressure measurements were limited to a small range of vapor pressures, from 1.0 to 30.0 mm. of mercury, because of the small size of the sample. Since then an apparatus has been devised to measure vapor pressures up to the boiling point on samples as small as 3 cc. of gas at normal temperature and pressure. This apparatus has been incorporated into the previous system, with some minor changes. As a further aid in the identification of liquids, an adaptation has been made of a bromination titration to determine the olefin content.

Apparatus

The vapor pressure apparatus constitutes the only essentially new addition. The assembly is shown in Figure 1. A capillary manometer is connected to a bulb through capillary tubing, all of 2-mm. diameter. The total volume is estimated at 1.8 cc., so that at the boiling point a gas sample of 3 cc. at N. T. P. would be only 60 per cent in the vapor state. The manometer has the left end joined to a three-way stopcock, so that it can be pumped out with a vacuum pump and the absolute pressure read directly, or it can be opened to the air and the pressure read against the barometric pressure. This method enables one to use a manometer of only half the barometric length, and still cover a range of from 0 to 1000 mm.

The manometer is connected to the system through a three-way stopcock and a Toepler pump. The sample can be distilled into bulb C_1 (Figure 1), using liquid nitrogen, or it can be pumped into the bulb with the Toepler pump. Once it is in the bulb the leveling bulb is raised so that the mercury acts as a cut-off to keep the system free of stopcock grease in which the hydrocarbons dissolve at the higher pressures. Measurements made on *cis*-2-butene using the stopcock showed that as much as 8 per cent of the hydrocarbon dissolved in the stopcock grease in the course of a determination.

Dewar flasks containing freezing mixtures at various temperatures are placed around bulb C_1 containing the sample and the vapor pressure is read. Because of the small volume of the system, equilibrium is established very quickly, usually in 5 to 7 minutes, and it is possible to make a complete set of readings up to the boiling point in about 45 minutes.

Freezing mixtures used were absolute methyl alcohol (−96° C.), solid carbon dioxide in acetone (−78.5° C.), chloroform (−67° C.), *m*-xylene (−55° C.), cyclohexanone (−45° C.), mercury (−38.9° C.), carbon tetrachloride (−22° C.), ice-salt (−10° C.), ice (0.0° C.), benzene (5.5° C.), ethylene bromide (10.0° C.), and water from 10° C. to room temperature. These baths keep their temperatures to within 3° C. over a period of a

Table I. Densities and Molecular Weights

Compound	Temperature ° C.	Density G./cc.	Molecular Weight
Ethylene	−78.5	0.540	28.0
	−96.0	0.560	28.0 (theoretical)
	−80.0	0.534 (*8*)	...
Cyclobutane	−78.5	0.802	55.6
	−67.0	0.788	56.0 (theoretical)
	−46.0	0.761	...
	−37.0	0.731	...
	−26.0	0.715	...
	0.0	0.698	...
	0.0	0.703 (*4*)	...
cis-2-Butene	−78.5	0.724	56.0
	−78.5	0.711[a]	56.0 (theoretical)

[a] Extrapolated from data of Coffin and Maass (*3*).

Table II. Vapor Pressures of *cis*-2-Butene

t ° C.	*P* (Author) *Mm.*	*P* (Kistiakowsky) *Mm.*	Deviation %
−78.5	6.9	6.5	+ 6.2
−66.6	16.1	20.1	−20.0
−48.0	60.9	62.0	− 1.8
−38.9	107.5	106.8	+ 0.7
−22.5	234	251	− 6.8
−11.0	413	420	− 1.7
0.0	659	658	+ 0.2
2.7	730	730	0.0
3.5	752	751	+ 0.1
5.5	813	816	− 0.3

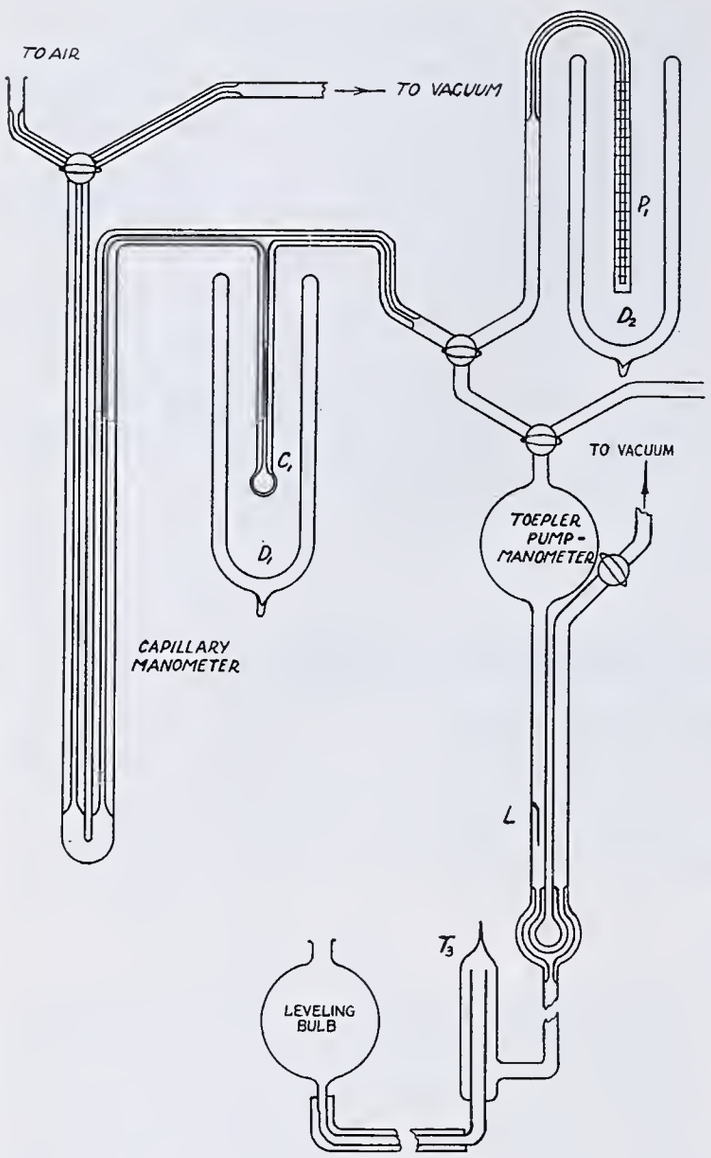

FIGURE 1. ANALYTICAL APPARATUS

C_1. Bulb
D_1, D_2. Dewar flasks
P_1. Micropyenometer
L. Constant volume indicator
T_3. Trap

half hour, larger drifts being due to impurities. The temperature was read with an alcohol thermometer calibrated before each run with solid carbon dioxide in acetone (−78.5° C.), melting mercury (−38.9° C.), and ice (0.0° C.) and a linear interpolation used between these points. Above 0.0° C. a mercury thermometer was used, calibrated at the ice point.

Pressures were read, starting at the lower temperatures and going up to the boiling point. Each pressure reading was taken from both sides of the temperature to ensure the establishment of equilibrium. Although no measurements were made above room temperature, the system is adaptable to use at higher temperatures by wrapping it in a Nichrome spiral heating element.

Materials

cis-2-Butene was obtained from G. B. Kistiakowsky, who used it in his determinations of the heat of hydrogenation of olefins (*5*). It was distilled into the system under vacuum, using a magnetic break-off, and put through two isothermal bulb-to-bulb distillations.

Ethylene and cyclobutane were obtained together with carbon monoxide from the photochemical decomposition of cyclopentanone and separated by fractional distillation through a series of traps. The separations were complete in the case of carbon monoxide and there was about a 1 per cent contamination of the cyclobutane fraction with ethylene. This was removed by further fractionation. Microcombustions were made on the gases to determine their purity, using the apparatus described by Blacet and Leighton (*2*), and showed the samples to be 99.5 per cent pure. Further evidence of the purity was obtained from the molecular weight determinations.

As a final check on the identity of the cyclobutane, microtitrations were carried out using a modification of the method described by Uhlig and Levin (*9*).

Densities and Molecular Weights

The densities and molecular weights were measured by the method described in the first paper (*1*). Several determinations were made on different samples (Table I).

Vapor Pressures

As a check on the accuracy of the vapor pressure apparatus, measurements were made on pure *cis*-2-butene. The vapor pressures obtained are shown in Table II, together with the values of Kistiakowsky (*5*). The boiling point as measured here was 3.8° C. The boiling point determined by Kistiakowsky was 3.7° C., while the boiling point as determined by Roper (*6*) is 3.5° C., all at 760 mm. of mercury. The data obtained by Roper, Kistiakowsky, and the author have been plotted in Figure 2, and the best straight line has been drawn through the points. The data of Roper lie closest to this line. The points at lowest temperatures fall off from the curve most, because of the large relative error in these low vapor pressure readings.

TABLE III. VAPOR PRESSURE OF CYCLOBUTANE

P Mm.	T ° K.	$1/T \times 10^3$	log P	P Calculated[a]	Deviation %
5.8	194.6	5.139	0.763	5.2	+11.5
50.5	227.6	4.394	1.703	50.3	+ 0.4
55.2	229.1	4.365	1.715	55.0	+ 0.4
73.8	234.2	4.270	1.868	73.4	+ 0.5
160	250.2	3.997	2.204	165	− 3.1
255	260.2	3.843	2.407	270	− 5.5
474	273.1	3.662	2.675	469	+ 1.0
583	278.6	3.589	2.766	586	− 0.6
706	283.5	3.527	2.849	708	− 0.3
765	285.8	3.499	2.883	771	− 0.8
832	288.1	3.471	2.920	840	− 1.0

[a] Determined from experimental curve.

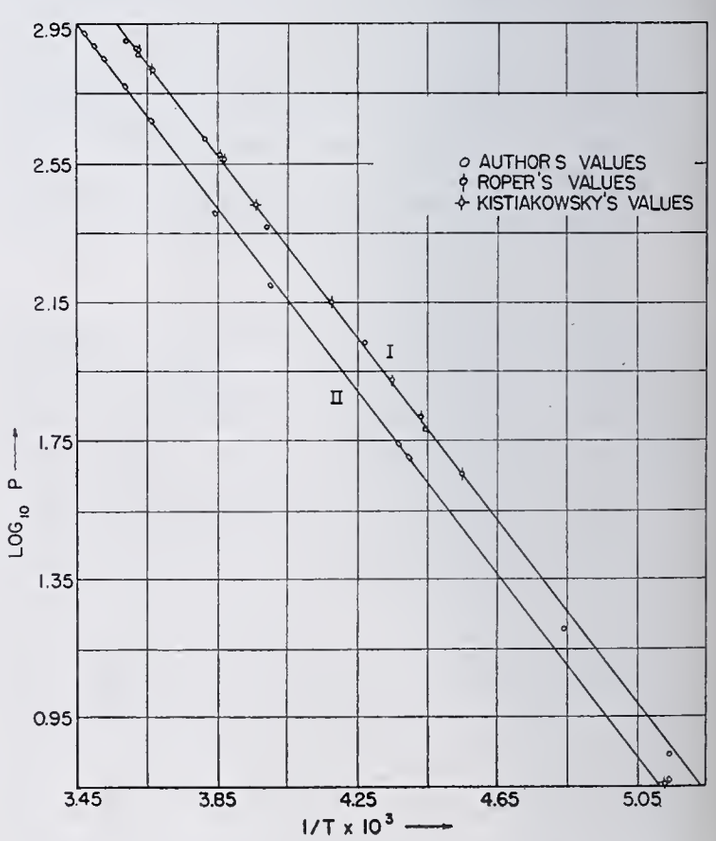

FIGURE 2. VAPOR PRESSURE MEASUREMENTS
Curve I. *cis*-2-Butene. Curve II. Cyclobutane

The data for cyclobutane have been assembled in Table III and plotted in Figure 2. As can be seen, they lie very close to a straight line.

The general equation for the vapor pressure–temperature dependence is given by the Clausius-Clapeyron equation:

$$\log P = -\frac{a}{T} + b$$

Table IV. Clausius-Clapeyron Constants and Heats of Vaporization

Compound	a	b	$H_{vap.}$ $\left(\frac{Kcal.}{Mole}\right)$
Cyclobutane	1324.5	7.5215	6.054
cis-2-Butene	1320.7	7.6630	6.037
			5.900 (*6*)
			5.810 (*5*)

In Table IV are given the experimentally determined constants for cyclobutane and *cis*-2-butene, as well as the heats of vaporization. These constants are good to about 1 per cent.

Titration

A direct adaptation of the method of Uhlig and Levin (*9*) was used. Previous attempts had been made using the method of Lewis and Bradstreet (*7*) but the results were not better than 5 to 9 per cent.

A solution of bromine in C. P. glacial acetic acid was made, containing 0.045 millimole of bromine per cubic centimeter (0.045 molar). It was stored in the dark in a brown, glass-stoppered bottle. It was used in a calibrated, 10-cc. buret graduated in 0.05 cc. The concentration of the bromine solution changed very little from day to day, never more than one part in a thousand, but it was nevertheless daily restandardized with a 0.1 molar standard solution of sodium thiosulfate.

The titrations were carried out in a 25-cc. glass-stoppered, Erlenmeyer flask. A small sealed bulb was dipped into liquid air, so that the sample was frozen out in the bottom. The end was cut off, and the tube and contents were quickly dropped into the precooled flask, containing 5 cc. of chloroform. The flask was swirled for a few seconds in an ice-salt bath at −10° C. and the contents were titrated. The total time of titration was about 5 minutes. The end point was distinct when a blue-white lamp was used for illumination and the flask held against a white background. Various samples of olefinic hydrocarbons brominated immediately, while the cyclobutane and samples of cyclohexane and cyclopentane showed no bromination at all, even after 30 minutes at −10° C. The cooling was found necessary, not in order to slow down the bromination of the olefins, but to lower the vapor pressure of the hydrocarbons and prevent losses. The results of the brominations are given in Table V.

Table V. Bromination Experiments

Compound	Sample	Olefin Found	Olefin Present
	Millimoles	*Millimole*	*Millimole*
Chloroform	50.00	0.000	0.000
Cyclohexane	0.560	0.002	0.000
Cyclopentane	0.493	0.002	0.000
Cyclobutane	0.413	0.003	0.000
Cyclopentene	0.330	0.326	0.330
Cycloheptene	0.340	0.337	0.340
cis-2-Butene	0.534	0.529	0.534
2-Pentene	0.335	0.330	0.335

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Apparatus for Microevaporations

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IN MICROPROCEDURES. it is frequently necessary to evaporate small quantities of solution. It is desirable to carry out these evaporations in the original tube or cone, but boiling is out of the question, since the formation of bubbles ejects the solution from the cone. Evaporation on a steam bath is slow because of condensation on the sides of the tube and the absence of convective currents above the solution. The usual procedure, which calls for transferring the solution to a watch glass or slide by means of a capillary pipet, is undesirable since it involves the loss of some of the material.

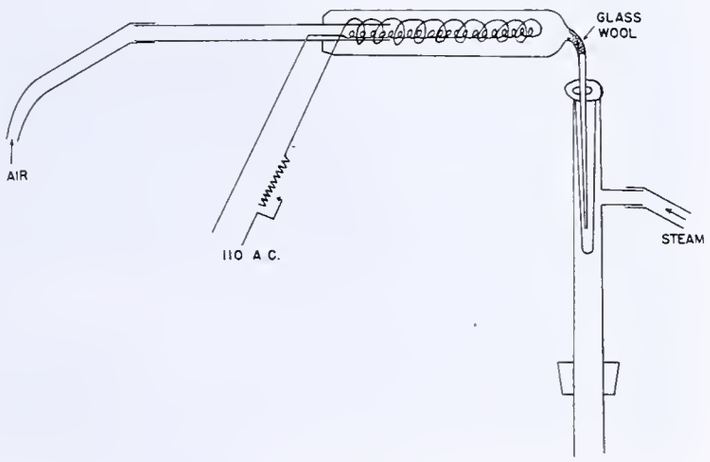

Figure 1. Diagram of Apparatus

The desired evaporation can be carried out by blowing a blast of hot air on the surface of the solution, which at the same time is being heated in a steam jacket. An air current from an atomizer bulb or compressed air connection is passed through a heater which is made by enclosing a coil of 80 cm. of 20 mil (No. 24 B. and S. gage) Nichrome wire in a piece of Pyrex tubing, about 15 mm. in diameter and 15 cm. long (Figure 1). Since permanent seals cannot be made around Nichrome wire, the leads are brought out through small holes in the side of the tube. The coil is connected to the 110-volt current through a resistance of 25 to 30 ohms, which may take the form of a slide-wire resistance or electric glow cones. The hot air is introduced into the microcone by an extension of the heater tube which is drawn to a bore of about 1.5 mm. This extension contains a wad of glass wool to catch tiny pieces of oxide which may be given off from the Nichrome wire after prolonged heating. The heater unit is clamped above the steam jacket, which in turn is clamped loosely in a vertical position. The microcone, containing the solution to be evaporated, is placed in the steam jacket which is raised until the delivery tube is just above the surface of the liquid. For convenience in adjusting the steam jacket, a one-hole cork is slipped on it to serve as a handle. The steam should move rapidly through the jacket, so that the sides of the microcone will not be fogged by condensate.

This device has been found very efficient; a volume of 0.3 ml. may be evaporated in 2 or 3 minutes.

Acknowledgment

The writer wishes to acknowledge technical assistance from R. W. Waggener and P. A. Roth, as well as criticisms of this paper by J. H. Reedy.

MODERN LABORATORIES

Socony-Vacuum's New Engineering Building

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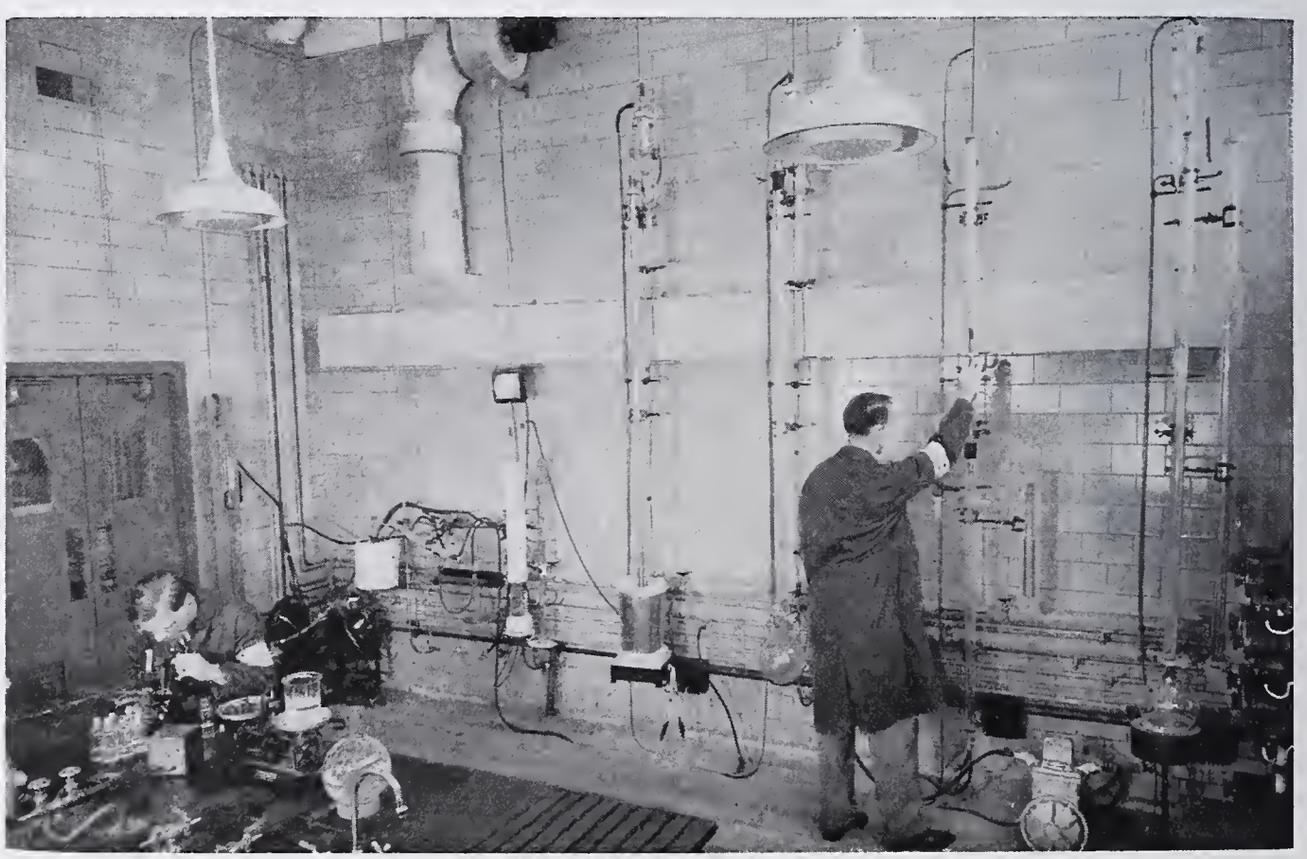
IN RECENT years leading architects have familiarized themselves with the requirements of chemical laboratory buildings, so that the design of suitable laboratory space is fairly well organized. The same attention has not been given to structures intended for laboratory engineering use, since relatively few large buildings of this type are constructed in a single year. For this reason, the design of the new chemical engineering building erected by Socony-Vacuum, based upon a broad knowledge of requirements as well as a study of some more modern existing engineering buildings, should be of interest.

In an engineering building provision must be made for handling bulky objects of considerable weight, for moving large quantities of materials and products, and for supplying utilities in quantities not generally associated with laboratory work. Attention must also be given to the elimination of accident hazards, which are intensified by the need for working during 24 hours of each day.

In designing the building, safety was considered of paramount importance, flexibility of layout and adaptation to future needs being next in importance. The lowest practicable operating cost was sought, recognizing that a substantial part of the operating personnel would consist of experienced chemical engineers and other skilled research technicians. The appearance of laboratory buildings should be as attractive as possible without increasing the capital investment unduly, since customers and field men are frequent visitors.

A flat-roof structure of steel construction with a high central bay to provide the necessary head room was selected, as this would provide an unobstructed floor area for engineering work. By limiting the clear roof span to 28 feet, it was possible to avoid the use of deep roof trusses which create



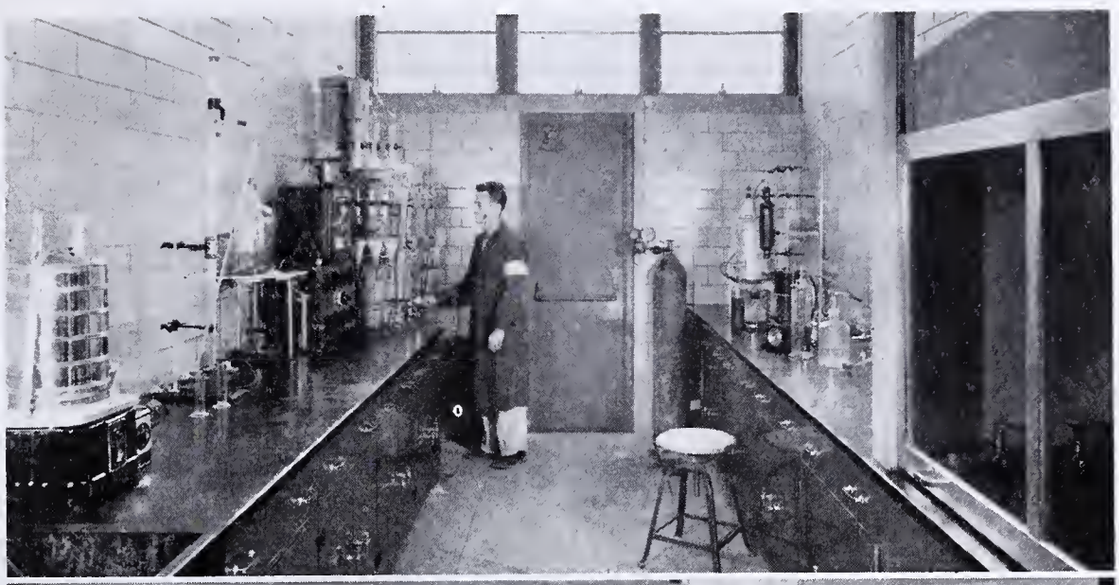
DISTILLATION ROOM

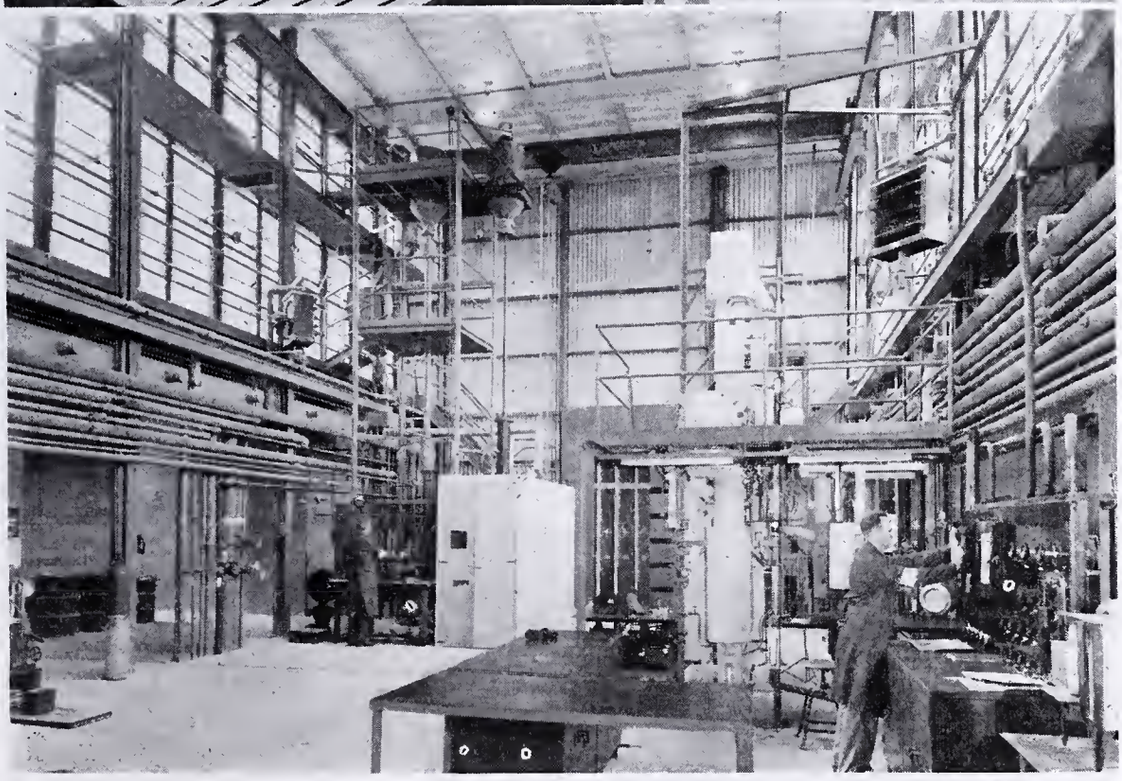
difficult maintenance problems and are less flexible and satisfactory than flat-roof structures for laboratory buildings.

The design requirements were met with a brick and steel structure 208 feet long and 78 feet 6 inches wide. In view of present emergency conditions, only the front half of the building has been completed and a temporary rear wall placed. Adequate utility capacity in the present structure will permit extending the building to any desired length.

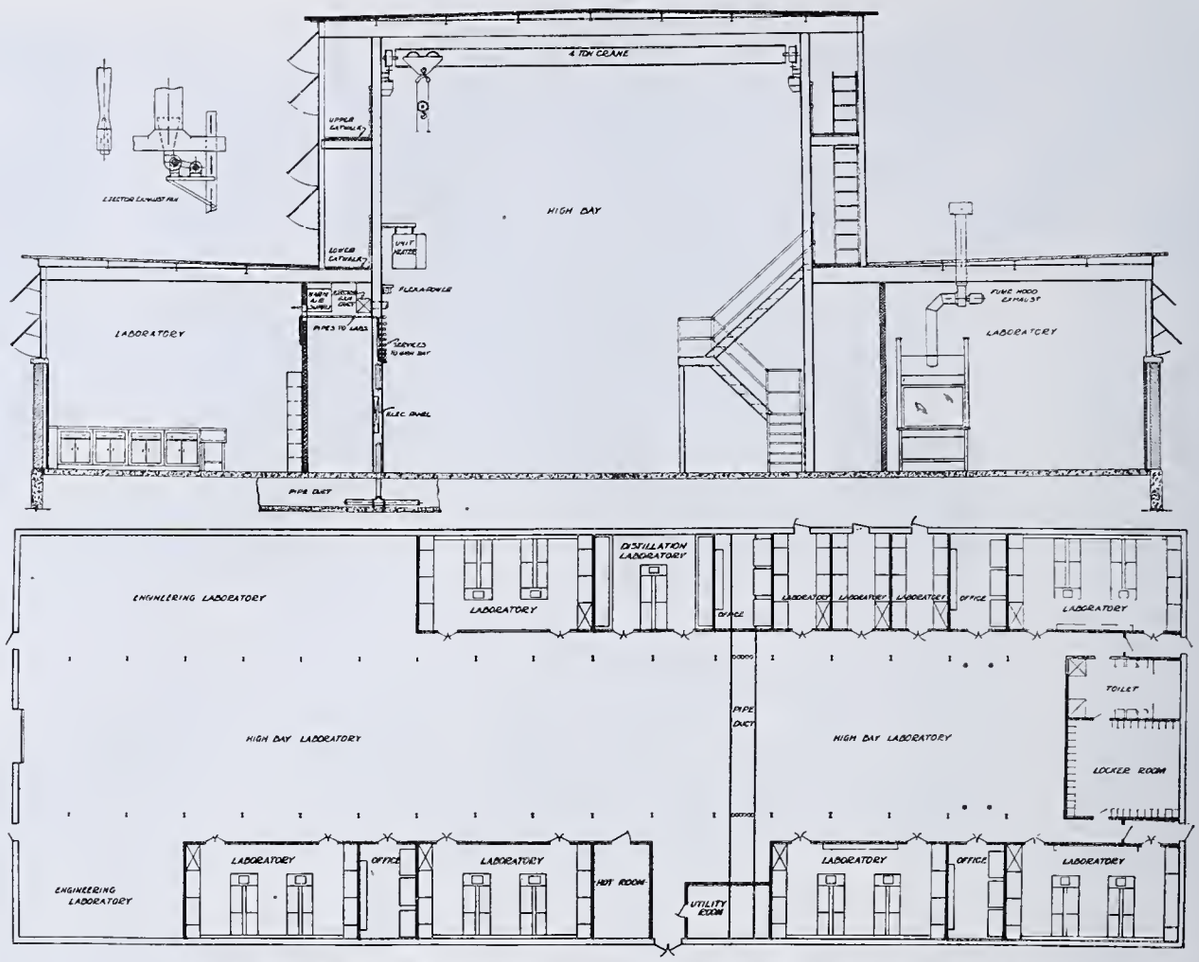
In chemical engineering development work it is necessary to follow pilot-plant operations with analytical work and small-scale testing, carried on adjacent to the actual units as far as practicable. The necessity of erecting bubble towers and similar structures dictated head room under the crane of about 30 feet, while laboratory work in some engineering operations required only 13 feet of head room. This led to the construction of the step type of structure, which appears to offer several very definite advantages, since test work can be located adjacent to the unit while utilities can conveniently be supplied from headers placed on the columns. Experience with laboratory construction indicated that windows below a 7-foot level are a liability in an engineering building, as they interfere with the use of walls for the support of apparatus.

In a building as large as this the routing of material, utilities, and foot traffic assumes great importance. The personnel enter the building through two small front entrances.





(*Above*) Small Laboratory (*Center*) Large Laboratory
(*Below*) High Bay Engineering Laboratory



(*Above*) Cross Section of Engineering Laboratory; (*below*) Floor Plan

Shower and locker rooms are adjacent to the entrances. Within the building the movement of personnel is channelized by two well defined longitudinal aisles. Samples, small packages, and drums are received at a door in the center, which is not ordinarily used by foot traffic. Heavy trucks and bulky pieces of equipment can be brought in through a large door in the rear.

Steam, compressed air, water, and electrical services enter the building and pass directly across it by means of an underfloor pipe trench. Branches rise along the line of the main columns to a utility rack running the length of the building on each side of the main engineering bay. Laterals to the various laboratories and to the large chemical engineering units run overhead. All large electrical power loads are fed from a Flexa power system located along the utility rack.

All lighting loads, laboratory benches, and small units are fed from small distributor panels spaced periodically on columns, which also serve to locate safety equipment, such as gas masks, fire blankets, fire extinguishers, heating and vent controls, telephones, and other general purpose equipment.

Tall chemical engineering equipment is erected in the central bay. Floor drain outlets are provided at frequent intervals. Flue outlets are located along the utility racks. The flues connect to two vertical stacks which normally provide draft. A motor-driven blower forces a powerful jet of air through a throat in the stack if it is desired to augment the draft. This eliminates the corrosion and explosion hazard difficulties encountered where the stack gases pass through the blower, and avoids back pressure when the blower is not in use.

As bubble towers and similar engineering equipment may rise 30 feet from the floor line, catwalks have been provided along each side of the bay at the 15 and 22 foot levels. These catwalks connect by platforms and stairs with the various units, provide secure operating platforms, and also provide for escape of the personnel in case of an emergency. Along each side of the chemical engineering bay space has been provided at the rear for small-scale operations which can be carried out in normal head room.

The main aisles also serve the laboratories. Laboratory doors have always been a problem. Doors provide means for isolating areas in the event of fire or when toxic gases are being handled, but are otherwise best left open. A happy solution has been reached by providing double doors which fold back against the wall in the aisle and are held in this position by fusible links. The doors may be released at any time and are small enough to avoid obstructing the aisles.

The chemical laboratories are of a conventional design, a buff glazed tile being employed for the walls to reduce maintenance expense. Metal furniture was chosen, since wooden furniture sometimes gives trouble in this climate, and metal drawers are better able to carry the heavy loads imposed upon them in an engineering laboratory. Alberene stone has been found ideal in petroleum work for table tops; it can be cut, patched, and refinished as required with a minimum of loss.

Fireproof construction has been used throughout, an explosion venting type of window has been specified, and provision has been made for fog-type automatic sprinklers in hazardous areas.

Considerable experience is required before an accurate appraisal of the value of a new structure can be made. However, a building of the present design seems ideally suited to the needs of a rapidly growing chemical engineering organization. The structure is expected to show a low maintenance cost and make production efficiency possible without departing from the high standards of safety requirements which are common in the petroleum industry.

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Factors to Be Considered in Quantitative Polarography

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IN A RECENT monograph (*3*) the field of voltammetry (polarography) and of amperometric titrations was comprehensively reviewed. Since the literature was covered in this monograph until the end of 1940, the author has not endeavored to give a condensed review of the entire subject here, but proposes to concentrate on those factors which have to be considered in quantitative (and sometimes qualitative) polarography. Some of these factors are often overlooked in the literature. This may not be of any consequence in a particular study, but neglect of these factors may bring polarography into discredit as a general analytical method. Some of the factors have been discussed at length by Kolthoff and Lingane (*3*), and these will only be summarized in the present paper, which will concentrate on those factors that are of primary importance when dealing with mixtures of electroreducible substances.

Factors of Primary Importance

1. The diffusion current measured is an apparent diffusion current, i_{d_a}. In order to find the true diffusion current, i_d, which is proportional to the concentration, C, the residual current of the medium, i_r, at the potential where the apparent diffusion current is measured must be subtracted from i_{d_a}:

$$i_d = i_{d_a} - i_r = kC \qquad (1)$$

The residual current in a medium free of oxygen and other reducible substances should be zero at a potential of −0.55 volt (against S. C. E.). It increases with increasing negative potential, but should never be larger than a few tenths of a microampere. When the concentration of the electroreduced substance is greater than about 0.001 N, i_d becomes very large with respect to i_r, and the latter can be neglected from a practical viewpoint. However, the correction becomes increasingly more important the smaller the concentration of the reducible substance and the more negative its reduction potential. The most exact way of finding the residual current is by determination of the current-voltage curve of the supporting electrolyte in the absence of the reducible substance(s). The residual current can be found with a fair degree of approximation by extrapolating the part of the current voltage curve before the wave starts to the potential at which the apparent diffusion current is read.

2. Quite generally, oxygen should be removed from the solution before electrolysis is started. This can be done by passing nitrogen or hydrogen through the solution in the cell for 10 to 20 minutes. In neutral or alkaline medium oxygen is completely removed by the addition of some solid sodium sulfite. Oxygen yields two waves. The first, corresponding to a reduction to hydrogen peroxide, is fairly steep and has a half-wave potential of about +0.15 volt *vs.* the S. C. E. The second wave, corresponding to a reduction of peroxide to hydroxyl ions, is very flat, and extends over a potential range between −0.6 and −1.2 volt, the half-wave potential being −0.94 volt. Since a solution which is saturated with air is about 0.001 N in oxygen, it is evident that oxygen waves will interfere with the determination of the diffusion current of another reducible substance. This interference becomes very pronounced at low concentrations of the substance being determined.

3. The factors which determine the diffusion current are given by the fundamental Ilkovic (*1*) equation:

$$i_d = 605nD^{1/2}Cm^{2/3}t^{1/6} \qquad (2)$$

in which i_d is the diffusion current in microamperes, n is the number of faradays of electricity required per molar unit of the electrode reaction, D is the diffusion coefficient of the reducible (or oxidizable) substance in cm.^{2}/sec.$^{-1}$, C is its concentration in millimoles per liter, m is the weight of mercury in milligrams flowing out of the capillary per second, and t is the drop time in seconds. Experimentally, the Ilkovic expression has been found to hold when the drop time is greater than 3 seconds.

4. In order to get reproducible results with the same capillary and the same solution the pressure on the dropping mercury should be kept constant. From the Ilkovic equation and Poiseuille expression it follows that

$$i_d = k'\sqrt{h}$$

where h is the height of the mercury column above the tip of the dropping electrode.

5. With all other factors constant Equation 1 follows from Equation 2. From a practical viewpoint it is of importance to know how the value of k in Equation 1 is affected by a change of the concentration of the indifferent electrolyte in the supporting medium. The relative change of k is equal to the relative change of the square root of the diffusion coefficient of the reducible (or oxidizable) substance. It is the author's experience that, in general, the concentration of the indifferent electrolyte can be varied between about 0.02 and 0.5 M without affecting the value of k, provided that the indifferent electrolyte does not form a complex with the reducible substance or react with it in some other chemical way. When an aquo

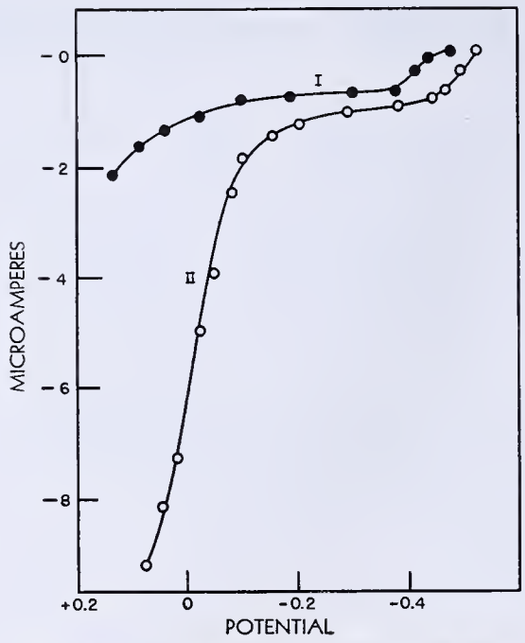

FIGURE 1. CURRENT-VOLTAGE CURVES OF CYSTEINE IN A BUFFER SOLUTION WITH A pH OF 6.0

I. 2.5×10^{-4} M cysteine (true diffusion current)
II. 2.0×10^{-3} M cysteine (false diffusion current)

TABLE I. VALUE OF $k = i_d/c$

(Obtained by E. F. Orlemann in electrolysis of lead nitrate in potassium nitrate solutions)

Concentration of $Pb(NO_3)_2$ M	Value of k In 0.1 N KNO_3	In 1 N KNO_3	In 0.1 N KCl
1×10^{-3}	7.02	7.00	7.10
5×10^{-3}	7.32	7.02	7.42
1×10^{-2}	7.60	7.06	..

ion is transformed into a complex ion, the diffusion coefficient (and therefore k) is affected. Metal ion complexes with tartrate or nitrate, for example, have a smaller D than the aquo ions. On the other hand, some ammino ions have a larger D than the corresponding aquo ions.

6. The value of k (D) is considerably affected by a change of the solvent. In water-alcohol mixtures k is much smaller than in water.

7. The diffusion currents of most substances increase by 1.3 to 2.0 per cent per degree increase in temperature, owing chiefly to the increase in the diffusion coefficient with increasing temperature.

8. In order to find the true diffusion current the migration current has to be eliminated. This is done by making the concentration of the indifferent electrolyte about 50 times greater than that of the reduced ion. If the migration current is not eliminated, there is no longer proportionality between i_d and the concentration; in other words, Expression 1 no longer holds. The complete elimination of the migration current may be easily overlooked when the concentration of the reduced ion becomes relatively large. As a demonstration of the effect, some results obtained in the electrolysis of lead nitrate in solutions of potassium nitrate or chloride are given.

If Equation 1 is valid, the value of k in a particular supporting electrolyte should be constant and independent of the lead concentration. From Table I it is seen that the value of k obtained with a 0.001 M solution of lead in 0.1 N potassium nitrate is the same as in 1 N nitrate (see 5 above). Even in the 0.1 N nitrate solution the migration current was eliminated when the lead concentration was 0.001 M. However, the value of k was found to increase markedly in the 0.1 N nitrate solution when the lead concentration was increased. This is due to an incomplete elimination of the migration current. On the other hand, the value of k was found to be independent of the lead concentration in the 1 N nitrate solution, since the concentration of the indifferent salt was large enough to eliminate the migration current even in the 0.01 M lead solution.

9. When the electrolysis product forms an insoluble film at the surface of the mercury drop, the film may have a large resistance and interfere badly with further electrolysis. In such instances a false diffusion current may be obtained which is no longer proportional to the concentration, and may even become independent of the concentration. A false diffusion current was found in the determination of anodic waves of cysteine (*2*). This substance can be determined polarographically by measuring the anodic diffusion current in 0.1 N perchloric acid. When the pH becomes greater than about 2, a film of mercurous cysteinate is formed at the electrode, which interferes with the further electrolysis. This is demonstrated by the current voltage curves in Figure 1, which were obtained in a buffer solution with a pH of 6.0. At cysteine concentrations smaller than about 3×10^{-4} M a true diffusion current is found. At higher concentrations a false diffusion current is found which is independent of the cysteine concentration. It was not possible to eliminate the film by the addition, for example, of a little gelatin.

In Figure 2 are shown anodic current voltage curves obtained with solutions of 0.002 N bromide in 0.1 N potassium nitrate (*6*); curve I refers to the solution in the absence of gelatin, curve II to the same in the presence of 0.01 per cent of gelatin. The anodic wave starts out in a normal way. However, when the current has become equal to about 4 microamperes a film of mercurous bromide interferes with the further electrolysis. The current increases only slightly as the potential is made more positive, until it becomes equal to the diffusion current at a potential of about +0.3 volt.

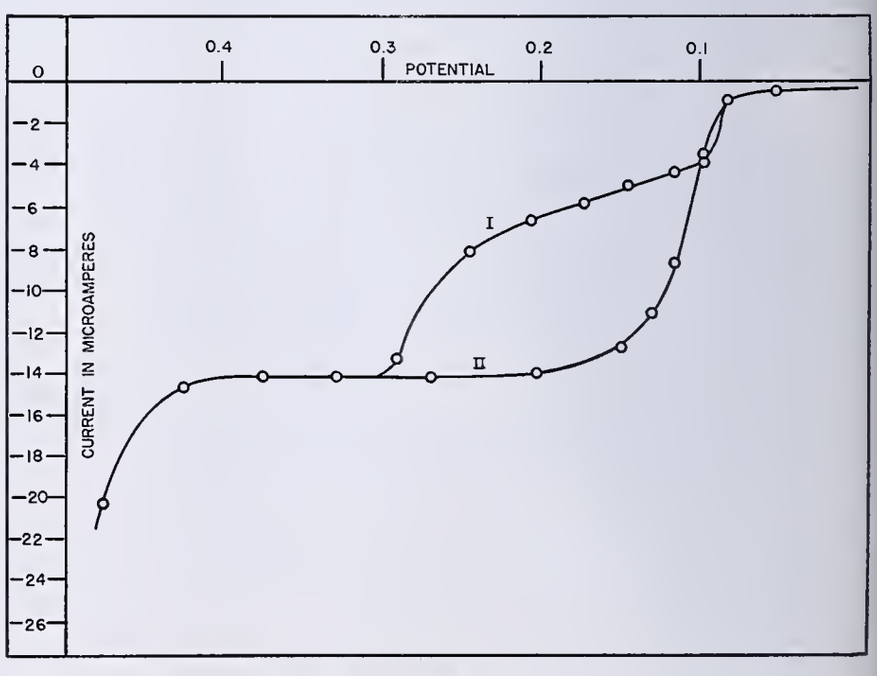

FIGURE 2. CURRENT-VOLTAGE CURVES OF 2×10^{-3} N BROMIDE IN 0.1 N POTASSIUM NITRATE

I. No gelatin added
II. In presence of 0.01 per cent gelatin

The addition of a trace of gelatin eliminates the interference by the film, as is evident from curve II.

10. The importance of a proper reference electrode is not generally appreciated. Heyrovský recommended the use of a pool of pure mercury in the cell as an internal reference electrode. In general, we (*3*) prefer an outside standard half-cell, ordinarily the saturated calomel electrode. Connection with the electrolysis cell is made by means of an agar salt bridge. This involves an extra resistance which has to be considered when the waves are analyzed for the relation between current and potential. Aside from this slight disadvantage, the use of an outside reference electrode is preferable to the internal pool of mercury for the following reasons:

The potential of the pool of mercury is often poorly defined. In the presence of depolarizers, like halides, and soluble hydroxides, the mercury adopts a potential close to that of the half-cell saturated with the particular mercurous halide or mercuric hydroxide. Relatively small amounts of oxygen in the solution are sufficient to oxidize enough mercury to yield the insoluble compound. In the presence of depolarizers which form soluble complexes or slightly dissociated compounds with mercury like cyanide and sulfide, the potential of the mercury will vary, dependent upon the amount of interaction between mercury and oxygen. When the indifferent electrolyte has no depolarizing effect the potential of the mercury varies and is of the order of +0.4 volt (*vs.* S. C. E.). The potential of the mercury referred to the S. C. E. has, roughly, the following values in different solutions: 0.1 *N* Cl^-, +0.09; 1 *N* Cl^-, +0.04; 0.1 *N* NaOH, −0.08; 1 *N* NaOH, −0.11; 0.1 *N* I^-, −0.3; 0.1 *N* CN^- (in NaOH), −0.6; 0.1 *N* sulfide, −0.85 volt. When half-wave potentials are measured it is necessary to determine the potential of the mercury pool separately. This is not necessary when a depolarized external half-cell is used.

When solution contains constituents which oxidize mercury—e. g., ferric chloride or vanadic acid—the internal mercury pool cannot be used. The diffusion current of these oxidizing agents can be found at the dropping electrode if the drops are collected in a suitable container (*1*) and prevented from interacting with the oxidant in the solution. When the solution contains constituents which form soluble stable complexes or slightly dissociated compounds with mercury—e. g., cyanide and iodide—the speed of interaction between mercury and oxygen is greatly enhanced. Thus, when the pool of mercury is introduced into the cell containing the solution to be electrolyzed, appreciable amounts of mercury (and usually of hydrogen peroxide) may be introduced into the solution when the mercury is left in contact with the solution before the oxygen is removed. This is well demonstrated (*9*) in Figure 3. The rate of interaction between mercury and oxygen can be studied conveniently by the polarographic method and in the case of interaction between mercury, oxygen, and cyanide, equivalent amounts of mercuric cyanide and hydrogen peroxide are formed:

$$Hg + O_2 + 2CN^- + 2H_2O \longrightarrow Hg(CN)_2 + H_2O_2 + 2OH^-$$

The first wave of the current voltage curve shows the reduction of mercury in the cyanide solution and the second flat wave that of hydrogen peroxide. The peroxide wave is very prominent in Curve VI of Figure 3. After the indicated times of contact, the oxygen was removed from the solution and then the current-voltage curves were determined.

In the interaction between mercury, oxygen, and iodide, only a mercury wave was found, since the peroxide reacts with the iodide to form iodine which in turn oxidizes mercury.

When a mercury pool is used as an anode in solutions containing cyanide, it is commendable to remove the oxygen from the solution before the mercury is added to the cell.

In connection with the above discussion a statement may be made regarding the addition of a "pilot" ion in cases where a mercury pool electrode is used. The pilot ion should be such that its half-wave potential is unaffected by the medium. Thallous thallium satisfies this requirement, its half-wave potential (−0.46 volt *vs.* S. C. E.) being the same in solutions containing hydroxide, tartrate, etc., as in solutions of ordinary indifferent electrolytes. Thus, upon addition of a small (but unknown) amount of thallous salt, a corresponding thallium wave is found and its known half-wave potential serves as a standard value on the potential axis. However, this method does not work, for example, when the solution contains cyanide. The anodic cyanide (0.1 *N*) wave starts at a potential of −0.58 volt and no cathodic wave can appear at potentials more positive than −0.58 volt. For example (*4*), in the electrolysis of a mixture which was 0.001 *N* in thallium, 0.1 *N* in sodium hydroxide, and 0.1 *N* in cyanide, an "apparent" thallium wave was obtained with a half-wave potential of −0.58 volt.

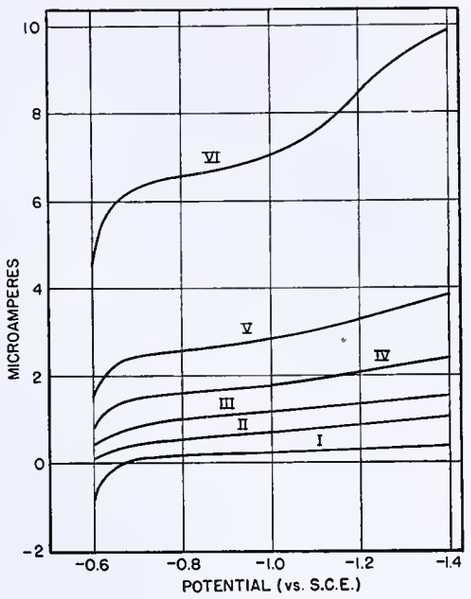

FIGURE 3. INTERACTION BETWEEN MERCURY AND AN AIR-SATURATED SOLUTION

Solution 0.1 *N* in potassium cyanide and 0.1 *N* in sodium hydroxide
I. Immediately air free
II. 10 minutes' contact
III. 30 minutes' contact
IV. 90 minutes' contact
V. 5 minutes' contact with stirring
VI. 30 minutes' contact with stirring

Mixtures of Reducible Substances

The following factors are of special importance in dealing with mixtures of reducible substances:

11. In the first place we briefly consider maxima on current-voltage curves. When, after the maximum is reached, the potential is made more negative the current decreases more or less rapidly, finally becoming equal to the diffusion current. When dealing with one reducible substance or with two reducible substances whose waves are widely separated it is not necessary to eliminate the maximum, as a potential range is easily found at which the diffusion current can be measured. However, when the waves of the two reducible substances are fairly close together it is necessary to eliminate the maximum of the first wave. If the maximum is not eliminated the diffusion current of the constituent which is reduced first may not be attained before the wave of the second constituent starts. In a mixture—e. g., of 0.001 *M* lead and cadmium ions in 0.1 *N* potassium chloride—a "false" diffusion current of lead is measured. This current is much greater than the true diffusion current which is found when the maximum is eliminated (*3*). Maxima are generally eliminated by the addition of small amounts of capillary-active substances, such as dyes, camphor, naphthalene, thymol, and especially gelatin and tylose.

12. When dealing with mixtures, the reaction product formed in the electrode reaction of the most easily reduced constituent may react with the second constituent; also, the reaction product of the second constituent may react chemically with the more easily reduced constituent. In the former case the diffusion current of the second constituent will be reduced by an amount which corresponds to the concentration of the second constituent which has been removed at the electrode. In the latter case the apparent diffusion current of the

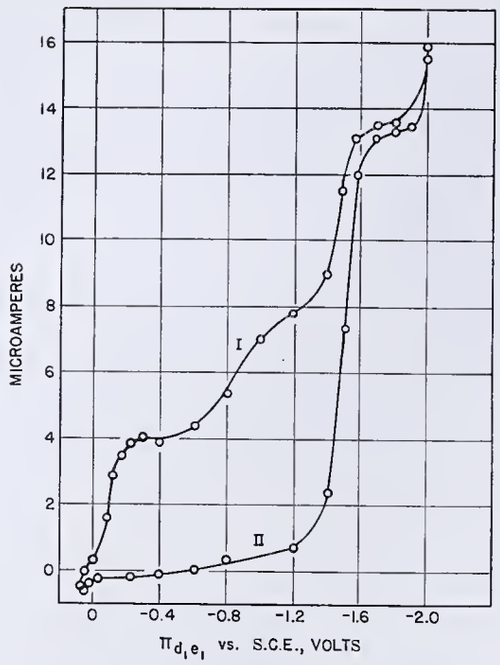

FIGURE 4. DECREASE OF DIFFUSION CURRENT OF HYDROGEN IONS BY HYDROXYL IONS PRODUCED IN REDUCTION OF OXYGEN

I. After saturating solution with air
II. Hydrogen wave from air-free 0.001 *N* HCl containing 0.1 *N* KCl and a trace of methyl red

second constituent also will be decreased, because chemical interaction does not take place until the second constituent is being reduced. The total diffusion current measured after reduction of the second constituent is equal to the sum of the first and the second, and from the polarogram it is impossible to decide whether the first or second diffusion current has been reduced.

An instructive example of interaction is found with a mixture of oxygen and very dilute hydrochloric acid in an excess of potassium chloride (*5*). The current-voltage curves reproduced in Figure 4 were obtained in the presence of a trace of methyl red to suppress oxygen maxima. Curve 2 is the current-voltage curve of an air-free, 0.001 *N* hydrochloric acid solution in 0.1 *N* potassium chloride. Curve 1 was obtained after saturating the solution with air. The two oxygen waves correspond to the reductions:

$$O_2 + 2H_2O + 2e \longrightarrow H_2O_2 + 2OH^-$$
$$O_2 + 2H_2O + 4e \longrightarrow 4OH^-$$

It is seen that in the presence of oxygen the diffusion current of the hydrogen discharge is greatly decreased and becomes equal to its original value minus the total diffusion current of oxygen. If the original diffusion current of the hydrogen ions had been smaller than the total one of oxygen, no hydrogen wave would have been found in the air-saturated solution. We make use of this decrease of the diffusion current of hydrogen ions to prove the formation of hydroxyl ions in some other electrode reactions.

The formation of hydroxyl ions in some electrode reduction is of importance when the solution contains metal ions which form slightly soluble hydroxides. Great interference by oxygen is encountered, for example, in the analysis of neutral air-saturated solutions containing copper, lead, cadmium, zinc, etc. The interference is eliminated when the electrolysis is carried out in weakly acid medium (say a suitable buffer) or in the presence of a complex former (ammonia, or tartrate) which dissolves the hydroxide formed. However, the author prefers to remove oxygen from the solution before the electrolysis is started (see 2 above).

An interesting example of interference by the formation of hydroxyl ions at the electrode is met with in the polarographic analysis of neutral mixtures of iodate or bromate and heavy metals.

The reduction of iodate or bromate occurs according to the net reactions:

$$IO_3^- + 6H^+ + 6e \longrightarrow I^- + 3H_2O$$
$$BrO_3^- + 6H^+ + 6e \longrightarrow Br^- + 3H_2O$$

Lingane (*8*) found that the diffusion current of iodate was apparently decreased, and under certain conditions almost eliminated by the preceding discharge of cadmium. In reality, however, it is the cadmium diffusion current which is decreased through the formation of cadmium hydroxide during the reduction of iodate. The cadmium is reduced at a more positive potential than the iodate. Hence the cadmium wave is unaffected by the iodate, but the diffusion current of the cadmium is decreased during the discharge of iodate. That the "apparent" decrease of the iodate current in the presence of cadmium is actually due to the formation of cadmium hydroxide at the surface of the mercury drop was proved by Orlemann (*10*). By means of a special microscopic arrangement he was able to observe a film (of cadmium hydroxide) on the surface of the mercury drop when a mixture of 0.001 *M* cadmium sulfate and 0.001 *M* potassium iodate was electrolyzed in a neutral unbuffered 0.1 *M* potassium chloride solution. The film did not appear until the iodate was also reduced.

To study the hydroxyl-ion effect from a quantitative viewpoint Orlemann (*10*) electrolyzed mixtures of cupric copper and iodate in neutral and ammoniacal solutions and in acid buffers. The results obtained are presented in Table II. All currents have been corrected for the residual current. The diffusion current of copper ($i_{d_{Cu}}$) was measured at a potential of −0.8 volt, the total diffusion current—(i. e., the sum of the diffusion currents of copper and iodate)—at a potential of −1.5 volts. In order to find the apparent diffusion current of iodate, the diffusion current of copper has to be subtracted from the total diffusion current. However, the diffusion current of copper is smaller at a potential of −1.5 volts than at −0.8 volt where it had been measured. This change is mainly due to a decrease of the drop time, as explained in section 13. The value of the diffusion current of copper at a potential of −1.5 volts has been calculated from the value measured at −0.8 volt in the way described in section 13. From Table II it is seen that the correct value of the diffusion current of iodate in the presence of copper is found in ammoniacal solution or in the buffer with a pH of 5. However, in neutral medium the apparent decrease of the iodate wave is approximately equal to the diffusion current of copper. Actually, the iodate reduction takes place to the same extent as in the absence of copper, but the hydroxyl ions produced in the reduction of iodate precipitate the cupric copper and prevent it from being reduced. If the iodate concentration in the mixture had been such that its diffusion current, when present alone, would have been less than that of the copper, no (apparent) iodate wave would be found at all. Actually, at potentials where iodate alone gives a wave a reduction of iodate occurs in the mixture, but the corresponding current would be equal to the decrease of the copper current.

The results indicate clearly that current-voltage curves obtained in mixtures of iodate or bromate or other substances, which upon reduction yield hydroxyl ions, and metals forming insoluble hydroxides, yield unambiguous results only when a medium is used in which the metal hydroxides are soluble.

13. The factors which determine a diffusion current are given by the Ilkovic expression (Equation 2, section 3).

TABLE II. DIFFUSION CURRENTS

[Obtained in mixtures of copper and iodate in various media at 25.0°. Concentration of iodate 5×10^{-4} *M* ($= 3 \times 10^{-3}$ *N*)].

Medium[a]	Concentration Cu × 10^3 *M*	$i_{d_{Cu}}$ at −0.8 Volt	$i_{d_{IO_3}}$ Apparent at −1.5 Volts	i_{total} at −1.5 Volts	Error $i_{d_{IO_3}}$ %
Neutral	0	0	11.2	11.2	0
Neutral	1.00	6.63	4.85	11.2	−57
0.5 *M* NH_3	1.00	7.80	11.2	18.7	0
Buffer, pH = 5.0	0.90	6.02	11.3	17.0	+1

[a] All solutions were 0.2 *M* in potassium nitrate.

Table III. Effect of Potential on i_d. I_d and $i_d/m^{2/3}t^{1/6}$ in Electrolysis of Cupric Copper

Potential (*vs.* S. C. E.)	−0.3	−0.5	−1.00	−1.30	−1.50	−1.60	−1.70
i_d, microamperes	6.12	6.14	6.10	5.98	5.88	5.84	5.70
$i_d/m^{2/3}t^{1/6}$	6.13	6.14	6.16	6.16	6.15	6.18	6.13

Table IV. Relative Values of $m^{2/3}t^{1/6}$ at Different Potentials

(Value at −0.5 volt is taken equal to 1)

π	0	−0.2	−0.5	−1.0	−1.1	−1.3	−1.5	−1.6	−1.7	−1.8	−1.9
Relative value (*r. v.*)	0.985	0.99	1.00	0.985	0.98	0.97	0.95	0.94	0.93	0.92	0.91

For our present purpose it is of interest to consider the change of the diffusion current of a given solution obtained with a capillary of known characteristics (m and t in Equation 2) with the applied potential.

The mass of mercury, m, flowing out per second is hardly affected by the applied potential. On the other hand, the drop time, t, which is roughly proportional to the surface tension of the mercury, is greatly dependent upon the potential. It has a maximum value at a potential of −0.55 volt (*vs.* S. C. E.), the so-called electrocapillary zero point. Its value decreases when the potential is made more positive or negative. For example, when the potential is zero, one may find a drop time—e. g., of 2.75 seconds—at −0.5 volt 3.10, at −1 volt 2.7, at −1.5 volts 2.2, and at −1.9 volts 1.6. Fortunately, the diffusion current is not proportional to t but to $t^{1/6}$. The result is that between potentials of zero and 1 volt, i_d changes only very slightly with the potential. However, the effect becomes more marked when the potential is made increasingly more negative than 1 volt. According to the Ilkovic equation i_d should change with the potential, but $i_d/m^{2/3}t^{1/6}$ should remain constant. This was verified again recently by Kolthoff and Orlemann (*7*), who measured the diffusion current of cupric copper at different potentials. From the known values of m and t of the capillary used they calculated the ratio $i_d/m^{2/3}t^{1/6}$. Table III shows that this ratio is constant within the experimental error.

Let us now consider a mixture of two reducible substances. In the proper supporting electrolyte a current-voltage curve is obtained as shown diagrammatically in Figure 5. The diffusion current, i_{d_1}, of substance 1 is measured at a potential π_1, while the total current i_t is measured at potential π_2 (all corrected for the residual current). The diffusion current, i_{d_2}, of substance 2 then is equal to the total current minus the diffusion current which substance 1 would have at a potential π_2. From the above, it is evident that the diffusion current of substance 1 at a potential π_2 [denoted as $(i_{d1})_{\pi 2}$] is:

$$(i_{d1})_{\pi_2} = (i_{d1})_{\pi_1} \frac{(m^{2/3}t^{1/6})_{\pi_2}}{(m^{2/3}t^{1/6})_{\pi_1}} \qquad (3)$$

and

$$i_{d_2} = i_t - (i_{d1})_{\pi_1} \frac{(m^{2/3}t^{1/6})_{\pi_2}}{(m^{2/3}t^{1/6})_{\pi_1}} \qquad (4)$$

Kolthoff and Orlemann (*7*) compared the relative value of $m^{2/3}t^{1/6}$ at different potentials for four different capillaries which had quite different characteristics. They found that the relative value was virtually independent of the characteristics of the capillary. The data given in Table IV allow the calculation of the change of i_d with the potential without determining the characteristics of the capillary. From Table IV it is seen that if the diffusion current of a substance measured at a potential of −0.5 volt is 10 microamperes it is equal to 9.4 at a potential of −1.6 volts and 9.1 at a potential of −1.9 volts.

Denoting the relative value at a certain potential π_1 by $(r.\ v.)_{\pi_1}$ Equation 4 can be rewritten as follows:

$$i_{d_2} = i_t - (i_{d1})_{\pi_1} \frac{(r.\ v.)_{\pi_2}}{(r.\ v.)_{\pi_1}} \qquad (5)$$

To illustrate the significance of Equation 5 some figures obtained in the polarographic analysis of a mixture which was 0.001 M in copper, 0.001 M in manganese, and 0.1 N in potassium chloride are given.

The diffusion current of copper measured at π = −0.5 volt was 6.19 microamperes. The diffusion current of manganese in the absence of copper at a potential of −1.7 volts was 6.00 microamperes. In the mixture a total current of 11.85 microamperes was measured at π = −1.7 volts. If we did not correct for the $m^{2/3}t^{1/6}$ effect we would find a value for the diffusion current of manganese in the mixture of 11.85 − 6.19 = 5.66, corresponding to an error of −5.6 per cent. However, if the value of i_d of copper at π = −1.7 volts is calculated with the aid of Equation 5 and Table IV we find for i_d of manganese: 11.85 − 5.75 = 6.10, corresponding to an error of +1.7 per cent. The larger the ratio of copper to manganese, the greater becomes the error if the $m^{2/3}t^{1/6}$ effect is not considered. For example, in a mixture which was about 0.002 M in copper and 0.001 M in manganese the following figures were found: i_d of copper at π of −0.5 volt = 12.76 microamperes; i_t at π of −1.7 volts = 18.00. Uncorrected for the $m^{2/3}t^{1/6}$ effect this would yield i_d of manganese = 18.00 − 12.76 = 5.24 microamperes, or an error of −12 per cent. However, if corrected for the effect we find i_d of manganese = 6.10 or an error of +1.7 per cent. It is not possible to eliminate the discussed effect by any of the empirical procedures which have been proposed in the literature for finding diffusion currents or the relation between i_d and the concentration.

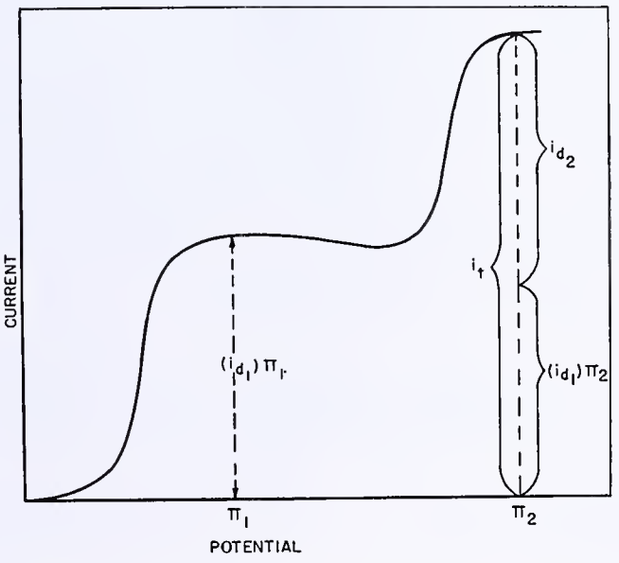

Figure 5. Schematic Picture of Current-Voltage Curve of a Mixture of Two Reducible Substances

14. Recently (*11*) the discovery has been made that an anomalous "wave" occurs in solutions with a relatively high concentration of indifferent electrolyte, approximately 0.5 M or larger, when a current due to the reduction of some constituent passes through the solution. For example, when a thallium solution is electrolyzed in 1 to 4 N potassium chloride we first obtain the diffusion current of thallium. At a potential of about −0.9 volt a new wave starts which is flat and reaches a maximum at −1.35 volts. When the potential is made more negative the decrease in current is much greater than corresponds to the decrease of $m^{2/3}t^{1/6}$. However, before it becomes equal to the diffusion current of thallium, the discharge of potassium sets in. The residual current in 1 to 4 N solutions of potassium chloride or other indifferent electrolytes does not show any irregularities, and is of the same order of magnitude as it is in 0.1 N solutions. The

anomalous wave is due to a discharge of water under the specified conditions:

$$H_2O + e \longrightarrow H + OH^-$$

The water current at a potential of −1.35 to −1.4 volts is proportional to the total current flowing. Suppose, for example, that under certain conditions the thallium wave is equal to 5 microamperes and the water wave to 4. When the thallium concentration is doubled, the water wave becomes equal to 8.

In addition to being dependent upon the total current, the concentration and kind of indifferent electrolyte present, the water wave depends greatly upon the characteristics of the capillary. The water current decreases markedly with increasing drop time.

From the analytical viewpoint the "water wave" can be the cause of serious errors in the interpretation of current-voltage curves, especially in the determination of constituents, the waves of which yield diffusion currents at potentials more negative than about 1 volt. Under such conditions only one wave is found, since the water wave overlaps with the wave of the reduced constituent. Consequently, the diffusion current measured is not equal to that of the reduced constituent, but is equal to the sum of the latter and the water wave. For example, in the electroreduction of zinc, iodate, or bromate a marked increase in the "apparent" diffusion current is found with increasing concentration of potassium chloride, if the concentration of the chloride is made greater than 0.5 *N*. Actually, the true diffusion current of the above species is hardly affected by the concentration of potassium chloride in solution. For example, in the electrolysis of a 5×10^{-4} *M* potassium iodate solution in 1 *N* potassium chloride using a capillary with a drop time of 3.60 seconds, Orlemann and Kolthoff (*11*) found an "apparent" diffusion current of 14.20 microamperes at −1.45 volts, whereas the true diffusion current was only 11.30. When the drop time was increased, the relative difference between the apparent and the true diffusion currents became much greater.

In quantitative polarographic work solutions which contain a high concentration (1 to 2 *N*) of indifferent electrolyte—(e. g., in steel analysis)—are often electrolyzed. When only one reducible constituent is present it is not essential to eliminate the water wave because a calibration line with known concentrations of the constituent can be obtained in the particular medium. However, when the solution contains a mixture of reducible substances, it is not easy to eliminate the effect of the water current upon the results by a set of calibrations, because the water current is proportional to the total current. In the polarographic analysis of a mixture in a medium of high salt concentration it becomes essential to eliminate the water wave entirely. This can be done by the addition of 0.01 per cent of gelatin to the solution. Other capillary-active substances, which are often used as maximum suppressors, also affect the water wave; they do not eliminate it completely as gelatin does, but shift the wave to more negative potentials. Next to gelatin the most effective of the capillary active substances investigated is tylose, which shifts the wave to a potential of −1.6 volts.

In order to avoid any complications by the water wave in work with the dropping electrode, the author recommends that 0.01 per cent of gelatin be added to solutions, the salt concentration of which is larger than 0.5 *N*.

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Determination of Mixed Aniline Points of Hydrocarbon Solvents

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DURING the past few years, considerable attention has been given to methods of determining the solvency characteristics of paint, varnish, and lacquer thinners. While the viscosity method represents an ultimate means of determining the relative value of various thinners, a more simple test, such as aniline point, probably will continue to be used for controlling the uniformity of a given thinner.

With the development of petroleum naphthas of substantial aromatic content, it has been necessary to use the mixed aniline point test. The mixed aniline point has been defined as the minimum miscibility temperature in degrees centigrade of a mixture of 10 cc. of anhydrous aniline, 5 cc. of the thinner under test and 5 cc. "of any naphtha whose aniline point is 60° C." (*3*). McArdle (*1*, *4*) states that the nonaromatic diluent shall be "a mineral spirit (regardless of boiling point)" having a 60° C. aniline point. Experiments described herein indicate the necessity for defining the diluent more closely.

The mixed aniline points, as defined above, have been determined on a number of experimental high-solvency naphthas of varying aromatic content. Two sets of tests were made, one using A. S. T. M. precipitation naphtha as the diluent and the other using mineral spirits. The inspection tests of the two diluent naphthas are shown in Table I.

Figure 1 shows the relationship obtained between the two sets of tests after classifying the high-solvency naphthas under

Table I. Inspection Tests

	A. S. T. M. Precipitation Naphtha	Mineral Spirits
Gravity, °A. P. I.	71.9	48.9
A. S. T. M. distillation D-86		
Initial b. p., ° F.	140	305
10% recovery, ° F.	150	323
50% recovery, ° F.	164	345
90% recovery, ° F.	195	388
End point, ° F.	234	423
Aniline point, ° C.	59.6	59.7

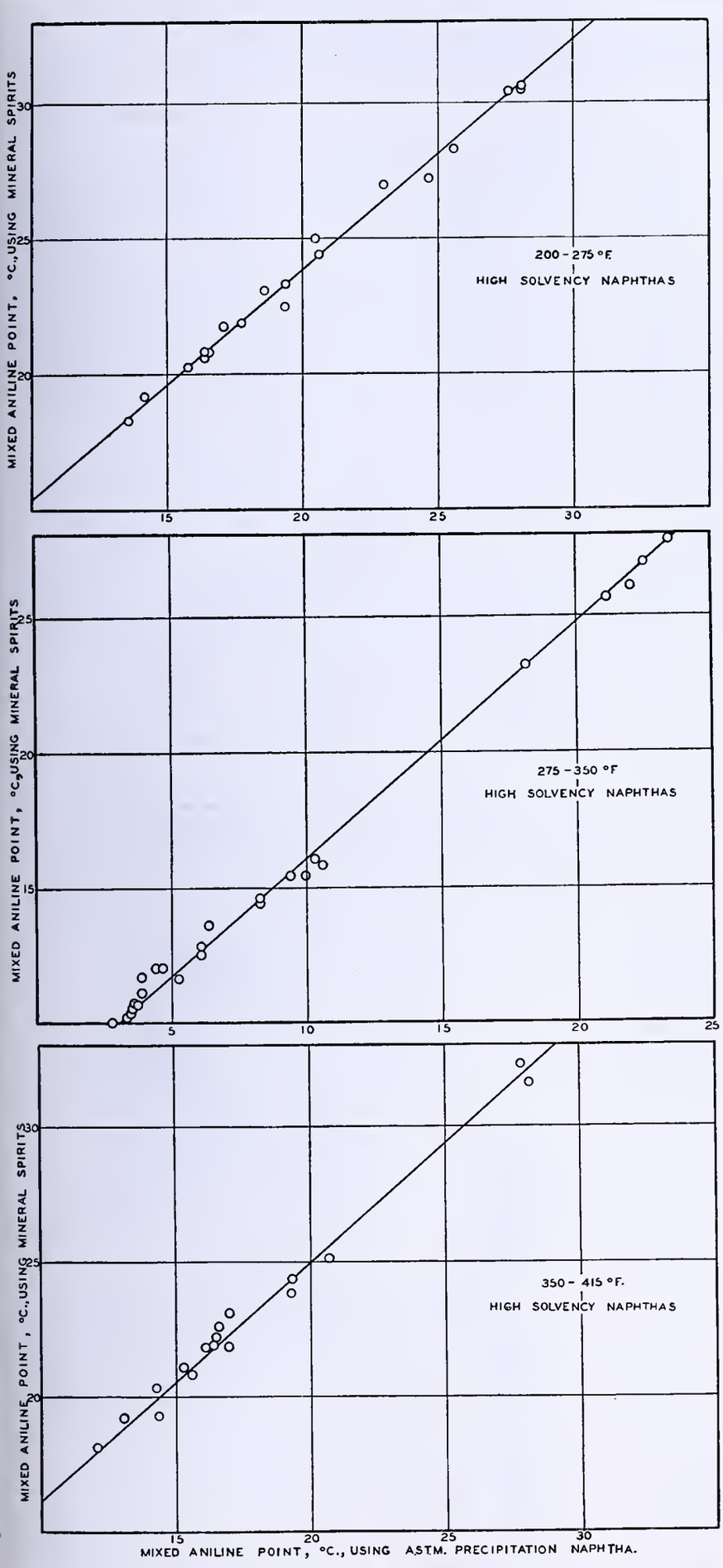
MIXED ANILINE POINT, °C., USING MINERAL SPIRITS
200 - 275 °F
HIGH SOLVENCY NAPHTHAS
275 - 350 °F
HIGH SOLVENCY NAPHTHAS
350 - 415 °F.
HIGH SOLVENCY NAPHTHAS
MIXED ANILINE POINT, °C., USING ASTM. PRECIPITATION NAPHTHA.

FIGURE 1. EFFECT OF DILUENT ON MIXED ANILINE POINTS OF HIGH-SOLVENCY NAPHTHAS

consideration into three boiling point ranges—200° to 275°, 275° to 350°, and 350° to 415° F., respectively. It will be noted that the mixed aniline points determined with A. S. T. M. precipitation naphtha are appreciably lower than when the mineral spirits are employed. These differences are not constant, but vary with the concentration of aromatics and with the boiling range of the solvent naphthas as shown by the following data taken from the curves:

Mixed aniline point with mineral spirits	10° C.	15° C.	20° C.
Mixed aniline point (° C.) with precip. naph.			
For 200–275° F. naphthas	..	9.5	15.5
For 275–350° F. naphthas	3	8.7	14.5
For 350–415° F. naphthas	..	9	14.4

Mixed aniline point with mineral spirits	25° C.	30° C.
Mixed aniline point (° C.) with precip. naph.		
For 200–275° F. naphthas	21.4	27.3
For 275–350° F. naphthas	20.3	
For 350–415° F. naphthas	20	25.7

This same effect of the variation of the paraffinic diluent on the mixed aniline point is observed with C. P. toluene. Baker's C. P. toluene, having an n_D^{20} of 1.4951 shows the following mixed aniline points:

	° C.
Using A. S. T. M. precipitation naphtha	−0.9
Using mineral spirits	+7.6

From the above discussion it is evident that the paraffinic diluent used for determining mixed aniline points should be defined more rigidly than it has been in the past. It is recommended that this diluent be identified by its aniline point and by its mixed aniline point with C. P. toluene. Some users employ a diluent equivalent to mineral spirits which has a mixed aniline point with C. P. toluene of about 7.5° C. McArdle (*2*) proposes that this diluent have an aniline point of 60° C. and a mixed aniline point of 10 ± 0.5° C. with C. P. toluene. It is suggested by the authors that the manufacturers and consumers of high-solvency naphthas cooperate to define the specifications for the diluent used to determine mixed aniline points.

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PRESENTED before the Division of Petroleum Chemistry at the 102nd Meeting of the AMERICAN CHEMICAL SOCIETY, Atlantic City, N. J.

Viscosity Determination of Cotton in Dimethyl Dibenzyl Ammonium Hydroxide

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IN AN EARLIER study (*6*) it was found that over a considerable viscosity range a nearly linear relation existed between the viscosities of 0.5 per cent solutions of cotton celluloses in dimethyl dibenzyl ammonium hydroxide and in cuprammonium hydroxide, respectively. The cotton cellulose samples in various states of degradation were produced by oxidation in alkaline medium. Recently Brownsett and Clibbens (*3*) have investigated cotton celluloses degraded by a number of different oxidizing and hydrolyzing agents and find for the group no unique relationship between dimethyl dibenzyl ammonium hydroxide and cuprammonium hydroxide viscosity. However, these authors find in most instances, in comparing celluloses degraded by a given chemical treatment, that substantially linear relationships exist between viscosities in the two solvents. More important still, they conclude that the dimethyl dibenzyl ammonium hydroxide viscosity or fluidity measures the total (apparent and latent) degradation which a cellulose has suffered while the cuprammonium value gives only the apparent degradation. The two methods yield comparable information if the cellulose sample is extracted with dilute sodium hydroxide solution prior to the cuprammonium hydroxide viscosity determination. This extraction is unnecessary in the case of the dimethyl dibenzyl ammonium hydroxide, presumably because of the greater basicity of the latter.

In order to secure more information about the properties of celluloses dissolved in the newer solvent the earlier studies (*6*) have been continued and are reported in this paper.

Apparatus

VISCOMETER. Cannon and Fenske viscometers were calibrated and used as in the earlier work (*6*).

APPARATUS FOR DISSOLVING THE CELLULOSE SAMPLE. The same principle has again been used to secure rapid solution of the sample but, as is apparent from Figure 1, a glass bell has been added whose lower end is sealed by the liquid of a constant-temperature bath in which the dissolving tube is immersed. This simple addition makes it possible to dissolve the sample in contact with any desired gas. The bell is secured to the stirrer shaft by a No. 5 rubber stopper through which a small-bore glass tube carrying a stopcock is passed. Through the latter the system is flushed and the desired gas atmosphere introduced. If it is desired to exclude water vapor from the water bath an additional cup for a mercury seal, shown in dotted lines in Figure 1, may be added. The glass bell and water seal have been used in all the present work unless otherwise noted.

Materials and Method

GASES. Cylinder oxygen and nitrogen of high quality were used without further purification.

SOLVENT. The source of dimethyl dibenzyl ammonium hydroxide was the commercial product Triton F, furnished through the courtesy of D. H. Powers of Röhm & Haas. Two different lots were used. As received, the first lot had a normality of 1.96, and at 25° C. a density of 1.072 and viscosity of 8.92 centipoises. When concentrated to 2.12 N the density rose to 1.078 and the viscosity to 11.24 centipoises. The second lot as received had a normality of 2.08, and at 25° a density of 1.081 and a viscosity of 13.90 centipoises. Comparable data for dilution of the second lot to 1.96 N are density 1.078 and 10.79 centipoises, and for concentration to 2.25 N, density 1.088 and 20.14 centipoises. Because of these small differences in Triton F specifications experimental data are compared only for the same lot of solvent unless otherwise noted.

COTTON SAMPLES. Samples of cotton of known cuprammonium fluidities were obtained from several different sources. The cuprammonium viscosity or fluidity values given in this paper always refer to a 0.50 per cent solution of cellulose whose viscosity was measured at 20° C. unless otherwise specified. The units are centipoises or reciprocal poises.

METHOD. The methods of dissolving and measuring the viscosity of the cellulose samples were in general the same as used earlier (*6*). However, the effects of varying certain conditions have been studied and are considered below. It has been found desirable to control the temperature during dissolving at 25° ± 0.5° C. and that during viscosity measurement to 0.02° C. The concentration of the Triton F solvent used was 1.96 N unless otherwise specified. In computing cellulose concentrations, a moisture content of 6 per cent has been assumed, and a 1 per cent solution defined as containing 100 mg. of anhydrous cotton per 10 ml. of solvent.

Results

EFFECT OF DISSOLVED GAS. Since in the present method dissolving is accomplished by rapid stirring of the cellulose-solvent mixture in contact with a gas, it seemed desirable to try to ascertain any effects of such gas. To this end several cellulose solutions were momentarily pumped (just until a crop of bubbles rose from the solution) and their viscosities compared with similar but unevacuated solutions. While it was difficult to make the gas evacuation procedure highly reproducible, any effect was small, causing an average change in viscosity of less than 1 per cent.

EFFECT OF STIRRING IN OPEN SYSTEM DURING DISSOLVING. In order to ascertain what changes in solution concentration occurred during dissolving in an open tube, experiments were made in which the cellulose solution was weighed before and after the dissolving operation. When 10.66 grams of a 0.50 per cent cotton (cuprammonium viscosity 19) in 1.96 N solvent was stirred at 650 r. p. m. at 30° C. in a tube open to the air, the average weight loss per hour was 15 mg. Lowering the temperature to 22° C. caused a gain of 6 mg. per hour, the atmospheric relative humidity being 80 to 90 per cent. Adding the glass bell (Figure 1) with a water bath seal at 21° C. caused a loss of 8 mg. per hour. This loss dropped to 6 mg. per hour of stirring when the water bath was at 25° C. and this was the dissolving temperature adopted. This loss causes the cellulose concentration to increase to about 0.501 per cent during 3.5 hours of stirring with a calculated viscosity increase in this case of a little more than 0.5 centipoise. Comparable experiments with a cotton sample (cuprammonium viscosity 22) dissolved in 2.25 N solvent to 0.45 per cent concentration in nitrogen showed an average gain of 5 mg. per hour.

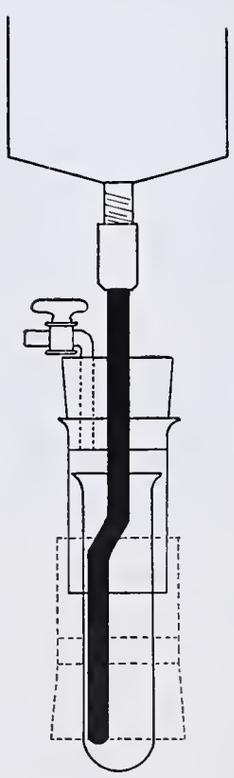

FIGURE 1. APPARATUS FOR DISSOLVING COTTON SAMPLE

EFFECT OF SOLVENT CONCENTRATION. In the earlier work (*6*) a solvent concentration of 1.96 N was found adequate for the celluloses studied. In extending the work to less degraded celluloses produced by various means, a somewhat larger concentration between 2.1 and 2.25 N has been

found to possess greater dissolving capacity for very slightly modified cellulose—e. g., cuprammonium viscosity 30 to 50. In spite of their greater solvent capacity these more concentrated liquids do not swell cellulose as quickly as the 1.96 *N* solvent. The effect of increasing solvent concentration upon the viscosity of the cellulose solution produced is shown graphically in Figure 2, in which viscosity in centipoises is plotted vertically and solvent normality horizontally. In the viscosity region up to 500 centipoises it is clear that the viscosity of the cellulose solutions rises with increasing solvent concentration at a rate which is greater the more viscous the solution. The rising solvent viscosity, however, causes the specific viscosities of the cellulose solutions to fall with increasing solvent normality.

Effect of Air and Oxygen during Dissolving. The ease with which cellulose is oxidized in the presence of alkalies made it of interest to ascertain the oxygen sensitivity of the present solutions of cellulose. Although the bell attachment (Figure 1) made it possible to vary the gas present during the

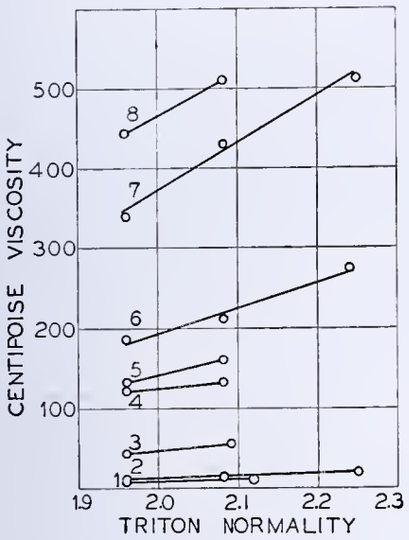

Figure 2. Effect of Solvent Concentration upon Viscosity

1. Triton F solvent, first lot
2. Triton F solvent, second lot
3. Sample (cuprammonium viscosity 19) at 0.25% concentration
4. Sample (cuprammonium viscosity 5.4) at 0.75% concentration
5. Sample (cuprammonium viscosity 11) at 0.50% concentration
6. Sample (cuprammonium viscosity 19) at 0.50% concentration
7. Sample (cuprammonium viscosity 22) at 0.45% concentration
8. Sample (cuprammonium viscosity 31) at 0.45% concentration

dissolving of the cellulose, the viscosity measurements were always made in contact with air. Experiments with a cotton yarn sample (cuprammonium viscosity 19) at 0.50 per cent concentration in 1.96 *N* solvent showed, when nitrogen was present during the dissolving period of 3.5 hours, a viscosity of 189.0 centipoises. With air present the value was 182.0, while oxygen still gave a viscosity of 181.8. For the same sample at 0.25 per cent concentration the figures were 45.4 (air) and 47.7 (nitrogen). This behavior is in marked contrast to cellulose dissolved in cuprammonium solution which deteriorates rapidly upon exposure to air or oxygen (*2*). In view of the above and other similar evidences of some atmospheric attack upon the cellulose during dissolving, nitrogen has been used in all the work reported here, unless otherwise noted. Because the nitrogen used was not specially purified, and some contamination from foreign gases dissolved in the water of the seal may have occurred, and furthermore exposure to air took place during viscosity measurements, it is

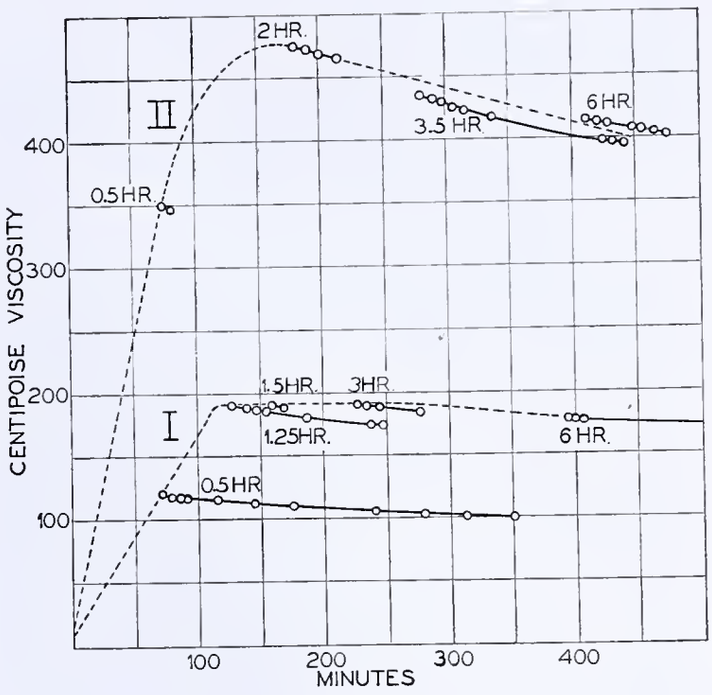

Figure 3. Effect of Time upon Viscosity

Duration of stirring given in hours

I. Sample (cuprammonium viscosity 19) at 0.50% concentration in 1.96 *N* Triton F

II. Sample (cuprammonium viscosity 22) at 0.45% concentration in 2.08 *N* Triton F

possible that if oxygen is rigidly excluded in all operations evidence of greater oxygen sensitivity will be found.

Effect of Temperature. In certain experiments the sample was dissolved to 0.25 per cent concentration in contact with air at different water bath temperatures. Thus a sample (cuprammonium viscosity 19) gave a centipoise viscosity of 50.6 when the dissolving temperature was 20° C., and 45.4 at 25° C. A less degraded sample showed a somewhat greater viscosity loss between 25° and 30° C. In other experiments the viscosity of a cellulose solution was measured at both 20° and 25° C. in order to determine the temperature dependence. In Table I are given data for four cellulose samples whose cuprammonium viscosities range from 5.3 to 31. The temperature coefficient increases with viscosity, varying from 1.23 with no cellulose present in the 2.08 *N* solvent to 1.30 for the solution of greatest viscosity. The average centipoise change per degree in viscosity between 20° and 25° C. ranges from 2.42 to 28.74 for the samples and concentrations studied, thus indicating a marked temperature sensitivity for the more viscous solutions.

Effect of Time. As was pointed out earlier (*6*) the viscosity of a solution of cellulose in dimethyl dibenzyl ammonium hydroxide decreases with time. This factor becomes the more noticeable the greater the viscosity of the solution examined and/or the smaller the viscometer constant. Carrying out the dissolving and the viscometer measurements in an atmosphere of not specially purified nitrogen did not

Table I. Effect of Temperature upon Viscosity of Triton F Solvent and Solutions of Cellulose

Sample	Cuprammonium Viscosity	Triton Normality *N*	Cellulose Concentration	Triton Solution Viscosity 25° C.	Triton Solution Viscosity 20° C.	Ratio $\frac{\eta\ 20°}{\eta\ 25°}$
	Centipoises		%	*Centipoises*		
11	31	2.08	0.45	485.2	628.9	1.30
13	19	2.08	0.50	210.5	270.6	1.29
15	5.4	2.08	0.75	133.2	171.0	1.28
16	5.3	1.96	0.50	51.35	63.45	1.24
		2.08	0	13.90	17.14	1.23
		1.96	0	10.79	13.20	1.22

considerably change the rate of viscosity decrease with time (*1*).

The viscosity of a given solution is, therefore, dependent upon the time during which the cellulose and solvent have been in contact. To obtain more information about this effect, stirring times and total times of contact have been deliberately varied.

In Figure 3 the total times of contact are plotted horizontally while viscosity is plotted vertically, and the stirring time for each solution is noted. Any given solution was stirred only once. Curve I shows the behavior of 5 cellulose samples (cuprammonium viscosity 19) at 0.50 per cent concentration in 1.96 *N* solvent when stirred for various lengths of time in contact with air. The cellulose was incompletely dissolved after only half an hour's stirring but almost dissolved after 1.5 hours' stirring. When necessary, solutions were forced through a sintered-glass filter to remove undissolved cellulose prior to viscosity measurements. Similarly, curve II shows the behavior of 4 cellulose samples (cuprammonium viscosity 22.2) at 0.45 per cent concentration in 2.08 *N* solvent when stirred for various times in nitrogen. This sample was completely dissolved only upon 3.5 hours' stirring.

Both curves show that viscosity at first increases rapidly with time and then falls off slowly. A maximum viscosity may occur before the sample is completely dissolved. This maximum occurs earlier with the more degraded sample and will probably also be affected by stirring conditions and the state of subdivision of the cellulose. The viscosity of the more degraded sample remains substantially constant for stirring times from 1.25 to 3 hours. This is probably explained by more cellulose dissolving to offset the aging effect. The sharper maximum in curve II is probably due to the slower dissolving and more rapid aging of the less degraded cellulose sample. The experimental points on curves I and II show the aging effects with the individual solutions as given by successive viscometer measurements. In general the viscosity decreases at a nearly linear rate for an hour or so, but on long standing the rate of aging decreases. This is observed in following the aging of the various solutions shown in curves I and II. The slope of curve I as it passes beyond the last point was determined by measuring the viscosity of the last solution after 12 hours when its viscosity had dropped to 140 centipoises.

The aging rate of a cellulose solution was found to be greater the greater the concentration of a given cellulose in solution, and the greater the viscosity of a solution of a given concentration. Thus the initial linear viscosity decreases, in centipoises per minute, varied from 0.03 to 0.90 when the concentration of cellulose (cuprammonium viscosity 44) varied from 0.10 to 0.45 per cent. With a more degraded cellulose (cuprammonium viscosity 5.4) this decrease varied from 0.01 to 0.08 as the cellulose concentration varied from 0.15 to 0.75 per cent. In 0.50 per cent cellulose solution this decrease was 0.02, 0.11, 0.33, and 3.1 for solutions whose centipoise viscosity was 43, 135, 269, and 1540, respectively. Dissolving the cellulose in a nitrogen atmosphere, to which the preceding figures refer, instead of in air, produced a lower rate of aging when the former gas was used. Thus the viscosity of a 0.50 per cent cellulose (cuprammonium viscosity 19) solution decreased at the rate of 0.2 centipoises per minute (air) against 0.12 (nitrogen).

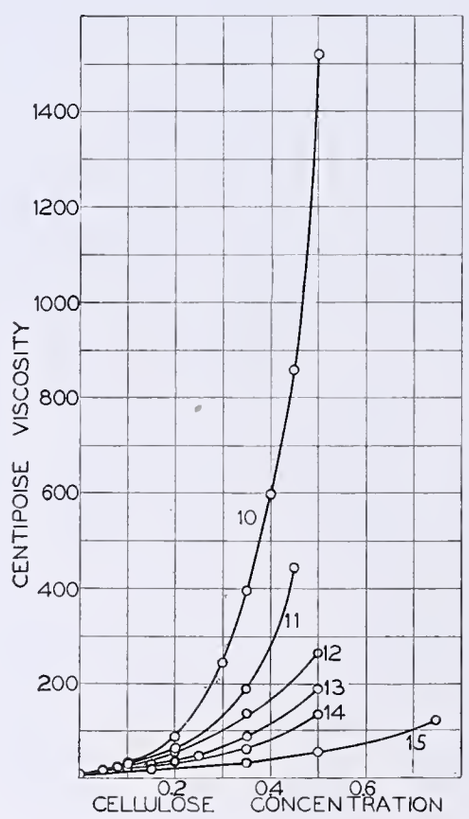

FIGURE 4. RELATION BETWEEN VISCOSITY AND CELLULOSE CONCENTRATION
1.96 *N* Triton F
Concentration of cellulose in per cent

TABLE II. DEPENDENCE OF VISCOSITY UPON CELLULOSE CONCENTRATION AND QUALITY

[Viscosity in centipoises (η). Cuprammonium viscosity (η_{Cu}) for 0.50 per cent cotton at 20° C. Triton F (1.96 *N*) viscosity (η_T) at 25° C.]

Sample (η_{Cu})	10 (43.3[a])	11 (31)	12 (25)	13 (19[a])	14 (11)	15 (5.4)
Cotton concentration	η_T	η_T	η_T	η_T	η_T	η_T
%						
0.05	17.3	...	...	...	...	...
0.076	23.9	...	...	...	...	...
0.10	31.8	30.1[b]	27.4[b]	...	20.1[b]	...
0.15	...	...	...	30.7[b]	...	19.6[b]
0.20	88.5	66.3[b]	53.6[b]	...	35.8[b]	...
0.25	...	...	...	47.7	...	...
0.30	246	...	...	...	...	...
0.35	397	189	136	88.1	61.7	30.6
0.40	598	...	...	...	...	...
0.45	861[b]	454[b]	...	...	...	...
0.50	1520	...	267	189	135[b]	54.2
0.75	...	...	...	...	...	123[b]

[a] Cuprammonium viscosity determined at 25° instead of 20° C.
[b] Second lot of Triton F.

Some aging or decrease in viscosity has been observed under whatever conditions successive viscosity measurements have been made. It is hoped to study further the nature of the interaction between cellulose and dibenzyl dimethyl ammonium hydroxide which appears responsible for the aging. Because of this interaction it is necessary to specify the age of a solution in considering its viscosity. In this paper all data have been interpolated (or sometimes extrapolated) to the same "age" before being compared.

VARIATION OF VISCOSITY WITH CELLULOSE CONCENTRATION. Since the manner in which viscosity changes with cellulose concentration affords an insight into the nature of cellulose solutions, several cellulose samples of very different viscosities and prepared by different methods have been studied in 1.96 *N* solvent. All samples were dissolved by stirring in contact with nitrogen for 3.5 hours and the viscosity has been interpolated (occasionally extrapolated) to an age of 275 minutes. The data are recorded in Table II and shown graphically in Figure 4, in which cellulose concentration is plotted horizontally and viscosity in centipoises vertically. It is clear that the viscosity increases exponentially with solute concentration. A similar relation has been found for cuprammonium solutions of cellulose (*5*) and nitrocelluloses in organic solvent (*4*).

In Figure 5 the dotted curves indicate how the specific viscosity of these samples changes with cellulose concentration. It appears that a linear relation between specific viscosity and solute concentration will occur only below a cellulose concentration of 0.05 per cent for the least degraded sample (10) although an essentially linear relation already occurs below 0.30 per cent for the most degraded sample (15). This low concentration region is of interest because of its possible theoretical significance (*7*). In Figure 5 the solid curves show fluidities of these samples in reciprocal poises plotted

vertically and solute concentrations horizontally. This plot emphasizes the nearly linear character of the fluidity variation for sample 15 and the increase of curvature with decreasing cellulose degradation. Also it is clear from this plot that the fluidity range of the celluloses studied varies widely with solute concentration, and passes through a maximum near 0.25 per cent.

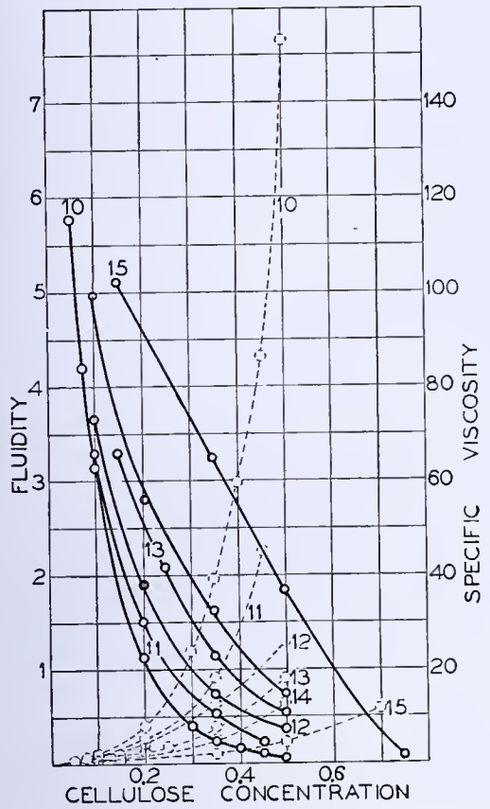

FIGURE 5. RELATION OF FLUIDITY AND SPECIFIC VISCOSITY TO CELLULOSE CONCENTRATION IN 1.96 *N* TRITON F
Solid curves, fluidity
Dotted curves, specific viscosity

In view of the recent work of Brownsett and Clibbens (*3*), no unique relation between cuprammonium viscosity and Triton F viscosity is to be expected except in the cases of celluloses modified by the same or equivalent chemical attack. In the present work samples 10 and 13 were chemically unmodified celluloses, sample 12 was bleached, while samples 11, 14, and 15 were bleached with sodium chlorite–sodium hypochlorite, peroxide, and sodium hypochlorite, respectively, so that no unique relation would necessarily be expected. The simple "ten" relation found earlier (*6*) involved a series of celluloses similarly degraded—namely, by a succession of commercial laundry treatments—to cuprammonium fluidities between 4 and 25. However, present samples 12 to 15, inclusive, fit into the former series. Brownsett and Clibbens (*3*) find for hydrocellulose, and oxycelluloses prepared with dichromate–oxalic acid, periodic acid, and alkaline bromate, respectively, that for 0.5 per cent solutions the cuprammonium fluidity is approximately 6.5 times the Triton F fluidity. This figure is 5.8 and 4.6 for oxycelluloses prepared by dichromate–sulfuric acid and neutral hypochlorite, respectively. After extraction with dilute sodium hydroxide, however, all the celluloses give the 6.5 ratio. The discrepancy between this lower value and value of 10 found in the earlier work (*6*) is probably explained for the most part by the considerable aging which occurred during the long dissolving time of 18 hours used by Brownsett and Clibbens.

Summary and Analytical Considerations

From the foregoing certain conclusions can be drawn as to conditions which should be favorable to viscosity analyses for cellulose quality when carried out with dimethyl dibenzyl ammonium hydroxide as solvent. When slightly degraded celluloses (cuprammonium viscosity 25 or greater) are to be analyzed the solvent concentration can advantageously be raised to 2.1 to 2.2 *N*. From Figure 2 it appears that so raising the solvent concentration should not decrease the sensitivity of the method even for moderately degraded samples. It is desirable to control the temperature of the dissolving sample to a few tenths of a degree and the temperature of the viscometer measurements to a few hundredths. This becomes the more important the more viscous the solution.

To reduce changes in solution weight (concentration) to a few milligrams per hour during dissolving it is an easy matter to employ a glass bell (Figure 1) to ensure an atmosphere of fixed water vapor pressure. Using a mercury or oil seal eliminates any water vapor. If a nitrogen atmosphere is maintained in the bell the viscosity values are some 4 to 5 per cent higher and the solution ages somewhat more slowly. Because the viscosity of cellulose in dimethyl dibenzyl ammonium hydroxide solution changes with time it is necessary to know how long the cellulose and solvent have been in contact and it is desirable to employ known stirring times. Since after dissolving this aging occurs at an approximately linear rate, for an hour or so, extrapolations are easy.

In general, unless the cellulose has a cuprammonium viscosity in excess of 20 and/or the cellulose concentration is greater than 0.5 per cent the solution ages no faster initially than about 0.2 centipoise per minute. Also under these conditions a considerable variation in dissolving time appears permissible in view of the very flat maximum in curve I of Figure 3. While a cellulose concentration of 0.5 per cent is satisfactory for samples with a cuprammonium viscosity of 25 or less, for less degraded celluloses a smaller concentration is desirable to avoid excessively viscous solutions. From the fluidity curves in Figure 5 it appears that a cellulose concentration of 0.25 per cent should give the analysis maximum sensitivity for celluloses with cuprammonium viscosities falling between 5 and 43 centipoises. In technical analysis a cellulose concentration of 0.35 per cent may prove most generally useful.

If it is desired to compute cuprammonium viscosities (or fluidities) from measurements made upon celluloses dissolved in dimethyl dibenzyl ammonium hydroxide, it becomes necessary to have conversion data. A certain amount of such data (*3*, *6*) is now available. Thus far conversion relations have proved simple over considerable ranges of cellulose degradation, making possible the use of conversion factors. However, the present method of itself appears to give different information about cellulose quality than the cuprammonium method, and according to Brownsett and Clibbens (*3*) the dimethyl dibenzyl ammonium hydroxide method measures total cellulose (apparent and latent) degradation while the former method measures only apparent degradation. The present method, therefore, appears to merit consideration upon additional grounds.

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Analytical Possibilities of Some Hydroxybenzalrhodanines

o-, *m*-, and *p*-Hydroxybenzalrhodanines and 3,4-Dihydroxybenzalrhodanine

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SINCE the use of an organic compound for inorganic analysis by Griess (*5*) in 1879 and especially since the excellent results obtained with dimethylglyoxime (*9*) in 1905, the use of organic reagents in inorganic analysis has increased steadily.

Rhodanine was first prepared by Nencki (*7*) in 1877 and the analytical applications of a number of its derivatives were recently investigated by Feigl (*2*), who found that rhodanine itself gave yellow precipitates with silver, mercury, gold, palladium, and platinum, and that *p*-dimethylaminobenzalrhodanine was the most sensitive reagent yet discovered for the detection of silver (*3*). Because of its aqueous insolubility, alcohol or acetone solutions of the reagent are used. Rhodanine itself is precipitated from such solutions when added to appreciable quantities of water, which restricts its wider use as an analytical reagent.

Table I. Physical Constants

	Color	Solubility[a] Water	Solubility[a] 95% Alcohol	Melting Points Cited	Melting Points Found
		G./100 cc.		° *C.*	° *C.*
Rhodanine	Yellow	0.4032	3.1444	269.70	267.69
o-Hydroxybenzalrhodanine	Yellow	0.0136	2.1932	200 and 218–219	213–14
m-Hydroxybenzalrhodanine	Greenish yellow	0.0292	1.2436	244–45	245–46
p-Hydroxybenzalrhodanine	Orange	0.0216	2.0620	260	270–75
3,4-Dihydroxybenzalrhodanine	Yellow	0.0115	0.5280	270–80	265 decomposed

[a] Grams per 100 cc. of solvent at 25° C. Values are averages of three parallel determinations, made by making a saturated solution at 60° C., allowing to cool to 25° C. in presence of undissolved solid, pipetting off 25-ml. portions, evaporating solvent, drying residue at 110° C., weighing, and recalculating on basis of 100 ml. of solvent.

Since the introduction of a hydroxyl group into benzaldehyde increases the water-solubility from 4- to 8-fold and the introduction of a second hydroxyl makes the product even more water-soluble; and since a number of aromatic hydroxy compounds themselves possess analytical possibilities, the present investigation was undertaken to find whether or not both of these properties might be carried over into the condensation products.

A wide variety of methods for the condensation of rhodanine and aldehydes is given in the literature. Concentrated sulfuric acid (*11*), acetic acid (*6*), and an alkaline medium (*11*) seemed to be most generally employed. All three methods were used by the present authors in the preparation of their reagents and in every instance the alkaline medium gave better yields and a purer initial product. Continued agitation throughout the period of condensation was found to be very advantageous.

o-Hydroxybenzalrhodanine was made by Zipser (*11*). All three of the monohydroxybenzalrhodanines were made by Borgellini (*1*), and the 3,4-dihydroxybenzalrhodanine was made by Rosemand and Boehm (*8*). No analytical applications of these compounds were reported.

Of the several methods for the preparation of protocatechualdehyde listed in the literature, that proposed by Fettig and Remsen (*4*) gave the authors only a 10 per cent yield of purified product, while the procedure of Wegscheider (*10*) gave a 75 per cent yield.

The following compounds are discussed below: rhodanine (I), *o*- (II), *m*- (III), and *p*- (IV)-hydroxybenzalrhodanines, and 3,4-dihydroxybenzalrhodanine (V).

Solutions of the five reagents used in all subsequent tests were made by dissolving in 100 ml. of 95 per cent alcohol the following amounts of each compound: I 0.82, II 0.1, III 0.23, IV 0.05, and V 0.28 gram. Three drops of these reagents added to 3 ml. of water produced no cloudiness. One molar aqueous solutions of the cations were used to produce the results reported in Tables II and III. No differences were noted when the tests were conducted in the presence of Cl^-, Br^-, I^-, $C_2H_3O^-$, NO_3^-, ClO_3^-, SO_4^{--}, CNO^-, CNS^-, $S_2O_3^{--}$, $C_2O_4^{--}$, PO_4^{---}, AsO_4^{---}, CO_3^{--}, and $C_4H_4O_6^{--}$. Tests were conducted on all the cation solutions using alcoholic solutions of the four hydroxyaldehydes, but no distinctive colors or precipitates were formed.

Wherever feasible, solutions of the following cations were tested under the conditions specified in Table II: Al^{+++}, As^{+++}, Ba^{++}, Ca^{++}, Cr^{+++}, Mg^{++}, Mn^{++}, Ni^{++}, Sn^{++++}, and Zn^{++}. No precipitates were formed. Very little difference was obtained when dilute hydrochloric or acetic acid solutions of the cations were used.

The only reagent producing a distinctive deep red color in alkaline solution was 3,4-dihydroxybenzalrhodanine (Table III). One drop of the alcoholic reagent solution was added to 3 cc. of the aqueous cation solution and the mixture made basic with an excess of sodium hydroxide, ammonium hydroxide, or pyridine. In some cases an excess of ammonium salts interfered with the test.

3,4-Dihydroxybenzalrhodanine was by far the most sensitive of the reagents tested but it was also the least water-soluble. The alkaline nickel test was definite and characteristic.

Table II. Color and Sensitivity[a] of Precipitates from Neutral[b] Solutions of Cations

Cation	Rhodanine Color	Rhodanine Sensitivity	*o*-Hydroxybenzalrhodanine Color	*o*-Hydroxybenzalrhodanine Sensitivity	*m*-Hydroxybenzalrhodanine Color	*m*-Hydroxybenzalrhodanine Sensitivity	*p*-Hydroxybenzalrhodanine Color	*p*-Hydroxybenzalrhodanine Sensitivity	3,4-Dihydroxybenzalrhodanine Color	3,4-Dihydroxybenzalrhodanine Sensitivity
Cd^{++}	...	...	...	...	...	...	...	...	Red	9,000
Ag^+	Dark green	10,000	Red brown	10,000	Yellow	10,000	Brown	10,000	Greenish black	100,000
Co^{++}	...	...	...	...	...	...	...	...	Orange	17,000
Cu^{++}	Green	16,000	Red brown	160,000	Red brown	160,000	...	...	Brown	160,000
Au^{+++}	Cream	5,000	Red brown	5,000	Red brown	50,000	Red brown	5,000	Red brown	50,000
Fe^{+++}	...	...	...	...	Yellow	18,000	Yellow	18,000	Brown	180,000
Pb^{++}	...	...	...	...	Yellow	50,000	...	...	Red brown	50,000
Hg^+	Cream	5,000	Yellow	5,000	Yellow	5,000	...	...	Brown	5,000

[a] Limiting sensitivities were calculated from data obtained by adding known volumes of distilled water to a definite amount of molar solution of cations until a dilution was reached at which test was still distinctive but beyond which it became questionable. Figures express number of grams of solution in which 1 gram of cation was detectable.

[b] Salt of cation dissolved in distilled water.

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Table III. Lakes[a] and Colors of 3,4-Dihydroxybenzalrhodanine in Alkaline Solutions of Cations

Cation	Excess NaOH	Excess NH_4OH	Excess Pyridine
Al^{+++}	.....	Red-brown lake	Brown lake
Co^{++}	Purple-red lake	.....	.....
Mg^{++}	Purple-red lake	Red lake	.....
Mn^{++}	Purple-red lake	Pink ppt.	.....
Ni^{++}	Lavender lake	Lavender lake	Deep blue soln.
Ag^{+}	.....	Violet ppt. Purple soln.	Violet ppt. Blue soln.

[a] Lake formation was taken to be affixing of characteristic color to precipitate, so that a clear solution resulted upon filtering.

Summary

The analytical possibilities of *o*-, *m*-, and *p*-hydroxybenzalrhodanines and 3,4-dihydroxybenzalrhodanine were studied. More metals were precipitated by the hydroxybenzalrhodanines than by rhodanine itself. The very slight water-solubility of the compounds materially restricts their use as analytical reagents.

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Electrolytic Determination of Iron

A Modified Moore Method

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MANY of the volumetric methods for iron are highly satisfactory for routine determinations. However, they all require standard solutions, and, when iron is determined only occasionally, considerable time is often spent in standardizing the required solution. The electrolytic method presented here requires stock solutions which can be stored indefinitely, and should be particularly convenient in laboratories where electroanalysis of other metals is routine. Both accuracy and precision of 1 to 2 parts per thousand are readily attained in the presence of the usual substances in iron ores. Only the customary silica separation, followed by decomposition with hydrofluoric and sulfuric acids to recover the iron combined as silicate, is necessary; chloride does not interfere.

The method is based on the deposition of metallic iron from the complex formed by the addition of excess ammonium carbonate solution to an acid solution containing ferric iron and orthophosphate ions. The hot solution is electrolyzed at a high current density without stirring.

Moore (*3*) suggested the use of a ferric phosphate–ammonium carbonate solution for the electroanalysis of iron. Brand (*2*) used pyrophosphate instead of phosphate. He stated that the last traces of iron were difficult to deposit and that the solution dissolved the metal when current was not flowing.

Avery and Dales (*1*) criticized Moore's method. They found that 5 hours were required to deposit 200 mg. of iron. Their deposits were high in weight, although deposition was incomplete. They found a slight amount of carbonaceous material and some phosphorus in their deposits.

Experimental

Selection of Ammonium Carbonate Stock Solution. The addition of excess ammonium carbonate solution to an acidic ferric phosphate solution forms a soluble complex, which is much more soluble in cold than in hot, and in concentrated than in dilute ammonium carbonate. Since electrolysis of the cold solution is unsatisfactory, it is necessary to prepare a solution sufficiently concentrated to hold moderate amounts of the ferric complex in solution at 70° C. (just below the ebullition temperature). When it precipitates before or during electrolysis, many hours are usually required for resolution and complete deposition of the iron. This precipitation is possibly the cause of the criticism of Moore's method (*1*).

When the concentration of ammonium carbonate in the electrolyte is at least 3.5 *M*, the solubility of the complex is such that no precipitation of iron occurs at 70° C. from a solution containing as much as 200 mg. of iron in a volume of 200 ml. In order to prepare conveniently an electrolyte of such concentration, a 5.0 *M* stock solution is necessary.

Preparation of Stock Solutions. The 5.0 *M* ammonium carbonate stock solution, NH_3/CO_2 = 2.05–2.1, was prepared by dissolving 1570 grams of Eimer & Amend reagent quality lump ammonium carbonate, assay 31.8 to 32.5 per cent ammonia, in 700 ml. of clear reagent quality concentrated ammonium hydroxide, specific gravity 0.90, and sufficient water to make 4 liters. A higher concentration of free ammonium hydroxide is undesirable. With only occasional shaking, several days were required for complete solution. Heating to 65° C. and/or stirring facilitate solution but must be employed with suitable precautions because of the volatile nature of ammonium carbonate.

The 2.0 *M* ammonium dihydrogen phosphate stock solution was prepared by dissolving 230 grams of the low-arsenic salt in water and diluting to 1 liter. Other sufficiently pure alkali phosphate salts or phosphoric acid also were found to be satisfactory.

Preparation and Standardization Ferric Ammonium Sulfate Test Solution. In 2 liters of water containing 278 ml. of concentrated sulfuric acid 890 grams of ferric alum were dissolved. After filtering, the solution was diluted to 10 liters and used in all the analyses reported; it was 1.0 *N* with sulfuric acid. It was standardized by the stannous chloride–potassium dichromate procedure of Willard and Furman (*4*). The reduction and titration were carried out at 25° C. The averaged result of four determinations was 0.1033 gram of iron in 10 ml.

Apparatus. The cathode was a 10-gram cylindrical platinum wire gauze, surface area 80 sq. cm. The anode was a 1.25-mm. diameter platinum wire loop, surface area 4 sq. cm. The electrolytic stand was arranged so that the electrodes could be removed without interruption of the current. Current was taken from a lead storage bank capable of supplying 6 amperes at 10 volts. Larger electrodes and a higher current are desirable.

A 400-ml. Pyrex brand tall-form beaker was used as electrolyte container. A 1.5 × 15 cm. indented upright glass column (similar to that of the familiar Claisen distillation flask) sealed just below the rim served as a spray trap, while evolved ammonia was drawn off by gentle suction through a side tube near the top. This device was not necessary, but prevented escape of ammonia into the laboratory during electrolysis, since it was not convenient to use a hood. The beaker was covered with a suitably notched split watch glass.

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Table I. Examination of Anodic Depolarizers

Depolarizer Added		Time of Electrolysis	$(NH_4)_2S$ Test	Weight of Fe Deposit	Error in Fe
Grams		*Min.*		*Gram*	*Mg.*
5	Welch's Na_2SO_3	35	Negative	0.2104	+3.8
5	E. & A. c. p. Na_2SO_3	30	Green	0.2094	+2.8
5 ml.	30% SO_2 NH_4HSO_3	35	Negative	0.2098	+3.2
5	Merck's $Na_2SO_3.7H_2O$	35	Green	0.2073	+0.7
5	E. & A. reagent $NaHSO_3$	35	Negative	0.2115	+4.9
7	Baker's $K_2SO_3.2H_2O$	45	Pale green	0.2078	+1.2
5	Hydrazine sulfate	80	Negative	0.2074	+0.8
5	Ammonium oxalate[a]	75	Pale green	0.2063	−0.3
5	Urea + 1 gram KI	105	Negative	0.2069	+0.3
5	Sodium formate	60	Pale green	0.2066	0.0
5	Sodium hypophosphite	40	Negative	0.2123	+5.7
5	Ammonium phosphite	75	Pale green	0.2071	+0.5

[a] Did not completely dissolve during electrolysis.

General Procedure

A sample containing 100 to 200 mg. of iron was weighed and dissolved in the clean electrolytic beaker. All iron was oxidized to the ferric condition with persulfate or hydrogen peroxide, the excess being destroyed by boiling. Nitrate must be absent. The solution was cooled, and 15 ml. of stock phosphate solution were added to the cold weakly acidic solution and mixed thoroughly; the volume should now be 50 ml. or less. There must be 3 to 5 moles of phosphate for every gram-atom of metal other than the alkalies.

Then 150 ml. of stock ammonium carbonate solution were added, the first part cautiously from a buret to avoid spattering. Not more than 10 ml. should be required for neutralization. The clear tan solution was covered with the split watch glass and heated to 67–70° C. (not higher). Both electrodes were placed against the bottom of the beaker. If this is not done, the slowness of upward diffusion of metal ions into the effective electric field will greatly prolong the time required for complete deposition. The solution was electrolyzed for 90 minutes with a current of 6 or more amperes ($N.\ D._{100}$ = 5 to 10) or until deposition was complete. Incomplete deposition is indicated by a green or black color formed upon addition of clear ammonium sulfide to a small portion of the electrolyte. Electrolysis was continued 15 minutes after the first negative test, the deposit being kept covered during electrolysis by use of wash water on the cover glass and sides of the beaker.

The current was reduced to 1 ampere and the electrodes were removed without interruption of the current while being continuously washed with a heavy stream of water. The cathode was washed thoroughly with water, then with 95 per cent ethyl alcohol, dried 30 to 35 cm. above a spread burner flame, and weighed.

Results of Analyses

In all the analyses described below, unless otherwise noted, the same conditions were employed—namely, 20 ml. of standard ferric alum solution (0.2066 gram of iron), 15 ml. of stock phosphate solution, 150 ml. of stock carbonate solution, a 200-ml. volume of electrolyte, and 90 minutes of electrolysis at 67–70° C. with a current of 6.0 amperes at 5.2 volts. The temperature, current, and voltage remained nearly constant during electrolysis. The ammonium sulfide test was made on the entire electrolyte after removal of the electrodes. Under these conditions, it is sensitive to 0.1 mg. of iron.

The iron deposits obtained, unless otherwise stated, were always bright, dense, adherent, highly pure, and readily washed and dried without rusting. Hydrogen sulfide must be absent from the laboratory atmosphere to avoid contamination of the deposit with sulfur. When present, arsenic partly codeposits with iron.

Examination of Anodic Depolarizers. Since nitrate formed by anodic oxidation of ammonia depolarizes the cathode and retards iron deposition, various reducing agents were examined in an attempt to eliminate this difficulty. The results are shown in Table I.

Of all the depolarizers examined, only sulfite and hypophosphite were effective in reducing the time required for complete deposition, but neither was satisfactory because impure iron deposits were obtained, sulfide being present in the sulfite and arsenic in the hypophosphite. The deposits from the sulfite solutions were dark, contained sulfur, and were slightly rusty. Obviously, the depolarizers were added after the ammonium carbonate, since many of them would reduce ferric ion in acidic solution.

Separation of Iron from Other Materials by Electrolysis. Table II shows that the method is satisfactory in the presence of large amounts of ammonium chloride and ammonium sulfate. The upper limits for noninterference of aluminum, chromium, and titanium are 50 mg. of aluminum trioxide, 40 mg. of chromium, and 20 mg. of titanium dioxide.

In Table III it is shown that moderate amounts of manganese interfere. Crystals of manganese ammonium phosphate, formed during electrolysis, in part adhere to the cathode, causing high results. The time when these crystals were first detected is shown. Ten minutes elapsed between the addition of carbonate and start of electrolysis. It is obvious that it is necessary to deposit the iron completely and stop electrolysis before this crystallization starts in order to obtain accurate results for iron. The presence of other metals, such as aluminum, chromium, and titanium, apparently helps in holding manganese in solution. This effect was not studied quantitatively. An iron ore containing up to about 1 per cent of manganese may be analyzed without interference.

Table II. Separation of Iron from Other Materials by Electrolysis

Material Added		$(NH_4)_2S$ Test	Weight of Fe Deposit	Error in Fe
Mg.			*Gram*	*Mg.*
....	[a]	Green	0.2062[b]	−0.4
....	[a]	Negative	0.2066[c]	0.0
10,000 NH_4Cl	[a]	Negative	0.2066[c]	0.0
10,000 NH_4Cl	[a]	Negative	0.2065	−0.1
10,000 $(NH_4)_2SO_4$	[a]	Green	0.2060[c]	−0.6
10,000 $(NH_4)_2SO_4$	[a]	Negative	0.2065	−0.1
70 Al_2O_3	[d]	Negative	0.2059	−0.7
50 Al_2O_3	[a]	Negative	0.2066	0.0
50 Al_2O_3		Negative	0.2065	−0.1
50 Cr	[d]	....	0.2058	−0.8
40 Cr		....	0.2066	0.0
40 Cr		....	0.2067	+0.1
20 TiO_2	[e]	Negative	0.2069	+0.3
10 TiO_2		Negative	0.2066	0.0
10 TiO_2		Negative	0.2068	+0.2

[a] 10 ml. of stock phosphate used.
[b] Electrolyzed 60 minutes.
[c] Electrolyzed 75 minutes.
[d] Added as potassium alum solution.
[e] Added as 5% sulfuric acid solution.

Table III. Separation of Iron from Manganese by Electrolysis

Mn Added (as $MnSO_4$)	Fe Added	$MnNH_4PO_4$, Appeared after:	Time of Electrolysis	$(NH_4)_2S$ Test	Weight of Fe Deposit	Error in Fe
Mg.	*Gram*	*Min.*	*Min.*		*Gram*	*Mg.*
10	0.2066	50	90	Neg.	0.2104	+3.8
5	0.2066	75	90	Neg.	0.2078	+1.2
5	0.1033	60	60	Neg.	0.1034	+0.1
5	0.1033	60	60	Neg.	0.1033	0.0
4	0.2066	75	90	Neg.	0.2069	+0.3
4	0.2066	75	75	Neg.	0.2065	−0.1

It is probable that magnesium would not interfere to the same extent as manganese, since magnesium ammonium phosphate is appreciably soluble in hot ammoniacal solution.

If it is desired to determine other metals remaining in the electrolyte after separation of the iron, the ammonium carbonate may be boiled off and the precipitated metallic phosphates dissolved by cautious addition of mineral acid. Any silica is removed by filtration before proceeding.

Codeposition of Iron and Other Metals

In Table IV data are given for the codeposition of iron with nickel, cobalt, molybdenum, and tungsten. With nickel or cobalt, the iron alloy deposited more rapidly than nickel or

Table IV. Codeposition of Iron and Other Metals

Weight of Second Metal Added[a]	Weight of Fe Added	Dimethylglyoxime Test	$(NH_4)_2S$ Test	Weight of Deposit	Total Error
Gram	*Gram*			*Gram*	*Mg.*
0.0126 Ni	0.1033	Positive	Negative	0.1153	− 0.6
0.0084 Ni	0.1033	Negative	Negative	0.1116	− 0.1
0.0084 Ni	0.1033	Negative	Negative	0.1114	− 0.3
0.0042 Ni	0.1033	Negative	Negative	0.1077	+ 0.2
0.1091 Co	..	..	Negative	0.1037	− 5.4
0.1091 Co	0.1033	..	Negative	0.2114	− 1.0
0.1091 Co	0.2066	..	Negative	0.3154	− 0.3
0.1091 Co	0.2066	..	Negative	0.3159	+ 0.2
0.0218 Mo	0.1033	[b,c]	Black	0.0950	−30.1
0.0109 Mo	0.2066	[c]	Negative	0.2181	+ 0.6
0.0109 Mo	0.1033	[c,d]	Negative	0.1144	+ 0.2
0.0075 W	0.1033	[c,e]	Negative	0.1072	− 3.6
0.0075 W	0.1033	[c,e]	Negative	0.1068	− 4.0

[a] Second metals added as $NiSO_4$, $CoSO_4$, Na_2MoO_4, and Na_2WO_4 standard solutions to ferric alum before addition of phosphate.
Electrolyzed:
[b] 140 [d] 75, and [e] 60 min., respectively.
[c] Used 10 ml. of stock phosphate.

cobalt alone. Ammonium sulfide is still only a test for iron; it does not precipitate small amounts of the other metals. The quantitatively codeposited alloys may be dissolved in acid and one metal determined by another procedure; the second may then be calculated by difference.

Up to 10 mg. of molybdenum are quantitatively codeposited with 100 mg. or more of iron. Amounts of molybdenum above about 20 mg. depolarize the cathode and prevent complete deposition of iron. The range between 10 and 20 mg. of molybdenum in 200 ml. of electrolyte was not examined. In this range, the transition from the platable to the depolarizing concentration occurs. It might be profitable to examine the possibility of plating molybdenum alone from a suitable electrolyte containing a very low concentration of molybdate.

Summary

Iron can be quantitatively determined as metal by electrodeposition from a hot complex ferric phosphate–ammonium carbonate solution at a high current density without stirring. The method is accurate and reasonably fast. The highly sensitive ammonium sulfide test is available to prove completeness of deposition. The method may be applied to low manganese iron ores without removal of hydrochloric acid.

Small amounts of nickel or molybdenum are quantitatively codeposited with large amounts of iron, and cobalt with at least twice as much iron. Tungsten must be absent.

Large amounts of chloride, sulfate, and phosphate do not interfere. Nitrate and all strong oxidizing agents must be absent.

Acknowledgment

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From a thesis presented by William H. Armistead, Jr., to the Graduate School of Vanderbilt University in partial fulfillment of the requirements for the degree of doctor of philosophy.

Determination of Ash in Coals Unusually High in Calcite and Pyrite

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In determining ash in coals containing unusually large amounts of calcite and pyrite, difficulty in obtaining satisfactory results by the standard A. S. T. M. procedure (*3*) may be experienced, because of the varying amounts of sulfur that are retained as calcium sulfate in the ash. Variations in the heating procedure used in the ash determination influence the amount of sulfur retained. Lower ash and lower sulfur in the ash are obtained by slow rates of heating.

This paper is a report of cooperative work done by the Analytical Laboratory of the Illinois State Geological Survey and the Pittsburgh Laboratory of the United States Bureau of Mines for the purpose of studying modified procedures for the determination of ash in such troublesome coals.

Experimental Tests

For this work five sizes of coal prepared by screening from a large sample of $1^1/_4$ inch × 0 screenings from the No. 2 bed, Woodford County, Ill., were used. The larger sizes were crushed to pass a No. 4 sieve and after mixing and riffling all sizes, two 1-quart samples of each were cut out. One set of samples was sent to the U. S. Bureau of Mines Laboratory and one set was retained in the Illinois State Geological Survey Laboratory.

The samples are designated as follows:

Sample	Size	Sample	Size
1	$1^1/_4$ × $^3/_4$ inch	4	10 × 48-mesh
2	$^3/_4$ × $^3/_8$ inch	5	−48-mesh
3	$^3/_8$ inch × 10-mesh		

Each laboratory prepared its own samples for analysis by the usual A. S. T. M. procedure (*1*). Analyses in the two laboratories indicated that the two samples of each size were satisfactory duplicates, with the exception of sample 3. Because the two 1-quart portions differed in ash content by too large an amount, the ash values reported by the Geological Survey laboratory were obtained on the 60-mesh coal prepared in the Pittsburgh laboratory.

The coals were analyzed for total sulfur (Eschka method), and for forms of sulfur and carbon dioxide by methods of the U. S. Bureau of Mines (*7*). Moisture at 105° C. was determined by each laboratory on the samples according to the A. S. T. M. standard method (*2*).

Bureau of Mines Determinations

Method A. The dried samples were heated on the hearth of a hot muffle furnace for 8 minutes to drive off volatile matter, then heated at 725° C. to constant weight (within 0.001 gram).

Method B. The dried samples were placed in a cold furnace and heated to 725° C. in 1.5 hours. The temperature was kept at 725° C. to constant weight.

Method C (Parr's sulfated ash method, *5*, *6*). The dried samples were burned by Method A and after cooling were moistened with a few drops of 1 to 1 sulfuric acid and dried on an air bath until the fumes were largely driven off. The samples were then heated at 725° C. to constant weight. This treatment produces a sulfated ash in which it is assumed that all calcium is present as the sulfate. To correct to a calcium oxide basis the percentage of sulfur trioxide, coal basis, determined in the sul-

fated ash is subtracted from the sulfated ash or 1.82 times the mineral carbon dioxide, coal basis, is subtracted from the sulfated ash. In the latter method of correction it is assumed that the mineral carbon dioxide is a measure of the calcium carbonate present in the original mineral matter of the coal.

Table I. Sulfur Forms and Carbon Dioxide

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
	%	%	%	%	%
Sulfate sulfur					
U. S. B. M.	0.06	0.08	0.07	0.11	0.12
I. G. S.	0.01	0.02		0.04	0.07
Pyritic sulfur					
U. S. B. M.	0.67	1.03	1.30	2.28	3.14
I. G. S.	1.02	1.10		2.56	3.50
Organic sulfur					
U. S. B. M.	0.72	0.69	0.58	0.55	0.52
I. G. S.	0.53	0.49		0.40	0.19
Total sulfur					
U. S. B. M.	1.45	1.80	1.95	2.94	3.78
I. G. S.	1.56	1.61		3.00	3.76
Carbon dioxide					
U. S. B. M.	0.29	0.53	1.59	4.24	3.63
I. G. S.	0.47	0.64		4.10	3.64

Table II. Determination of Ash

(Per cent of dry coal)

Sample	Ash	SO_3 in Ash	Sulfide in Ash	SO_3- and S-Free Ash	Ash	SO_3 in Ash	Sulfide in Ash	SO_3- and S-Free Ash
	Data by Method A				Data by Method E			
1	9.28	0.46	..	8.82	9.40	0.29	0.04	9.09
	9.27	0.40	0.00	8.87	9.50	0.30	0.02	9.19
Av.	9.28			8.85	9.45			9.14
2	14.37	0.62	..	13.72	13.80	0.22	0.02	13.57
	14.20	0.57	0.03	13.60	13.66	0.31	0.03	13.34
Av.	14.29			13.66	13.73			13.46
3	18.59	1.52	..	17.06	18.06	..	..	..
	18.70	1.56	0.01	17.13	17.87	..	..	..
Av.	18.65			17.10	17.97			
4	31.27	3.64	..	27.61	29.26	1.73	0.02	27.52
	31.15	3.49	0.02	27.64	28.92	1.28	0.02	27.63
Av.	31.21			27.63	29.09			27.58
5	34.93	3.14	..	31.73	34.83	1.86	0.32	32.81
	35.12	3.03	0.06	32.03	34.65	1.59	0.27	32.92
Av.	35.03			31.88	34.74			32.87
	Data by Method B				Data by Method F			
				SO_3-Free Ash				SO_3-Free Ash
1	9.13	0.29		8.84	9.32	0.23		9.09
	9.09	0.26		8.83	9.33	0.24		9.09
Av.	9.11			8.84	9.33			9.09
2	14.09	0.40		13.69	13.77	0.23		13.54
	14.06	0.34		13.72	..	..		..
Av.	14.08			13.71	13.77			13.54
3	17.87	0.85		17.02	17.53	..		..
	17.80	0.68		17.12	17.85	..		..
Av.	17.84			17.07	17.69			
4	29.04	1.39		27.65	28.81	0.70		28.11
	28.91	1.07		27.84	28.14	0.68		27.46
Av.	28.98			27.75	28.48			27.79
5	33.19	1.01		32.18	34.73	1.45		33.28
	33.01	0.83		32.18	34.03	1.24		32.79
Av.	33.10			32.18	34.38			33.04
	Data by Method D							
1	9.12	0.29		8.83				
	9.10	0.24		8.86				
Av.	9.11			8.85				
2	14.03	0.38		13.65				
	14.05	0.34		13.71				
Av.	14.04			13.68				
3	17.58	0.53		17.05				
	17.71	0.61		17.10				
Av.	17.65			17.08				
4	28.53	0.86		27.67				
	28.49	0.76		27.73				
Av.	28.51			27.70				
5	32.95	1.02		31.93				
	33.16	0.95		32.21				
Av.	33.06			32.07				

Method D. The dried samples were placed in a cold furnace and heated to 400° C. in 0.5 hour. They were held at this temperature for a further 0.5 hour, then transferred to another furnace at 725° C. and heated to constant weight.

One ash sample from each test was analyzed for total sulfur by the sodium carbonate fusion method (*7*). The duplicate ash was tested for sulfide sulfur by the evolution method used for coke (*8*). Sulfate sulfur was determined in the hydrochloric acid solution from the sulfide tests. Ammonium hydroxide was added in slight excess and the precipitated iron together with any insoluble ash was removed by filtration. The filtrate was made slightly acid and the sulfur precipitated in the regular manner.

Geological Survey Determinations

Method E. The dried samples were heated on the hearth of a hot muffle furnace for 10 minutes to drive off volatile matter, moved just inside the furnace for 5 minutes, and then moved back to the hot portion of the furnace where they were heated to constant weight at 750° C.

Method F. The dried samples were placed in a cold muffle furnace and heated to 750° C. in 1.75 hours. They were heated to constant weight at this temperature.

Method G (Parr's sulfated ash method). The dried samples were burned by Method F and after cooling were treated with 1 to 1 sulfuric acid, the excess acid was fumed off on a hot plate, and the samples were heated to constant weight at 750° C.

The ashes obtained by all three methods were analyzed for sulfur trioxide by extraction with dilute hydrochloric acid, precipitation and removal of R_2O_3 with ammonium hydroxide, with subsequent precipitation of sulfate with barium chloride in acid solution. Sulfide sulfur determinations were made on the ashes obtained by Methods E and F by the procedure mentioned above (*8*).

The effect of using furnaces of different sizes, in which the rate of change of atmosphere varied, for duplicate determinations was studied in the Geological Survey laboratory. One duplicate was ashed in the larger Hoskins F. D. 204 furnace whose heating chamber is $7^1/_2$ inches wide, $5^1/_4$ inches high, and 14 inches long while the other was ashed in the smaller Hoskins F. D. 202 furnace whose heating chamber is $4^1/_4$ inches wide, 3 inches high, and 10 inches long. Both furnaces were equipped with thermocouples placed loosely through the back, so that air flow through the furnace would take place.

All tests in both laboratories were made in electrically heated muffle furnaces.

Results of Tests

Results of analyses of the coals for forms of sulfur and mineral carbon dioxide are given in Table I. Ash values as determined by the various methods, together with amounts of sulfur as sulfur trioxide retained in the ashes, are listed in Tables II and III. Sulfide sulfur was found only in ashes obtained by Methods A and E. Table IV presents additional data showing the effect of a slower rate of heating and ashing in furnaces of different sizes in which air circulation was different. Table V presents a comparison of average results obtained by different methods.

Discussion

The tolerances for permissible differences between ash determinations in the same laboratory and between different laboratories by the A. S. T. M. standard method (*4*) are 0.3

TABLE III. DETERMINATION OF ASH

(Per cent of dry coal)

Sample	Sulfated Ash	SO_3 in Sulfated Ash	SO_3-Free Ash	Sulfated Ash Less 1.82 × Mineral CO_2	Sulfated Ash	SO_3 in Sulfated Ash	SO_3-Free Ash	Sulfated Ash Less 1.82 × Mineral CO_2
	Results by Method C				Results by Method G			
1	9.81	1.05	8.76	9.28	10.15	0.76	9.39	9.29
	9.82	1.00	8.82	9.29	10.23	0.84	9.39	9.37
Av.	9.82		8.79	9.29	10.19		9.39	9.33
2	15.15	1.54	13.61	14.22	14.86	1.21	13.65	13.70
	15.18	1.50	13.68	14.25	14.99	1.25	13.74	13.83
Av.	15.17		13.65	14.24	14.93		13.70	13.77
3	20.43	3.43	17.00	17.56	...	...	...	...
	20.49	3.49	17.00	17.62	...	...	...	...
Av.	20.46		17.00	17.59	...	...	...	...
4	35.62	8.09	27.53	27.88	35.95	7.85	28.10	28.49
	35.68	8.15	27.53	27.94	35.71	7.77	27.94	28.25
Av.	35.65		27.53	27.91	35.83		28.02	28.37
5	39.38	7.42	31.96	32.78	40.68	7.16	33.52	34.06
	39.59	7.50	32.09	32.99	40.67	7.21	33.46	34.05
Av.	39.49		32.03	32.89	40.68		33.49	34.06

TABLE IV. COMPARISON OF ASH VALUES

Obtained by varying time necessary for furnace to reach 750° C., by ashing in different sized furnaces, and by applying the modified Parr method where first burning off took place in different sized furnaces. Data by Illinois Geological Survey.

Sample	Per Cent of Ash Starting with Cold Furnace, Heating to 750° C. 1.5 hours	2.25 hours	Per Cent Ash, Starting with Cold Furnace, Heating to 750° C. in 1.75 Hours Large furnace, Hoskins F. D. 204	Small furnace, Hoskins F. D. 202	Per Cent Ash, Sulfated Modified Parr Method Large furnace, Hoskins F. D. 204	Small furnace, Hoskins F. D. 202	Per Cent Ash, Modified Parr, Sulfated Ash Less 1.82 × Mineral CO_2
1	9.14	9.08	9.00	9.24	9.59	9.53	9.03
2	14.08	14.05	14.02	...	14.76	14.89	13.87
3	17.89	17.60	17.46	17.73	20.08	20.15	17.23
4	29.07	28.52	28.66	29.91	35.46	35.70	27.87
5	33.28	32.98	32.76	33.16	39.16	39.39	32.68

TABLE V. COMPARISON OF AVERAGE ASH VALUES OBTAINED BY METHODS A, B, D, E, AND F WITH METHODS C AND G

(Per cent of dry coal)

Sample	Data by U. S. Bureau of Mines: Method A, hot muffle	Method B, cold muffle	Method D, two muffles	Method C, Parr's method[a]	Difference from Method C: Method A	Method B	Method D
1	9.28	9.11	9.11	9.29	−0.01	−0.18	−0.18
2	14.29	14.08	14.04	14.24	+0.05	−0.16	−0.20
3	18.65	17.84	17.65	17.59	+1.06	+0.25	+0.06
4	31.21	28.98	28.51	27.91	+3.30	+1.07	+0.60
5	35.03	33.10	33.06	32.89	+2.14	+0.21	+0.17

Sample	Data by Illinois Geological Survey: Method E, hot muffle	Method F, cold muffle	Method G, Parr's method[a]	Difference from Method G: Method E	Method F
1	9.45	9.33	9.33	+0.12	0.00
2	13.73	13.77	13.77	−0.04	0.00
3	17.97	17.69	17.23	+0.74	+0.46
4	29.09	28.48	28.37	+0.72	+0.11
5	34.74	34.38	34.06	+0.68	+0.32

[a] Parr's sulfated ash less 1.82 × mineral CO_2.

and 0.5 per cent, respectively, on coals containing carbonates. For coals with more than 12 per cent ash containing carbonate and pyrite these are 0.5 and 1.0 per cent, respectively.

For the most part results obtained by the various methods checked within these tolerances, both in the same laboratory and between the two laboratories (Tables II and III), particularly the results corrected for retained sulfur. In a few cases the results did not check within these tolerances but, with one exception, these results were for the last two samples (samples 4 and 5) which are high in mineral matter containing large amounts of mineral carbon dioxide and pyritic sulfur. The ash of these samples is considerably higher than would normally be encountered in commercial samples.

The ash values obtained by Methods A and E, uncorrected for retained sulfur, are higher than those obtained by other methods with the exception of the Parr sulfated ashes. Methods A and E correspond to a more rapid rate of heating and the ashes contain considerably greater amounts of retained sulfur than those obtained by Methods B, F, and D. Results obtained by Method D probably show the best agreement between duplicate determinations, with comparatively lower amounts of sulfur being retained in the ashes. Results obtained by the Parr sulfated ash method check reasonably well for the most part, but this procedure is rather long and requires mineral carbon dioxide values for use in correcting the sulfated ash obtained.

In Table IV are presented further data on the effect of different rates of heating, not corrected for sulfur retained in the ash. Lower results are obtained with the slower rate of heating. In addition, information is presented on the use of different sized furnaces for ashing. The higher results obtained in the smaller F. D. 202 furnace indicate that sulfur trioxide as formed was not removed as rapidly as in the larger furnace and was therefore fixed in the ash to a larger extent. The adequate removal of sulfur trioxide therefore becomes important. Use of the Parr sulfated ash method appears to smooth out these differences to some extent.

Table V presents a comparison of average ash values obtained by the procedures tried. For those samples containing larger amounts of ash and mineral carbon dioxide the slower rates of heating give definitely better results when compared to those obtained by the Parr sulfated ash method. However, differences appear which are outside A. S. T. M. tolerances and in such cases the ash values should be corrected for retained sulfur or determined by the modified Parr method. It is not likely that such samples would be encountered in commercial samples but they might be encountered in certain special studies.

Summary

The preliminary hearth heating method (A and E) gave results within the A. S. T. M. tolerances for all duplicates obtained in the same laboratory. Checks between different laboratories within A. S. T. M. tolerances were obtained for coals containing up to about 3.6 per cent mineral carbon dioxide, but these ashes contained larger amounts of retained sulfur.

The determination of ash by the cold furnace method (B and F) gave duplicate results within A. S. T. M. tolerances for all samples in the U. S. Bureau of Mines laboratory and for samples up to about 1.6 per cent mineral carbon dioxide content in the Illinois Geological Survey laboratory. Checks between average values from the two laboratories were within A. S. T. M. tolerances for all samples.

Results obtained by the modified Parr method were all well within A. S. T. M. tolerances for duplicates in the same laboratory. With the exception of results on one sample (5), the checks between the two laboratories were within A. S. T. M. tolerances.

The determination of ash by Method D gives duplicates checking within the 0.3 per cent tolerance for all samples.

The adequate removal of sulfur trioxide from the furnace during ashing is important.

As compared to results obtained by the modified Parr procedure, Methods A and E gave results within the A. S. T. M. 0.3 per cent tolerance for samples containing up to 0.6 per cent of carbon dioxide; Method B for coals up to 3.6 per cent of carbon dioxide; Method F for coals up to 4.2 per cent of carbon dioxide (with one exception); and Method D for coals containing up to 3.6 per cent of carbon dioxide.

Sulfur trioxide–free ash values were similar for all methods.

The slow heating method starting with a cold furnace appears to be satisfactory for determining ash in commercial samples of coal containing unusually large amounts of calcite and pyrite.

Conclusions

Methods A and E are not recommended for determining ash in coals high in calcite and pyrite, because too much sulfur is retained in the ashes.

Methods B, D, and F give most consistent results, with Method D apparently giving the best results of the three.

Methods C and G appear to give good results, especially for coals high in calcite and pyrite where other procedures studied are not so satisfactory. This procedure requires more work than other procedures.

Adequate removal of sulfur trioxide from the furnace in which ashing takes place is necessary.

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Measurement of Color and Turbidity in Solutions of White Granulated Sugars

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A practical method for the measurement of color and turbidity in solutions of granulated sugars is presented, a modification of the method of Keane and Brice. The two assumptions on which their method is based are shown to be not entirely justified. Color measurements with the new method are free from the influence of turbidity, and vice versa.

A STRONG need for an adequate method for the measurement of color and turbidity in white granulated sugar solutions has been felt for some time. The best practical method has been that of Keane and Brice (*2*), which as presented, however, has suffered from certain errors arising from the basic assumptions made.

Mathematical Relationships

The transmittancy of a turbidity-free sugar solution will be represented by T_c and that of a colorless sugar solution by T_t. For a solution containing both color and turbidity, the transmittancy, T, is given by the fundamental expression

$$T = T_c \times T_t \tag{1}$$

If the transmittancy measurements are made with light passed by different filters, say a blue-green and a red filter, then

$$T_g = T_{gc} \times T_{gt} \tag{2}$$

and

$$T_r = T_{rc} \times T_{rt} \tag{3}$$

The notation is obvious.

Keane and Brice assume that T_{rc} is constant and equal to 1.00 for solutions of white sugars, stating that there is virtually no light absorption in the red part of the spectrum by the small amount of coloring matter present. From this assumption and Equation 3 they obtain

$$T_r = T_{rt} \tag{4}$$

and set the turbidity index equal to the per cent absorbency of red light:

$$I_t = 100(1 - T_{rt}) = 100(1 - T_r) \tag{5}$$

They also set the ratio T_{gt}/T_{rt} equal to 1.00, although they state that it is only an approximation. Then from Equations 2 and 3, they obtain

$$T_g/T_r = T_{gc}/T_{rc} \times T_{gt}/T_{rt} = T_{gc}/1 \times 1 = T_{gc} \tag{6}$$

The color index is thus taken as the per cent absorbency of the blue-green light in a turbidity-free solution and by the assumptions above is expressed as

$$I_c = 100(1 - T_{gc}) = 100(1 - T_g/T_r) \tag{7}$$

Nees (*3*) was not able to substantiate the assumption of Keane and Brice that both T_{rc} and T_{gt}/T_{rt} equal 1.00. Nees suggested that experimentally determined factors be applied to correct the difficulty and proposed expressing color and turbidity in terms of percentage absorption of blue light. Nees' method is not satisfactory, in that the use of an additive

TABLE I. TRANSMITTANCIES OF FILTERED SOLUTIONS

Sugar Sample	Run	T_{rc}	T_{gc}
1	1	0.954	0.786
	2	0.949	0.786
	3	0.950	0.783
2	1	0.890	0.769
	2	0.888	0.764
3	1	0.875	0.669
	2	0.875	0.676
4	1	0.852	0.625
	2	0.855	0.637
5	1	0.884	0.731
	2	0.877	0.723
	3	0.886	0.723

relationship of the absorbencies due to color and turbidity cannot be employed if a long cell is used. If A is the absorbency, from Equation 1

$$1 - A = (1 - A_c)(1 - A_t) \tag{8}$$

whence

$$A = A_c + A_t - A_cA_t \tag{9}$$

If the absorbencies are low—as was the case with the short cell used by Nees—the term A_cA_t can be neglected. However, if a long cell is used and the absorbencies vary from roughly 0.15 to 0.50, the term A_cA_t cannot be neglected. The absorbencies obtained with a cell of the length used by Nees are not large enough to permit adequate photometric accuracy to be obtained (*5*).

It would seem that the best solution to the problem is to determine experimentally the relationships between T_{rc} and T_{gc} and between T_{rt} and T_{gt}. By means of these relationships and Equations 2 and 3, the color and turbidity indices can be calculated and expressed as

$$I_c = 100(1 - T_{gc}) \tag{10}$$

$$I_t = 100(1 - T_{rt}) \tag{11}$$

Thus the indices are expressed by equations of the same form as employed by Keane and Brice.

Description of Apparatus

A Lumetron photoelectric colorimeter, Model 402E, manufactured by the Photovolt Corporation, New York, N. Y., was used for the transmittancy measurements. The instrument was altered to take a 25-cm. cell and the light source was replaced by a 6- to 8-volt, single-filament automobile headlamp which was lighted by storage batteries.

The two filters used were the same as employed by Keane and Brice (Corning light shade blue-green, No. 428, 3.4 mm. thick, and Corning traffic red, No. 245, 3.05 mm. thick. The No. 428 filter used in this work was from melt 194). Data supplied by the manufacturer indicate that the transmission curves for the No. 428 filter are practically identical for different melts of glass. The No. 245 filter covers a narrower spectral range and should be readily reproducible. If necessary, slight adjustments in filter thickness can be made to correct for any difference in the melts.

The color temperature of the light source was arbitrarily set at 2485° Kelvin. It was measured with an Eastman color temperature meter. For convenience in routine work, the lamp temperature adjustment is made by noting the galvanometer deflection with the No. 428 filter in position. This was checked from time to time against the Eastman meter. Experiments showed that the measured transmittancies were not dependent to any marked extent on the color temperature of the lamp. A change of 160° in the temperature did not change the measured transmittancy with the red filter a measurable amount and changed the blue filter reading 1.9 per cent. The color temperature can probably be adjusted to within 15°, which is equivalent to a transmittancy variation of 0.2 per cent.

Experimental Procedure

The primary transmission standard used in this work was a colorless, turbidity-free 50 refractometer dry substance (R. D. S.) sugar solution. It was prepared from confectioners' sanding sugar by adding Darco decolorizing carbon to the hot solution, allowing it to cool, filtering on a Büchner funnel through No. 40 Whatman paper, and filtering through asbestos according to the recommendations of Peters and Phelps (*4*). In this work "turbidity-free" is applied to any solution which was filtered through the specially prepared asbestos. There has been much discussion in the literature regarding color adsorption of asbestos and other filtering media (*1, 4, 6*). However, experiments made by the authors indicate that any adsorption of coloring matter from solutions of granulated sugar by asbestos is slight and can be neglected without introducing serious error.

For convenience, a secondary transmission standard was prepared and standardized against the primary standard. Two 5-cm. squares of thin glass from photographic plates were bound together with the inner surfaces separated by a border mask of thin cardboard. The transmittancies of this absorber for red light and blue-green light were determined, taking the transmittancy of the standard sugar solution in the 25-cm. absorption cell as 1.000 in each case. The secondary standard was checked in this manner several times during the course of the work. In routine runs, the circuit of the Lumetron was readily balanced by using the secondary standard without the necessity of having standard sugar solutions always on hand.

Solutions of granulated sugar (50 R. D. S.) were prepared by mixing equal weights of sugar and boiling double-distilled water. After cooling to room temperature, the transmittancies of the solutions were measured in the 25-cm. cell using first the blue-green and then the red filter. The solutions were next filtered through asbestos and the transmittancies again determined.

Duplicate and triplicate runs made with several sugar samples show that the transmittancies of the asbestos-filtered solutions are reproducible (Table I).

A considerable number of colorless but turbid 50 R. D. S. sugar solutions were also run in the colorimeter. The turbidizing agent was prepared by adding a little fuller's earth to a colorless sugar solution, stirring well, and allowing to settle overnight. The supernatant liquid was decanted and used to turbidize other colorless sugar solutions. A few measurements were carried out on solutions made turbid with finely divided amorphous sulfur.

Transmittancy Measurements

Table II contains the results of the transmittancy measurements made before and after filtration. A large number of

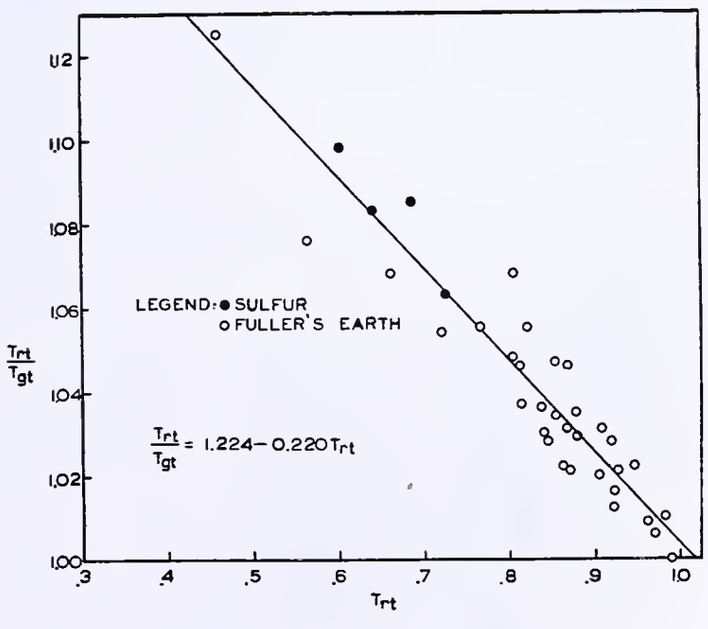

FIGURE 1. TRANSMITTANCY RELATIONSHIP FOR TURBID BUT COLORLESS SUGAR SOLUTIONS

samples of beet sugar from three beet factories and a lesser number of cane samples from a cane sugar refinery were used in the work. All the beet sugar samples were of white granulated sugar. The cane sugar samples were of varieties designated in Table II. In a number of cases transmittancy measurements were not made before filtration of the solutions through asbestos.

The ratio T_{rt}/T_{gt} was calculated from the relationship

$$T_{rt}/T_{gt} = \frac{T_r}{T_g} \times \frac{T_{gc}}{T_{rc}}$$

The results of the measurements on the turbid but colorless sugar solutions are given in Table III.

RELATIONSHIPS BETWEEN T_{rt} AND T_{gt}. The data in Tables II and III show plainly that T_{rt}/T_{gt} cannot be set equal to 1.00 if accuracy is desired. In cases of high turbidity the ratio departs markedly from 1.00, as is shown with samples 18, 20, and 32 from Factory 1, where the ratio is about 1.2. It is therefore necessary to establish from the experimental data the relationship between T_{rt} and T_{gt}. This relationship can be obtained from the data in Table II, but it is more advantageous to use the data in Table III which were obtained directly with artificial turbidity in colorless sugar solutions. In the first place, the use of artificial turbidity allows one to cover a greater range of turbidity and with greater uniformity. Secondly, it permits the use of a more nearly true turbidity rather than having to depend upon chance in using natural sugar turbidity. Often the low transmittancies observed with sugar solutions are due to fibers, large dust particles, and the like, especially if the samples have been stored in cloth bags. Even what might be called true turbidity is not constant in nature or particle size, but depends on its source and other factors.

It was found empirically that the relationship between T_{rt} and T_{gt} is best expressed by an equation of the form $T_{rt}/T_{gt} = k_1 + k_2T_{rt}$. The data in Table III were treated by the method of least squares and the equation obtained was

$$T_{rt}/T_{gt} = 1.224 - 0.220T_{rt} \qquad (12)$$

The average difference between the observed and calculated values of T_{rt}/T_{gt} is 0.008. The data have been plotted in Figure 1.

RELATIONSHIP BETWEEN T_{rc} AND T_{gc}. Early in this work it was realized by the authors that colored but turbidity-free solutions did not transmit all the light passed by the red (Corning No. 245) filter. It was at first thought that possibly some constant value of T_{rc} could be employed, but such did not prove to be the case. An analysis of the data in Table II showed that the relationship between T_{gc} and T_{rc} could be well expressed by an equation of the same type as was used for the turbidity ratio—namely, $\frac{T_{gc}}{T_{rc}} = k_3 + k_4 T_{gc}$. This relationship was obtained using only the data for the beet sugar samples, inasmuch as it was found that no significant difference was obtained between the equations for the individual beet plants, but that they did differ considerably from the equation obtained for the cane sugar samples. Thus it seems advisable to use one set of coefficients for beet sugar and one set for cane sugar. Most of the samples investigated in this work were beet sugar samples (107 beet, 16 cane) and the equation presented applies to beet sugar. Many more cane samples should be run to obtain a truly representative equation for cane sugar. The final equation obtained for beet sugar was

$$T_{gc}/T_{rc} = 0.310 + 0.673T_{gc} \qquad (13)$$

and the average difference between the observed and calculated values of the ratio is 0.007.

TABLE II. TRANSMITTANCIES

Sugar Sample	Transmittancies Before Filtration T_r	T_g	After Filtration T_{rc}	T_{gc}	T_g/T_r	T_{gc}/T_{rc}	$\frac{T_{gc}}{T_{rc}} - \frac{T_g}{T_r}$	T_{rt}/T_{gt}
			Beet Sugar Factory A					
1	....	....	0.888	0.688	....	0.775	....	...
2	....	....	0.889	0.703	....	0.791	....	...
3	....	....	0.893	0.682	....	0.764	....	...
4	....	....	0.916	0.720	....	0.786	....	...
5	....	....	0.924	0.775	....	0.839	....	...
6	....	....	0.937	0.775	....	0.827	....	...
7	....	....	0.906	0.704	....	0.777	....	...
8	....	....	0.880	0.661	....	0.751	....	...
9	....	....	0.926	0.771	....	0.833	....	...
10	0.807	0.632	0.925	0.759	0.783	0.821	0.038	1.05
11	0.680	0.465	0.901	0.670	0.684	0.744	0.060	1.09
12	0.807	0.637	0.931	0.752	0.789	0.808	0.019	1.02
13	0.672	0.486	0.911	0.730	0.723	0.801	0.078	1.11
14	0.722	0.527	0.897	0.693	0.730	0.773	0.043	1.06
15	0.630	0.437	0.874	0.669	0.694	0.765	0.071	1.10
16	0.726	0.561	0.912	0.735	0.773	0.806	0.033	1.04
17	0.811	0.660	0.909	0.697	0.740	0.767	0.027	1.04
18	0.601	0.383	0.876	0.678	0.637	0.774	0.137	1.22
19	0.687	0.517	0.854	0.612	0.753	0.717	−0.036	0.95
20	0.461	0.284	0.852	0.625	0.616	0.734	0.118	1.19
21	0.803	0.603	0.896	0.711	0.751	0.794	0.043	1.06
22	0.776	0.599	0.909	0.719	0.772	0.791	0.019	1.03
23	0.618	0.427	0.876	0.684	0.691	0.781	0.090	1.13
24	0.742	0.552	0.897	0.719	0.744	0.802	0.058	1.08
25	0.766	0.572	0.917	0.721	0.747	0.786	0.039	1.05
26	0.821	0.654	0.909	0.733	0.797	0.806	0.009	1.01
27	0.680	0.482	0.888	0.689	0.709	0.776	0.067	1.09
28	0.671	0.466	0.855	0.649	0.694	0.759	0.065	1.09
29	0.857	0.669	0.928	0.748	0.781	0.806	0.025	1.03
30	0.724	0.532	0.897	0.698	0.735	0.778	0.043	1.06
31	0.482	0.292	0.852	0.651	0.606	0.764	0.158	1.26
32	0.803	0.602	0.911	0.718	0.750	0.788	0.038	1.05
33	0.798	0.605	0.912	0.730	0.758	0.800	0.042	1.06
34	0.563	0.419	0.900	0.701	0.744	0.779	0.035	1.05
35	0.541	0.406	0.889	0.702	0.750	0.790	0.040	1.05
			Beet Sugar Factory B					
1	0.411	0.285	0.942	0.753	0.693	0.799	0.106	1.15
2	....	....	0.940	0.756	....	0.804	....	...
3	....	....	0.934	0.759	....	0.813	....	...
4	....	....	0.910	0.765	....	0.841	....	...
5	....	....	0.893	0.749	....	0.839	....	...
6	....	....	0.975	0.868	....	0.890	....	...
7	....	....	0.943	0.847	....	0.898	....	...
8	....	....	0.921	0.813	....	0.883	....	...
9	....	....	0.888	0.771	....	0.864	....	...
10	....	....	0.890	0.769	....	0.864	....	...
11	....	....	0.920	0.755	....	0.821	....	...
12	....	....	0.951	0.837	....	0.880	....	...
13	....	....	0.904	0.742	....	0.821	....	...
14	....	....	0.914	0.758	....	0.829	....	...
15	....	....	0.945	0.809	....	0.856	....	...
16	....	....	0.919	0.741	....	0.806	....	...
17	....	....	0.938	0.808	....	0.861	....	...
18	....	....	0.936	0.795	....	0.849	....	...
19	....	....	0.940	0.782	....	0.832	....	...
20	....	....	0.947	0.785	....	0.829	....	...
21	....	....	0.952	0.816	....	0.857	....	...
22	....	....	0.953	0.817	....	0.857	....	...
23	....	....	0.943	0.805	....	0.854	....	...
24	....	....	0.889	0.673	....	0.757	....	...
25	....	....	0.918	0.759	....	0.827	....	...
26	....	....	0.908	0.732	....	0.806	....	...
27	....	....	0.921	0.776	....	0.843	....	...
28	0.900	0.750	0.963	0.828	0.833	0.860	0.027	1.03
29	....	....	0.948	0.793	....	0.836	....	...
30	....	....	0.952	0.808	....	0.849	....	...
31	....	....	0.945	0.760	....	0.804	....	...

Derivation of Equations for Color and Turbidity Indices

If the color of a sugar solution is to be expressed as the per cent absorbency of blue-green light by the turbidity-free

OF VARIOUS SUGAR SOLUTIONS

Sugar Sample	Transmittancies: Before Filtration T_r	Before Filtration T_g	After Filtration T_{rc}	After Filtration T_{gc}	T_g/T_r	T_{gc}/T_{rc}	$\frac{T_{gc}}{T_{rc}} - \frac{T_g}{T_r}$	T_{rt}/T_{gt}
Beet Sugar Factory B (*Cont'd*)								
32	....	....	0.948	0.837	....	0.883	....	...
33	....	....	0.940	0.822	....	0.874	....	...
Beet Sugar Factory C								
1	0.849	0.688	0.931	0.764	0.810	0.821	0.011	1.01
2	0.862	0.702	0.933	0.772	0.814	0.827	0.013	1.02
3	0.846	0.682	0.932	0.763	0.806	0.819	0.013	1.02
4	0.871	0.711	0.944	0.767	0.816	0.813	−0.003	1.00
5	0.833	0.661	0.948	0.792	0.794	0.835	0.041	1.05
6	0.853	0.708	0.939	0.785	0.830	0.836	0.006	1.01
7	0.863	0.705	0.950	0.794	0.817	0.836	0.019	1.02
8	0.885	0.733	0.954	0.786	0.828	0.824	−0.004	1.00
9	0.876	0.707	0.942	0.773	0.807	0.821	0.014	1.02
10	0.842	0.672	0.930	0.762	0.798	0.819	0.021	1.03
11	0.846	0.695	0.923	0.757	0.822	0.820	−0.002	1.00
12	0.882	0.723	0.930	0.770	0.820	0.828	0.008	1.01
13	0.863	0.684	0.917	0.734	0.793	0.800	0.007	1.01
14	0.852	0.662	0.918	0.732	0.777	0.797	0.020	1.03
15	0.863	0.671	0.926	0.728	0.778	0.786	0.008	1.01
16	0.876	0.693	0.922	0.743	0.791	0.806	0.016	1.02
17	0.842	0.643	0.915	0.706	0.764	0.772	0.008	1.01
18	0.656	0.464	0.892	0.693	0.707	0.777	0.070	1.10
19	0.810	0.617	0.900	0.695	0.762	0.772	0.010	1.01
20	0.860	0.678	0.916	0.737	0.788	0.805	0.017	1.02
21	0.862	0.700	0.933	0.763	0.812	0.818	0.006	1.01
22	0.859	0.695	0.929	0.753	0.809	0.811	0.002	1.00
23	0.870	0.722	0.942	0.795	0.830	0.844	0.014	1.02
24	0.881	0.725	0.936	0.787	0.823	0.841	0.018	1.02
25	0.904	0.750	0.948	0.798	0.830	0.842	0.012	1.01
26	0.872	0.702	0.932	0.763	0.805	0.819	0.014	1.02
27	0.889	0.725	0.934	0.765	0.816	0.819	0.003	1.00
28	0.873	0.686	0.923	0.760	0.786	0.823	0.037	1.05
29	0.902	0.719	0.933	0.759	0.797	0.815	0.017	1.02
30	0.859	0.688	0.920	0.739	0.801	0.803	0.002	1.00
31	0.877	0.717	0.918	0.756	0.818	0.824	0.006	1.01
32	0.894	0.731	0.942	0.792	0.818	0.841	0.023	1.03
33	0.850	0.697	0.930	0.784	0.820	0.843	0.023	1.03
34	0.880	0.702	0.922	0.754	0.798	0.818	0.020	1.03
35	0.857	0.685	0.941	0.765	0.799	0.813	0.014	1.02
36	0.842	0.673	0.923	0.747	0.799	0.809	0.010	1.01
37	0.648	0.480	0.897	0.696	0.741	0.776	0.035	1.05
38	0.371	0.257	0.878	0.686	0.693	0.781	0.088	1.13
39	0.828	0.632	0.902	0.703	0.763	0.779	0.016	1.02
Cane Sugar Factory								
Cane granulated	....	....	0.884	0.731	....	0.827	....	...
Cane granulated	....	....	0.877	0.723	....	0.824	....	...
Cane granulated	....	....	0.886	0.723	....	0.816	....	...
Cane cubes	....	....	0.974	0.946	....	0.971	....	...
Extra hard cubes	....	....	0.981	0.945	....	0.963	....	...
Cocktail cubes	....	....	0.975	0.945	....	0.969	....	...
Tablets	....	....	0.985	0.962	....	0.977	....	...
No. 1. wet	0.885	0.773	0.962	0.870	0.873	0.904	0.031	1.04
No. 3 wet	0.849	0.703	0.935	0.797	0.828	0.852	0.024	1.03
No. 4 wet	0.822	0.602	0.916	0.717	0.732	0.783	0.051	1.07
Wet cube	0.948	0.928	0.983	0.968	0.979	0.985	0.006	1.01
Confectioners' A	0.886	0.827	0.967	0.933	0.933	0.965	0.032	1.03
Confectioners' AA	0.920	0.870	0.983	0.938	0.946	0.954	0.008	1.01
Sanding	0.907	0.868	0.985	0.953	0.957	0.968	0.011	1.01
Bar	0.768	0.624	0.895	0.747	0.813	0.835	0.022	1.03
Baker's	0.772	0.595	0.873	0.718	0.771	0.822	0.051	1.07

solution, it is necessary to calculate T_{gc} from the measured values of T_g and T_r, employing Equations 2, 3, 12, and 13. Similarly, the turbidity index can be calculated by finding T_{rt}.

After performing the algebra the following equations for T_{gc} and for T_{rt} are obtained:

$$T_{gc}^2 + T_{gc}[0.461 + T_g(0.220 - 1.819/T_r)] + 0.101T_g = 0 \quad (14)$$

$$T_{rt}^2 + T_{rt}[-5.564 - T_r(0.673 - 1.409/T_g)] + 3.744T_r = 0 \quad (15)$$

Only one root in each equation is significant. T_{gc} must be greater than or equal to T_g, but less than 1.00. Also T_{rt} must be greater than or equal to T_r, but less than 1.00.

A table was prepared by substituting various values of T_g and T_r in Equations 14 and 15 to obtain the transmittancies and then these were converted readily to per cent absorbencies, the units of the color and turbidity indices.

Comparison of Observed and Calculated Color and Turbidity Indices

A practical test of the method can be made by comparing the observed and calculated color and turbidity indices. From the data in Table II the color index is given as $100(1-T_{gc})$ and the turbidity index is $100(1 - T_r/T_{rc})$. In Table IV these indices are compared with the indices calculated from Equations 14 and 15, using the observed values of T_g and T_r.

The average difference between the calculated and observed color indices is 1.9 units; for the turbidity indices, it is 1.4 units. The agreement is especially gratifying in the cases of high turbidity.

Practical Application

The method has been applied for practical control work at the Woodland factory, and has functioned in a very satis-

TABLE III. TRANSMITTANCIES OF TURBID SUGAR SOLUTIONS

Type of Turbidity	T_{rt}	T_{gt}	T_{rt}/T_{gt}
Sulfur	0.726	0.682	1.064
	0.687	0.633	1.085
	0.641	0.592	1.083
	0.604	0.550	1.098
Fuller's earth	0.867	0.841	1.031
	0.841	0.817	1.030
	0.814	0.785	1.037
	0.804	0.767	1.048
	0.767	0.727	1.055
	0.983	0.973	1.010
	0.962	0.953	1.009
	0.947	0.927	1.022
	0.918	0.893	1.028
	0.854	0.826	1.034
	0.822	0.779	1.055
	0.742	0.704	1.054
	0.662	0.620	1.068
	0.564	0.524	1.076
	0.460	0.409	1.125
Fuller's earth (*Cont'd*)	0.990	0.990	1.000
	0.970	0.964	1.006
	0.908	0.881	1.031
	0.853	0.815	1.047
	0.805	0.754	1.068
	0.878	0.853	1.029
	0.868	0.830	1.046
	0.923	0.912	1.012
	0.927	0.908	1.021
	0.871	0.853	1.021
	0.863	0.844	1.022
	0.845	0.822	1.028
	0.813	0.777	1.046
	0.879	0.849	1.035
	0.924	0.913	1.012
	0.923	0.900	1.026
	0.838	0.809	1.036
	0.905	0.887	1.020

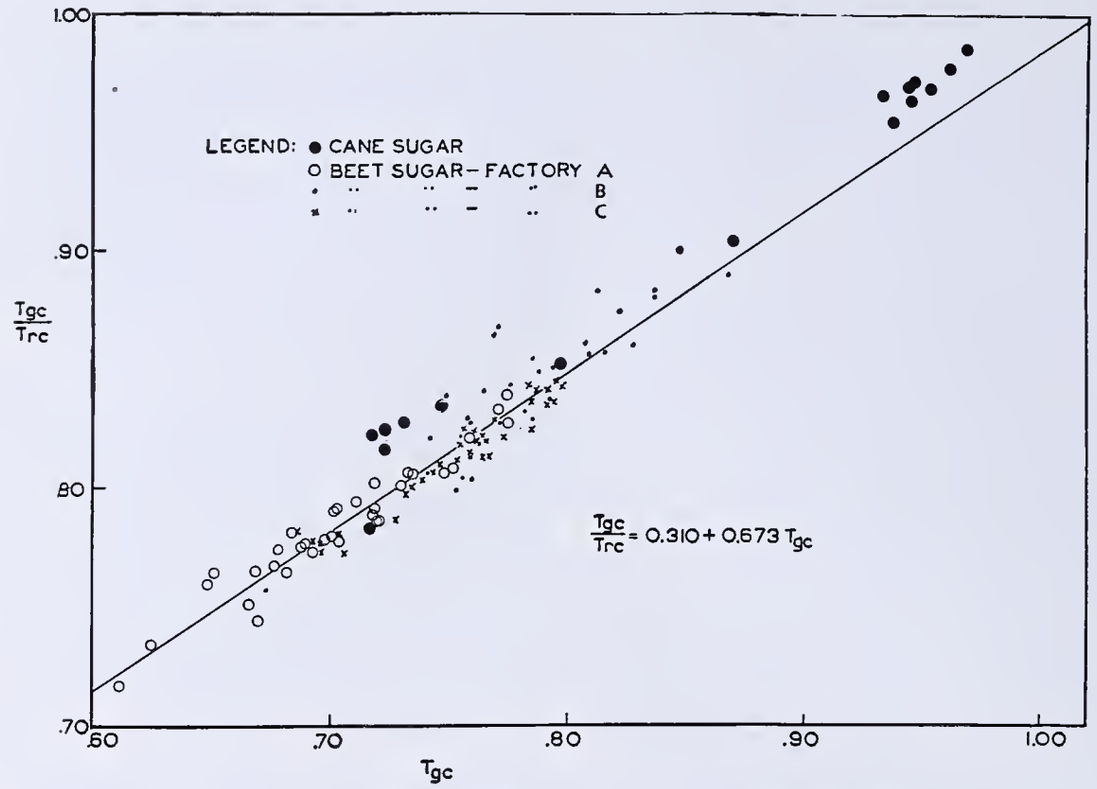

FIGURE 2. TRANSMITTANCY RELATIONSHIPS FOR SUGAR SOLUTIONS CONTAINING COLOR BUT NOT TURBIDITY

factory manner. About 7 minutes are required for each complete analysis.

One hundred and fifty grams of sugar are dissolved in an equal weight of hot distilled water, and stirred into solution. The hot solution is poured through a heat exchanger, from which it emerges at approximately room temperature. The cooled solution is poured into the absorption cell.

The color temperature of the light source is checked. The blue-green filter and secondary standard are inserted and the Lumetron is balanced to the required value. The secondary standard is removed, the absorption cell inserted, and a reading made of the transmission.

The same procedure is carried out with the red filter. From the two readings, the color and turbidity indices are obtained from tables. (Complete directions and copies of the tables will be furnished at cost to anyone interested.)

Discussion

The color and turbidity indices in this method are given as per cent absorbencies due to color or turbidity for an absorption cell 25 cm. in length. Naturally the values will differ for another cell length. It has been found, however, that a cell of this length is necessary for optimum absorption (*5*).

With control of the color temperature of the illuminant, and some care in duplication of light filters, there should be little difficulty in obtaining substantially the same results with different instruments.

In order to save time, the sugar samples are dissolved in

TABLE IV. COMPARISON OF OBSERVED AND CALCULATED COLOR AND TURBIDITY INDICES

Sugar Sample, Factory A	Color Index Observed	Color Index Calculated	Turbidity Index Observed	Turbidity Index Calculated
10	24	27	13	12
11	33	38	25	19
12	25	25	13	12
13	27	31	26	24
14	31	32	20	19
15	33	36	28	27
16	26	27	20	20
17	30	34	11	8
18	32	47[a]	31	25
19	39	28[a]	20	24
20	37	47[a]	46	42
21	29	32	10	10
22	28	28	15	14
23	32	36	30	28
24	28	31	17	17
25	28	33	17	14
26	27	25	10	11
27	31	36	23	21
28	35	36	22	22
29	25	29	7.7	5.4
30	30	32	19	19
31	35	48[a]	43	39
32	28	32	12	10
33	27	32	13	11
34	30	24[a]	37	38
35	30	22[a]	39	41
Factory C				
1	24	23	8.8	8.7
2	23	23	7.6	7.6
3	24	25	9.2	9.0
4	23	23	7.7	6.6
5	21	25	12	10
6	21	19	9.2	9.9
7	21	21	9.2	8.1
8	21	22	7.2	5.7

Sugar Sample, Factory C	Color Index Observed	Color Index Calculated	Turbidity Index Observed	Turbidity Index Calculated
9	23	24	7.0	5.4
10	24	25	9.5	8.7
11	24	21	8.3	10
12	23	22	5.2	5.5
13	27	27	5.9	6.2
14	27	29	7.2	5.8
15	27	29	6.8	4.7
16	26	28	5.0	4.0
17	29	31	8.0	5.8
18	31	37	27	25
19	30	29	10	9.7
20	26	27	6.1	5.5
21	24	23	7.6	7.6
22	25	23	7.5	7.2
23	20	21	7.6	7.4
24	21	21	5.9	6.0
25	20	21	4.6	4.3
26	24	25	6.4	5.7
27	23	22	4.8	4.0
28	24	27	5.4	4.2
29	24	26	3.3	1.6
30	26	25	6.6	6.7
31	24	22	4.5	5.5
32	21	22	5.1	3.6
33	22	21	8.6	9.3
34	25	26	4.6	3.7
35	23	25	8.9	6.9
36	25	25	8.8	9.0
37	30	29	28	28
38	31	29	58	54
39	30	31	8.2	6.7

[a] Solutions of these samples contained a variety of foreign matter, such as fibers and large and fine particles. Probably this is the reason for the large differences between calculated and observed values. Equations 14 and 15 cannot be expected to hold under such circumstances.

hot water. Tests have shown that this causes a slight increase in color, but as it is roughly constant, it is ignored.

There is no definite assurance that the exact relationships given in this paper will hold for coloring matter and turbidity present in beet sugars from other districts. If they do not, the correct relationships may readily be established by the methods described.

The problem is one of unusual complexity, and unfortunately no simple solution seems possible, barring the perfection of a method for rapid optical filtration of sugar solutions.

Acknowledgment

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Determination of K_2O in Commercial Fertilizers

Using 95 and 80 Per Cent Alcohol and Acid-Alcohol

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FOR several years many fertilizer chemists have believed that potash determinations made by the official method have given low results due to the use of 80 per cent alcohol and acid-alcohol in the determinations. The method to date has not been changed to correct this, even though the general referee on fertilizer of the A. O. A. C. has often recommended the investigation of the solubility of potassium chloroplatinate in acid-alcohol and alcohol.

Pierrat (*5*) gave the solubility of potassium chloroplatinate in various concentrations of alcohol at 14° C. but made no reference to the solubility at higher temperatures or under the conditions of the determination of the potash in fertilizers by the official method. Allen (*1*) reported a greater solubility of potassium chloroplatinate in 80 per cent than in 95 per cent alcohol but made no direct reference to the temperature, nor were the conditions identical with those involved in the determination of potash in fertilizers by the official method. Hughes and Ford (*4*) reported the solubilities of potassium chloroplatinate in 83 per cent alcohol and acid-alcohol at two different temperature levels, 18° and 38° C., but this work was done on potassium chloroplatinate precipitated from pure potassium chloride and was therefore not carried out under conditions exactly similar to those used in the determination of potash in fertilizers by the official method. Archibald, Wilcox, and Buckley (*2*) gave the solubilities of potassium chloroplatinate in alcohol-water mixtures at 20° C. but made no reference to acid-alcohol. Thus their results are not directly comparable to those obtained by the official method for potash in fertilizers. The above references, while important in themselves, are not general enough to include all the conditions to be met in the determination of potash in fertilizers by the official method.

The work reported in this study is a comparison of the potash contents of commercial fertilizers using 80 and 95 per cent alcohol. Since Hughes and Ford (*4*) reported increased solubility with rise of temperature, all work herein reported was done at 18° C. for both 80 and 95 per cent acid-alcohol and alcohol. Determinations were made on fertilizers of various analyses. A higher potash content was generally obtained when 95 per cent rather than 80 per cent acid-alcohol and alcohol were used.

Procedure

The study is divided into three steps:

1. Comparison of the K_2O values in mixed fertilizers by the official method (*3*, section 41, a), using aliquots of the same solution for both 80 and 95 per cent acid-alcohol and alcohol.
2. Comparison of the K_2O values in potash salts by two procedures.

Determination of the K_2O in potash salts by the method for potash salts (*3* section 41, b) using both 80 and 95 per cent acid-alcohol and alcohol.

Determination of the K_2O in potash salts, adding to the aliquot prepared for determination 1 gram of sodium chloride, by the method for potash salts (*3*, section 41, b) using both 80 and 95 per cent acid-alcohol and alcohol.

3. Comparison of the K_2O values in a high-analysis complete fertilizer using a collaborative check sample (*3*, section 41, a) with 80 and 95 per cent acid-alcohol and alcohol.

The same sintered-glass crucible (either Jena BG 3 or Pyrex M) was used in the filtration of the two aliquots of the same sample, using the concentrations of alcohol mentioned above. It was believed that this laboratory technique would avoid variations in filtration conditions that might occur had a padded Gooch crucible been used. All samples were washed with approximately the same amount of alcohol (125 ml.). All K_2O

Table I. Comparison of Averages of Potash Determinations of Mixed Fertilizers Using 80 and 95 Per Cent Alcohol

	Low Complete Fertilizers, 12–18 Units[a]		Medium Complete Fertilizers, 19–24 Units[b]		High Complete Fertilizers 25–40 Units[c]		Medium Phosphate and Potash Fertilizers, 19–24 Units[d]		High Phosphate and Potash Fertilizers, 25–40 Units[e]		Potash Salts 50 Units[f]	
	95% alcohol	80% alcohol	95% alcohol	80% alcohol	95% alcohol	80% alcohol	95% alcohol	80% alcohol	95% alcohol	80% alcohol	95% alcohol	80% alcohol
No. of analyses averaged	10	10	51	51	63	63	37	37	44	44	16	16
Average result, % K_2O	3.80	3.67	6.52	6.40	13.91	13.73	11.40	11.21	20.94	20.78	50.96	50.73
Average difference between alcohols, %	0.13	....	0.12	....	0.18	...	0.19	...	0.16	...	0.23	...
Indivdual sample difference between alcohols, %	0.02	....	0.00	....	0.02	...	0.03	...	0.02	...	0.17	...
	−0.33	....	−0.32	....	−0.31	...	−0.31	...	−0.34	...	−0.31	...
Percentage K_2O increase	3.54	....	1.88	....	1.36	...	1.70	...	0.77	...	0.45	...

Units distributed among the following analyses: [a] 3-8-6, 2-10-4, 2-12-2, 1-8-3, 2-8-6, 1-12-2, 1-14-2. [b] 2-12-6, 4-10-6, 4-12-4, 5-10-5, 6-8-6, 4-8-8, 4-16-4. [c] 3-12-12, 17-8-8, 2-12-12, 2-8-16, 3-6-18, 3-8-16, 3-9-18, 4-24-12, 3-18-9, 2-16-8. [d] 0-14-6, 0-12-12, 0-10-10, 0-8-16. [e] 0-21-9, 0-8-24, 0-20-20, 0-10-20, 0-16-24, 0-10-30. [f] 0-0-50.

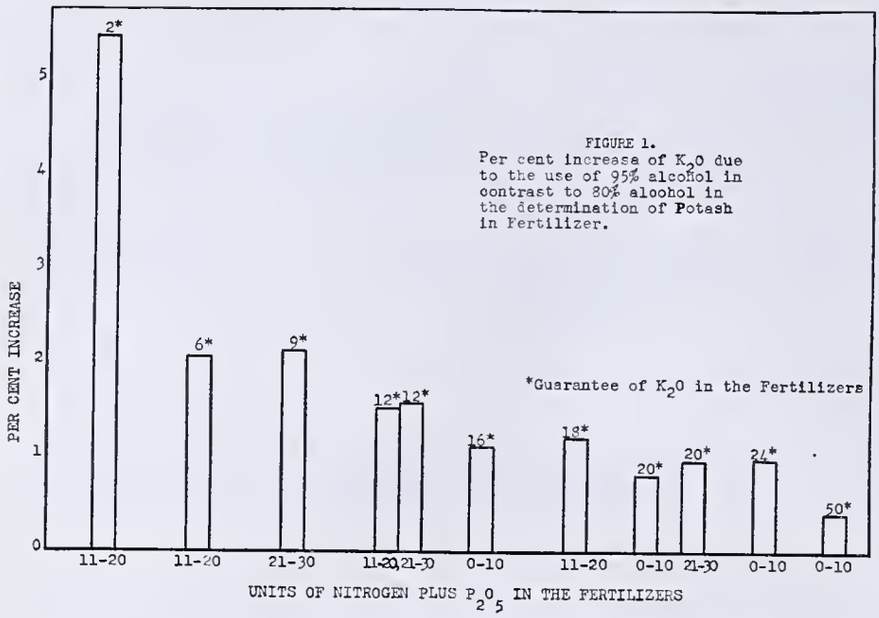

FIGURE 1.
Per cent increase of K_2O due to the use of 95% alcohol in contrast to 80% alcohol in the determination of Potash in Fertilizer.

Figure 1 shows the percentage increase of K_2O due to the use of 95 per cent acid-alcohol and alcohol in contrast to 80 per cent in the determination of K_2O in the fertilizers. Data reported in this graph show that there is a greater K_2O recovery with 95 per cent alcohol in the case of fertilizers having a low potash content than in the case of those having a high potash content. At the same time there is some indication that where the salt content is high in a potash determination the use of 95 per cent alcohol may give too high potash values, owing to the insolubility of the salts in the 95 per cent alcohol. This explanation is supported by the data in Table II, which gives the results obtained when sodium chloride is added.

Table II reports the comparison of the averages of determinations of potash in potash salts and on aliquots of the same salt to which has been added an equal amount of sodium chloride. Whereas a difference of 0.23 per cent greater was found when 95 per cent alcohol was used in place of 80 per cent, with the addition of the sodium chloride the difference increases to 0.84 per cent. This difference will usually be encountered unless the sample is washed with a larger volume of alcohol and it logically follows that it would require a larger volume of 95 per cent than of 80 per cent alcohol to wash out the excess sodium salt if the difference can be entirely attributed to some sodium salt.

Table III reports the averages of 12 potash determinations on a collaborative check sample representing a high-analysis complete fertilizer containing more than 40 units of plant food. In this case 0.16 per cent more potash is obtained with 95 per cent alcohol than with 80 per cent alcohol, in agreement with results obtained on fertilizers of similar analyses reported in Table I.

A study of the individual sample differences between alcohols of the three tables indicates that in some cases 95 per cent alcohol gave little if any increase in potash content when compared to 80 per cent alcohol, but in most cases a significant increase was evident.

TABLE II. COMPARISON OF AVERAGES OF POTASH IN POTASH SALT DETERMINATIONS

(With and without the addition of sodium chloride using 80 and 95 per cent alcohol)

	Potash Salts, 50 Units		Potash Salts, 50 Units, NaCl Added	
	95% alcohol	80% alcohol	95% alcohol	80% alcohol
No. of analyses averaged	16	16	16	16
Average result, % K_2O	50.96	50.73	52.03	51.19
Difference between alcohols, %	0.23	...	0.84	...
Individual sample difference between alcohols, %	0.17– 0.31	...	0.21– 1.05	...
Percentage K_2O increase	0.45	...	1.64	...

TABLE III. COMPARISON OF POTASH VALUES ON A COLLABORATIVE CHECK SAMPLE

	Collaborative Fertilizer, Check Sample, 42 Units	
	95% alcohol	80% alcohol
No. of analyses averaged	12	12
Average result, % K_2O	17.52	17.36
Difference between alcohols, %	0.16	...
Individual sample difference between alcohols, %	0.04– 0.28	...
Percentage K_2O increase	0.92	...

values were determined by dissolving the potassium chloroplatinate in hot water and reweighing the crucible.

Discussion of Results

Table I shows the comparison of the averages of determinations of potash, using 80 and 95 per cent acid-alcohol and alcohol. These results were obtained on low-, medium-, and high-grade complete fertilizers which contained 12 to 40 units of plant food; medium- and high-grade phosphate-potash mixtures which contained 20 to 40 units of plant food; and potash salts containing about 50 units of water-soluble potash.

The differences between results reported using 80 per cent as compared to those using 95 per cent alcohol range from 0.13 per cent in the case of low-grade complete analyses to 0.18 per cent for the high-grade complete analyses (Table I). A gain of 0.19 per cent was observed in the case of medium phosphate and potash fertilizers and of 0.16 per cent in the case of high phosphate and potash fertilizers due to the use of 95 per cent acid-alcohol and alcohol. An average difference of 0.23 per cent in favor of the use of 95 per cent alcohol was noted in the potash salt determinations.

Summary

There is definitely a greater potash value obtained with 95 per cent in place of 80 per cent alcohol in the determination of potash in fertilizers by the official method. This difference for fertilizers of 12 to 40 units of plant food ranges from 0.13 to 0.19 per cent K_2O. For muriates of potash there is an average difference of 0.23 per cent in favor of the use of 95 per cent alcohol.

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Derivatives of Amytal, Pentobarbital, and Dial

An Optical Crystallographic Study

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IN A PREVIOUS study the optical properties of certain derivatives of barbital and luminal were presented (*3*). Since chemical tests are not entirely satisfactory for the identification of some of the newer derivatives of barbituric acid, the optical crystallographic study has been extended to include three additional barbiturates: amytal (5-ethyl-5-isoamyl barbituric acid), pentobarbital (5-ethyl-5-alpha-methyl butyl barbituric acid), and dial (5,5-diallyl barbituric acid). Benzyl and phenacyl derivatives were prepared from each of the compounds under investigation. None of the derivatives presented in this paper, however, has been reported in the literature with the exception of *p*-nitrobenzyl compounds. These derivatives for amytal and dial have been prepared by Lyons and Dox (*5*), Hargreaves and Nixon (*2*), and Jesperson and Larsen (*4*), and for pentobarbital by Hargreaves and Nixon.

Each new compound was analyzed for halogens or nitrogen. The melting points were run by both the tube method and the "Bloc Maquenne" method. A determination for water of crystallization was made on each of the compounds prepared.

The *p*-nitrobenzyl, *p*-bromobenzyl, and *p*-chlorobenzyl derivatives were prepared with each of the medicinal substances. The analytical results are presented in Table I.

Optical Crystallographic Data

The optical properties of each compound listed in Table I were determined by methods used in the previous study for compounds of barbital and luminal (*3*), and are presented in Table II. The optical properties of the derivatives of the three substances differ sufficiently from each other to allow the use of the optical data in the identification of the original barbituric acid derivative. No previous optical crystallographic data have been reported for the derivatives of amytal, pentobarbital, or dial. Incomplete data on dial itself have been listed by Haas (*1*).

TABLE I. ANALYTICAL DATA

Compound	Formula	Crystal Habit	Color	Nitrogen Calcd. %	Nitrogen Found %	Halogen Calcd. %	Halogen Found %	Melting Points Uncorrected Tube	Melting Points Uncorrected Block
Amytal	$C_{11}H_{18}N_2O_3$	Rods	White	...	... ...	...	... ...	156.5	157
p-Nitrobenzyl derivative	$C_{25}H_{28}N_4O_7$	Plates	Pale yellow	11.29	11.18, 11.25	...	... ...	169	172
p-Bromobenzyl derivative	$C_{25}H_{28}N_2O_3Br_2$	Rods and plates	White	...	... ...	28.33	28.47, 28.29	133	134
p-Chlorobenzyl derivative	$C_{25}H_{28}N_2O_3Cl_2$	Rods and plates	White	...	... ...	14.92	14.87, 15.03	102–105	102
Pentobarbital	$C_{11}H_{18}N_2O_3$	Needles	White	...	... ...	...	... ...	130	130
p-Nitrobenzyl derivative	$C_{25}H_{28}N_4O_7$	Rods and plates	Pale yellow	11.29	11.28, 11.23	...	... ...	153	151
p-Bromobenzyl derivative	$C_{25}H_{28}N_2O_3Br_2$	Thick plates	White	...	... ...	28.33	28.28, 28.40	114	114
p-Chlorobenzyl derivative	$C_{25}H_{28}N_2O_3Cl_2$	Thick plates	White	...	... ...	14.96	15.02, 15.20	111	111
Dial	$C_{10}H_{14}N_2O_3$	Plates	White	...	... ...	...	... ...	171.5	173
p-Nitrobenzyl derivative	$C_{24}H_{24}N_4O_7$	Plates	Pale yellow	11.67	11.50, 11.64	...	... ...	191.5	192
p-Bromobenzyl derivative	$C_{24}H_{24}N_2O_3Br_2$	Rods and plates	White	...	... ...	29.16	28.95, 29.11	132.5	133
p-Chlorobenzyl derivative	$C_{24}H_{24}N_2O_3Cl_2$	Flat rods	White	...	... ...	15.45	15.60, 15.46	125–134	125–134

TABLE II. OPTICAL CRYSTALLOGRAPHIC DATA

Compound	Optical Sign	Elongation	Refractive Indices at 25° Alpha	Beta	Gamma	Rhombic Dispersion	Crystal System
Amytal	−	+	1.467	1.533	1.560	None	Monoclinic
p-Nitrobenzyl derivative	−	±	1.510	1.640	1.656	V > P	Monoclinic
p-Bromobenzyl derivative	+	±	1.559	1.573	1.662	V > P	Monoclinic
p-Chlorobenzyl derivative	+	±	1.539	1.574	1.694	None	Monoclinic
Pentobarbital	−	−	1.469	1.528	1.569	None	Monoclinic
p-Nitrobenzyl derivative	?[a]	+	1.548	?[a]	1.696	?[a]	Monoclinic
p-Bromobenzyl derivative	−	±	1.527	1.638	1.702	None	Monoclinic
p-Chlorobenzyl derivative	−	±	1.523	1.631	1.680	None	Monoclinic
Dial	−	−	1.518	1.567	1.610	None	Monoclinic
p-Nitrobenzyl derivative	−	+	1.511	1.658	1.699	V > P	Monoclinic
p-Bromobenzyl derivative	+	−	1.569	1.589	> 1.700	None	Monoclinic
p-Chlorobenzyl derivative	+	±	1.560	1.592	> 1.700	None	Monoclinic

[a] Since no optic axis interference figures could be seen, these values could not be determined.

Preparation and Analysis

The benzyl and phenacyl derivatives were prepared in the following manner:

Equivalent amounts of the barbituric acid derivative and potassium or sodium carbonate were dissolved in a little more than enough boiling water to make a saturated solution. This solution was added to a solution of the benzyl or phenacyl halide (two molecular quantities) dissolved in an amount of alcohol twice as large as the amount of water used to dissolve the barbituric acid salt. The resulting mixture was refluxed until the reaction was completed. Crystals of the derivative usually separated out of the solution during the heating. After being cooled, the derivatives were separated by filtration and purified by recrystallization from a mixture of chloroform and alcohol until there was no change in the melting point of the substance. The crystals were dried in air, and none of the compounds contained water of crystallization.

Acknowledgment

The authors wish to thank the Eli Lilly and Company for the amytal and pentobarbital, and the Ciba Company, Inc., for the dial.

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Type Analysis of Hydrocarbon Oils

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ORDINARILY, a sample of petroleum oil above the gasoline range contains so many individual hydrocarbons that their separate identification amounts to a lengthy program of research. However, such detailed knowledge is not necessary, since the usefulness of the oil is usually governed by the relative distribution of types—i. e., by average constitution. In industrial operations such as cracking or acid treatment the aim is the transformation of such types rather than of individual compounds. In the control of such operations, use of an accurate type analysis offers distinct advantages.

While there are in use a number of methods for analyzing gasolines and other light fractions in terms of paraffins, naphthenes, olefins, and aromatics, the only method which is generally applied to heavy oils—i. e., through the lubricant range—is that of Vlugter, Waterman, and van Westen (*15*). However, the relation of aromaticity to aniline point, whose assumed constancy is one of the bases of the original method, has been recently found to be variable (*16*). Moreover, the method is not intended for oils whose unsaturation is olefinic rather than aromatic, and hence is not applicable to many cracked oils.

The purpose of the present work was to attempt to provide a more accurate and widely applicable system of analysis by strictly defining the terms expressing composition and developing new correlations through which values of them may be derived from physical tests. The general procedure of analysis, which the method developed here shares with that of Waterman and his colleagues, is:

1. Evaluation of aromaticity or unsaturation of the sample.
2. Determination of the structure of the sample when completely saturated. This combined with 1 permits a segregation of the carbon atoms as aromatic, naphthenic, and paraffinic.

The more critical step is the evaluation of aromaticity or other unsaturation. If this is not correct, the predicted structure of the saturates, which depends on it, cannot be correct. It is shown below that unsaturation (whether olefinic or aromatic) may be calculated from physical measurements by the use of two empirically established relations. The analysis may then be completed by purely mathematical processes.

Unsaturation or Aromaticity

The Lorenz-Lorentz molecular refraction is defined as

$$Mr = M \times \frac{n^2 - 1}{n^2 + 2} \times \frac{1}{\mathrm{d}}$$

in which M is molecular weight, d is specific gravity (here at 20° C. referred to water at 4° C.), and n is refractive index (here at 20° C., for sodium D line).

A recent brief discussion of this function was given by Brode (*6*). Brühl (*3*), Eisenlohr (*4*), and others established from much evidence on pure compounds that the molecular refraction for any individual saturated hydrocarbon may be obtained by additively combining constant increments for the carbon and hydrogen atoms present, providing there are no strains from ring structures other than 5- or 6-membered. (These are believed to be the predominant ring structures in petroleum, *2*, *14*.) Further, there is a certain theoretical basis for this additivity, as discussed by Hückel (*7*), Nernst (*13*), etc. A mathematical statement of the relationship for a hydrocarbon C_nH_{2n+x} is

$$Mr = An + Bx \tag{1}$$

in which A and B are constants.

The usefulness of this relationship for our purposes depends on its validity when applied to actual oils—that is, multicomponent mixtures of hydrocarbons. It has therefore been tested and its constants established in such an application. For this to be done it was convenient to transform Equation 1 to

$$r = a + by \tag{2}$$

in which a and b are new constants and y is weight per cent of hydrogen, defined by

$$y = \frac{1.008\ (2n + x)}{M} \times 100$$

If a plot of r against y for a number of saturated oils yields points falling closely about a straight line then Equation 2, and hence also 1, are indicated to be generally valid.

Figure 1 shows such a plot; the data used were compiled from the work of a number of investigators, and represent a thorough search of the chemical literature. Sources are listed in the caption with the reasons, in brief, for accepting each group as fully saturated. As may be seen, the points fall within a narrow zone. It appeared justifiable, from the evidence presented in this figure, to select a line down the center of the zone of scatter as the practical locus of all saturates. The equation of this line (shown on the figure as a full line) is

$$r = 0.2084 + 0.008421y \tag{3}$$

The line derived from the slightly different molecular refraction constants used by Waterman is also indicated.

It was then necessary to extend the expression for specific refraction to double-bond unsaturation, and thus make it generally applicable to all hydrocarbons which occur in appreciable amounts in petroleum. It has been recognized that an additive increment of molecular refraction can be assigned to each double bond, though the value of the term varies with the kind of double bond. Denoting the variable increment by k, Equation 1 may be amplified by writing

$$Mr = An + Bx + kz$$

in which z is the number of double bonds per molecule. Then on dehydrogenating any saturated oil whose specific refraction is originally expressed, to a very close approximation, by Equation 3, the specific refraction changes to

$$r = 0.2084 + 0.008421y + \frac{kz}{M}$$

Or, expressing by h the unsaturation in grams of hydrogen per 100 grams of sample, so that

$$h = \frac{201.6z}{M} \tag{4}$$

then

$$r = 0.2084 + 0.008421y + \frac{hk}{201.6} \tag{5}$$

Because the quantity k has been left variable, with no restrictions imposed on it, Equation 5 is fully as accurate as 3 and is a defining equation for k.

As mentioned above, the first step in a type analysis is the determination of unsaturation or aromaticity. (Olefins are treated as partial aromatics for the present.) Equation 5 provides an expression for h in terms of r, y, and k. Of these three quantities the first two may be measured by familiar

TABLE I. VALUES OF k FOR REPRESENTATIVE HYDROCARBONS

Hydrocarbon	k at 20° C.
Cyclopentene	1.46
Alkyl cyclohexenes	1.40–1.85
Aliphatic monoolefins	1.60–1.90
Alkyl benzenes	1.60–1.90
Indene	1.89
Biphenyl	1.91
Alkyl naphthalenes	2.10–2.25
Alkyl anthracenes	2.60–2.90

methods. Only an evaluation of k is needed, then, to complete the determination.

Study of a large body of data from the literature on individual hydrocarbons shows each series to yield a characteristic range of values of k (as calculated from Equation 5). To each series a mean may be assigned which remains substantially constant with increasing M, except for generally low values for the first member or two members of a homologous series. Some of these values are given in Table I.

In the special case in which the class of unsaturates present in a sample is known with certainty, an appropriate value of k can be assigned from these values. However, in general, not enough is known about the composition of a sample to permit this assignment to be made. A means of making a separate measurement of k for each particular sample is needed.

In this connection, possible uses of the refractive dispersion were considered. A convenient expression of this property is that of Gladstone and Dale

$$S = \frac{n_F - n_C}{d} \times 10^4$$

Here n_F and n_C are refractive indices for the F and C hydrogen lines, and d is the specific gravity referred to water at 4° C. The temperatures of measurement should be the same, though the absolute value is not especially important.

Trends in various dispersion functions have long been recognized; Auwers (*1*) has exhaustively studied the dispersions of various hydrocarbon types. Some relation between dispersion and constitution has been indicated, but no satisfactory formulation of this relation has hitherto been made. In the course of the present work, it was found impossible to assign additive increments of molecular dispersion analogous to the additive increments of molecular refraction and the effort was

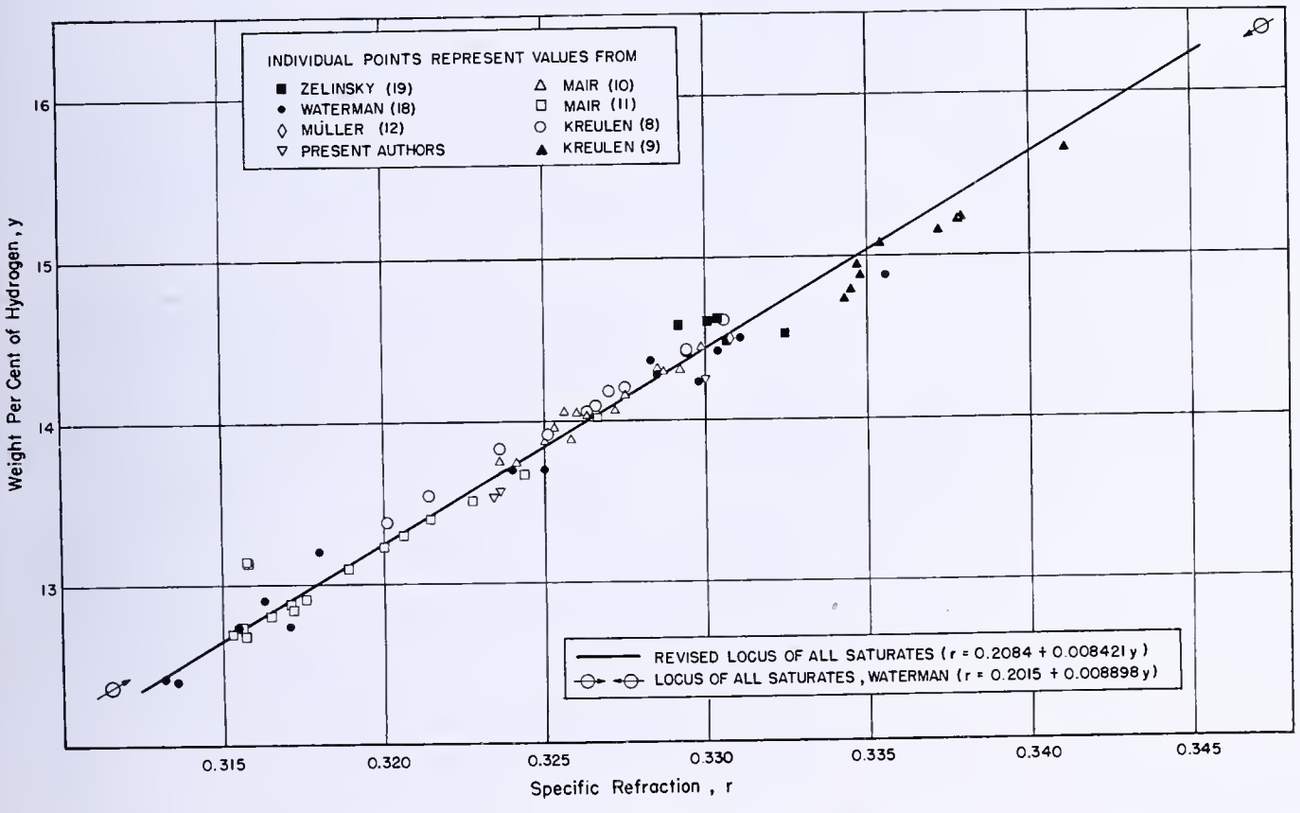

FIGURE 1. SPECIFIC REFRACTION–COMPOSITION RELATIONSHIP FOR SATURATED OILS

Zelinsky and Kazansky (*19*). Baku petroleum fractions were freed from aromatics by acid treatment, dehydrogenated, and acid-treated once more until, assertedly, only paraffins and 5-membered saturated rings remained. All fractions boiled within the range 160° to 200° C.

Waterman and Leendertse (*18*). Data on a large variety of hydrocarbon mixtures are summarized; all have been reported previously. Polymerization products from such varied hydrocarbons as isobutylene, tetralin, and pinene, as well as a number of natural oil fractions, were "completely hydrogenated". Complete saturation is stated to be proved by specific dispersions and, in some cases, by aniline points. The authors' rough conversions of the dispersions (stated for all but two samples), in yielding values of S consistently under 100, seem to confirm this. Molecular weights range from 238 to 645.

Müller and Neyman-Pilat (*12*). A Pennsylvania oil was repeatedly extracted with nitrobenzene and the raffinate was hydrogenated seven times, yielding a product of 479 molecular weight.

These laboratories (unpublished). Several oil fractions were exhaustively extracted, yielding raffinates with values of S under 100. Molecular weights range from 270 to 380. Source materials were Poso, Calif., and Rodessa, Texas, crudes.

Mair and Schicktanz (*10*). The water-white oil obtained from silica-gel absorption of a mid-continent crude fraction was subjected to fractional distillation and the resulting fractions were intensively extracted with acetone. All extraction cuts represented in Figure 1 are among those judged by the authors, on the basis of S values (100 or less), to be saturated. Molecular weights range from 385 to 537.

Mair, Willingham, and Streiff (*11*). Narrow cuts from the methyl cyanide extraction of an extract portion of the same crude were repeatedly hydrogenated to values of S of approximately 100. The molecular weights of the hydrogenated fraction range from 309 to 455.

Kreulen (*8*). A number of gas oils, of undesignated origins, were given "complete" hydrogenations. Authors' corrections of the dispersion data yield values of S varying between 96 and 100. Molecular weights range from 184 to 261.

Kreulen (*9*). Borneo paraffin was cracked ("care was taken that no cyclization occurred") in the presence of hydrogen, and the residue was separated into fractions by distillation. The value of S for each fraction (by authors' conversion) is 100 or 101. Aniline point–molecular weight data seem to confirm the fact that the samples are wholly paraffinic (they are asserted to be normal-paraffin mixtures). No data for y were obtained, but the evidence is considered good enough to justify calculating this quantity from molecular weights, on the assumption of the formula C_nH_{2n+2}. Molecular weights range from 138 to 498.

Table II. Specific Dispersions of Representative Hydrocarbons

Compound	S
1-Hexene	121
Benzene	189
Toluene	185
Indene	224
Biphenyl	269
Naphthalene	303
1-Methylanthracene	521

abandoned. Table II, giving values for certain unsaturated hydrocarbons, illustrates this difficulty.

Accordingly, another approach to the use of dispersion was tested. It is generally accepted that the Gladstone-Dale specific dispersion of all saturates is approximately 98 (*5*, *17*). For any unsaturate, then, the increase of molecular dispersion is $M(S - 98)$; the increment per double bond is $\frac{M(S-98)}{z}$. From Equation 4, this is a constant multiple of $\frac{(S-98)}{h}$, which we have designated the dispersivity. The increment of molecular refraction per double bond is, of course, k.

In Figure 2 the dispersivity is plotted against k for the large body of hydrocarbons for which values of k were tabulated earlier. Data of Mair on a series of extraction cuts in the lubricating oil range are also shown. These are the only data in the literature on oil samples considered complete and accurate enough to be included. As may be seen, the points, both for pure compounds and oils, fall with slight scatter about a unique line. Accordingly, a best mean line was drawn for all individual pure hydrocarbons shown on the figure and considered verified by the data on oil mixtures. This is the line shown; its equation was found to be

$$k = 0.811 \left[\frac{S - 98}{h}\right]^{0.292} \qquad (6)$$

Though the analogy between k and dispersivity pointed out above only suggests and does not require a relationship between them, a relationship is established empirically by the data of Figure 2.

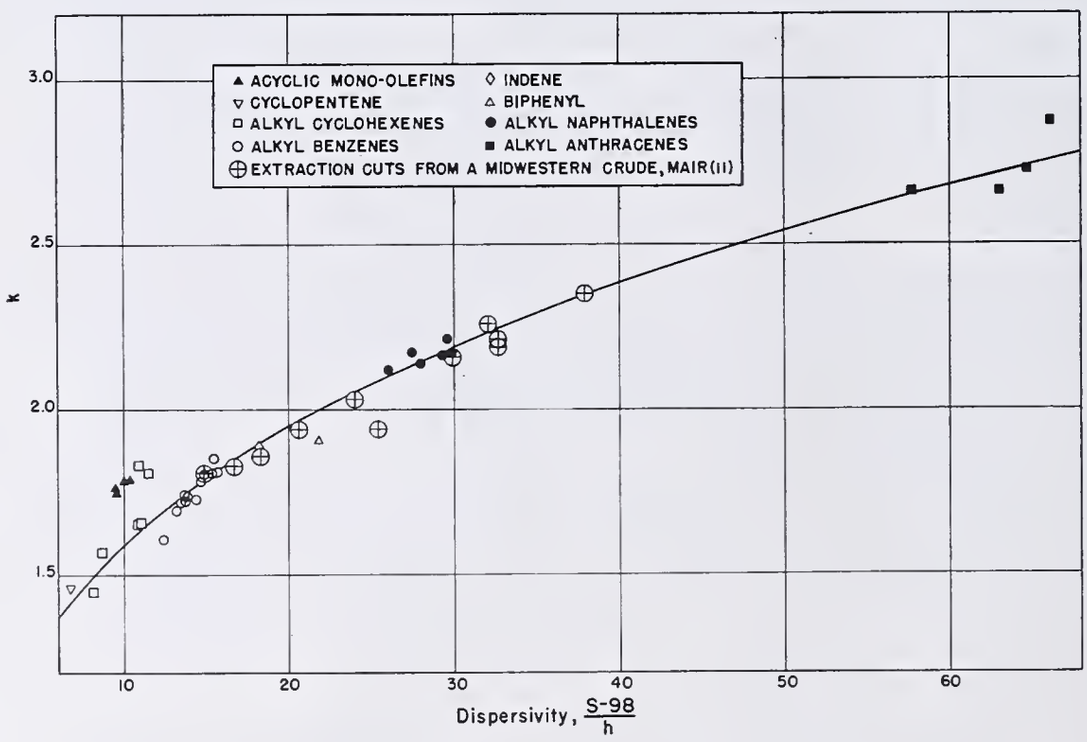

Figure 2. Relationship between k and Dispersivity for Unsaturated Hydrocarbons

For a complete expression of h in terms of measurable quantities r, y, and S, it is thus necessary only to eliminate k between Equations 6 and 5. Algebraic treatment is lengthy; a pair of nomographs by which the equations may be solved is provided in Figure 3. The analysis is hereafter designated the nomographic analysis.

Completion of Analysis

The preceding section developed a method of deriving the unsaturation, h, in terms of the measurable quantities, r, y, and S. To complete the plan of analysis, expressions of the percentages of aromatic, naphthenic, and paraffinic carbon atoms per molecule are required. For these a further measurement, that of molecular weight, is needed. Assuming for the moment that the unsaturates are wholly aromatic, the percentage of aromatic carbon atoms per molecule, for a hydrocarbon mixture of average formula C_nH_{2n+x}, is defined by $A = 100\left[\frac{2z}{n}\right]$. Then from the further simple constitutional relations (*4*) and

$$n = \frac{M(100 - y)}{1201.0}$$

there results

$$A = \frac{1191.5h}{100 - y} \qquad (7)$$

The number of aromatic carbon atoms per molecule is, of course, $An/100$.

The next step is to express the average number of rings per molecule in terms of quantities now known. Denoting this number by R,

$$2R + 2z + x = 2$$

or

$$R = \frac{2 - 2z - x}{2}$$

Substituting for z from Equation 4 and for x the expression (derived from the atomic weights for H, 1.008, C, 12.010, and CH_2, 14.026)

$$x = \frac{\frac{0.14026}{1.008}y - 2}{12.010}M$$

there results

$$R = 1 - \frac{Mh}{201.6} + \frac{M}{24.212} \times \left[2.016 - 0.14026y\right] \qquad (8)$$

At this point in the development an inevitable ambiguity arises. We can determine the number of rings per molecule but we cannot say how many carbon atoms there are per ring. If, for example, the molecule contains the naphthalene or decalin group there are ten carbon atoms in two rings or five per ring, while in benzene, diphenyl, or cyclohexane there are six per ring. While there is an accumulation of circumstantial evidence that petroleum contains no substantial

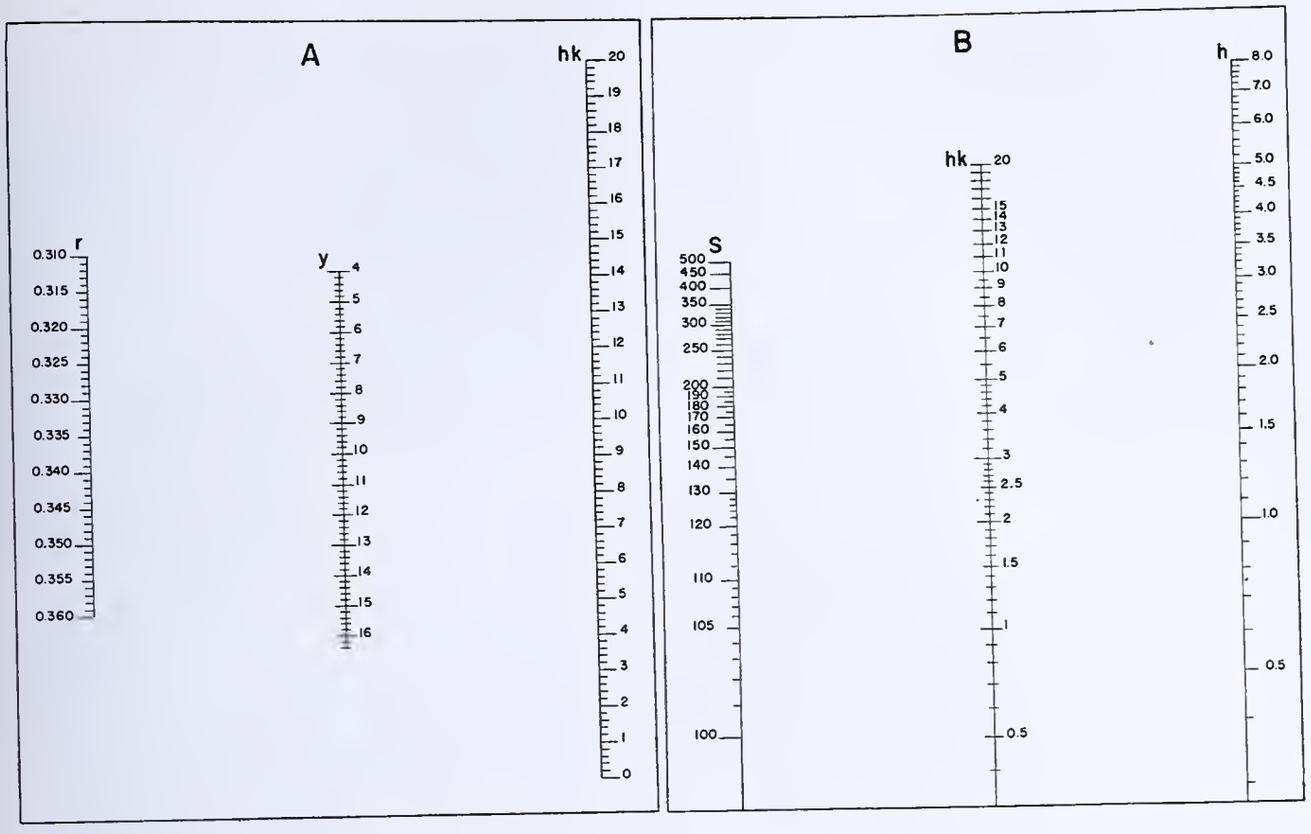

FIGURE 3. NOMOGRAPHIC CHARTS FOR SOLUTION OF GENERAL EQUATIONS

$$A,\ hk = 201.6r - 1.6978y - 42.02$$

$$B,\ h = \left[\frac{hk}{0.811\ (S - 98)^{0.292}}\right]^{1.412}$$

amount of other than 5-membered or 6-membered rings, we do not know the extent to which ring fusion and ring linkage occur. If, therefore, the analysis of the oil is to be formally completed an assumption of the number of carbon atoms per ring must be made at this point.

The number will be variable, obviously, depending on the ring types present. It was finally decided that it is best to make a separate assumption for each sample. In making this assumption, any available foreknowledge of the sample should be considered; likewise the value of k, which Figure 2 shows to be distinctly influenced by the degree of fusion, may be used—for example, values of about 2.5 denote a high degree of fusion, values below 2.0 a low degree.

In the method of Waterman the assumption is made that all the naphthenes present are 6-membered rings fused up to the limit imposed by the molecular weight of the sample. (It is assumed that no carbon atom is buried—that is, shared among more than two rings. In other words, the progression cyclohexane, decalin, perhydroanthracene . . . is assumed.) In effect this is an assumption of nearly the minimum possible number of carbon atoms per ring (if all are 6-membered rings), which is on the whole unlikely. It has the further disadvantage that it assumes that the number of carbon atoms per ring declines continuously in all oils as the molecular weight increases.

Having so ascertained the number of ring carbon atoms, the number of naphthenic carbon atoms can be calculated as the difference between this and the number of aromatic carbon atoms previously derived. This assumes, as noted previously, that all unsaturation is aromatic. Then, only the number of alkyl carbon atoms is needed further. This is, obviously,

$$n - \text{naphthenic C atoms} - \text{aromatic C atoms}$$

With these expressions of the numbers of aromatic, naphthenic, and alkyl carbon atoms converted to percentages, the analysis is complete.

Expression of Olefin Content

The presence of olefins in a sample is shown by a bromine number determination. The various techniques in use are well known. It is to be noted, however, that all methods become far less accurate as molecular weight increases. Expressed in terms of the total unsaturation, h, that portion due to olefins alone, h_0, equals the bromine number divided by 79.3, the ratio of atomic weights of bromine and hydrogen. Then the remaining portion, $h_A = h - h_0$, is due to aromatics alone. The true aromaticity is then

$$A = \frac{1191.5h_A}{100 - y}$$

R is calculated by Equation 8, as described above. It is important to note that this expression is independent of the distribution of unsaturation between h_0 and h_A. Then, including the aliphatic olefins with the saturated aliphatic part and the cyclic olefins with the naphthenes, we may write as before

$$\text{naphthenic C atoms} = \text{total cyclic C atoms} - \frac{nA}{100}$$

$$\text{alkyl C atoms} = n - \text{total cyclic C atoms}$$

The change from numbers to percentages of aromatic, naphthenic, and alkyl carbon atoms is readily made, and these quantities, together with h_0, comprise the solution of the nomographic analysis for the general case in which unsaturation is partly aromatic, partly olefinic. The olefins must be included with the naphthenes and paraffins, instead of being expressed separately, because it is impossible to distinguish cyclic from acyclic olefins. Thus, the percentage of naph-

Table III. Calculation Form for Nomographic Hydrocarbon Analysis

Sample	Recycle stock
d_4^{20} (vacuum)	0.9014
n_D^{20}	1.5009
$(nF - nC) \times 10^4$	140.2
Bromine No.	36.0
M (extrap.)	189.
C, wt. %	87.93
H, wt. %	11.47
C + H, wt. %	99.40
y	11.55
r	0.3268
S	155.5
hk from nomograph	4.22
h from nomograph	1.96
$k = \frac{hk}{h}$ (indicates kind of aromatics)	2.15
$h_0 = \frac{\text{bromine no.}}{79.3}$	0.45
$h_A = h - h_0$	1.51
$A = \frac{1191.5h_A}{(100 - y)}$ = aromaticity %	20.4
$n = \frac{M(100 - y)}{1201}$ = C atoms per molecule	13.9
$R = 1 - \frac{Mh}{201.6} + \frac{M(2.016 - 0.14026y)}{24.212}$ rings per molecule	2.25
C_R carbon atoms per ring[a]	5.0
$R.C_R$ number of ring carbon atoms per molecule	11.25
$C_A = \frac{nA}{100}$ aromatic C atoms per molecule	2.84
$C_N = R.C_R - C_A$ naphthenic C atoms per molecule	8.41
$C_P = n - C_A - C_N$ alkyl C atoms per molecule	2.65
% aromatic carbon atoms	20
% naphthenic carbon atoms	61
% alkyl carbon atoms	19

[a] Describing assumption used.

thenic carbon atoms, in the sense of including all cyclic olefins, is explicit.

An Example. Use of the method is illustrated in the analysis of a recycle stock from a refinery cracking furnace given in Table III, which shows the calculations in their proper sequence on a form convenient for routine use. In a sample contaminated slightly by nonhydrocarbon impurities, y is taken as one hundred times the ratio of hydrogen to hydrogen plus carbon, rather than the absolute weight per cent of hydrogen from combustion analysis. Other measurements are not altered to take account of the impurity. It is thus implied that the contaminants have average properties identical with those of the hydrocarbon mixture. This is certainly not exactly the case, in general, but no better assumption is apparent.

Reproducibility

When a Pulfrich refractometer is used for the optical measurements and the best combustion analysis technique is observed, determinations of the properties required by this analytical method are usually reproducible within the limits 0.0001 in r, 0.04 in y, and 2 in S. Assuming that the maximum values listed above re-enforce each other, the resulting maximum deviations in h, for values of k covering the usual range of variation for oil samples, are those shown in Table IV.

Since no method of obtaining bromine numbers is specified, it is not possible to discuss the reproducibility of h_0.

The quantity R depends on h and one additional measurement, M. From tests of routine methods used in these laboratories, M is usually reproducible within 4 per cent for a given sample. Then, using the extreme deviations of h noted above, the largest possible deviations in R, for some typical values of k, R, and M, are as shown in Table V. These figures represent the extremely unlikely case of simultaneous maximum deviations in all measurements, all re-enforcing.

Accuracy

Errors are inherent in the empirical relations of Figures 1 and 2 and there are, in addition, errors resulting from the limited accuracy of the measurements. The two types of error are difficult to dissociate, though with good techniques the contributions of errors of measurement may be confined to relatively small values—i. e., about the values of the reproducibilities discussed in the previous section. However, even supposing these could be made entirely negligible, it is very

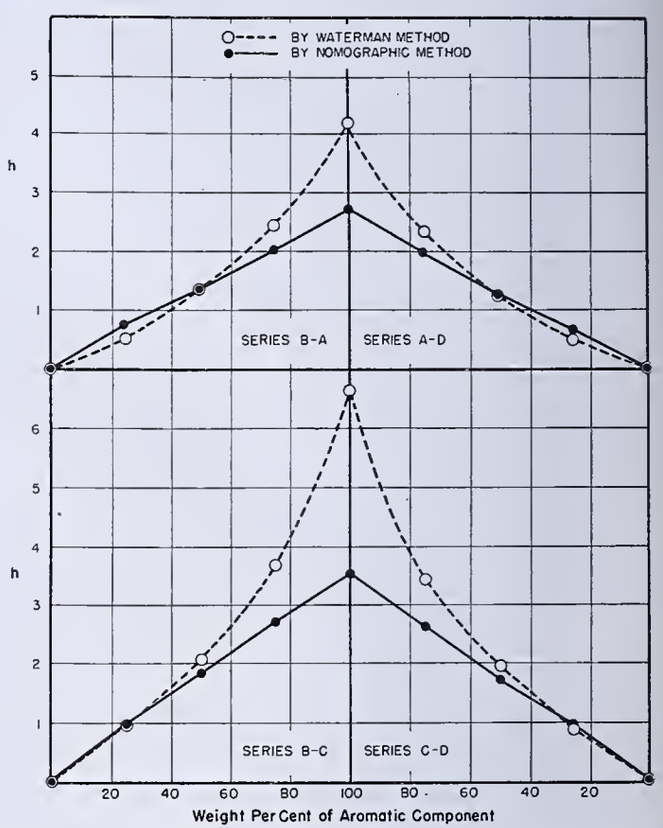

Figure 4. Deviations from Linear Blending of h
Calculated by Waterman and nomographic methods

Table IV. Maximum Deviations in h Due to Experimental Errors

k	δh
1.7	0.14
2.0	0.10
2.5	0.07

Table V. Maximum Deviations in R Due to Experimental Errors

k	R	M	δR
1.7	2	200	0.22
2.0	4	500	0.48
2.5	4	500	0.40

Table VI. Comparison of Values of h Obtained from Nomograph and by Hydrogenation

Oil	From Nomograph	By Hydrogenation	Difference
B-12	3.95	4.02	−0.07
B-16	3.35	3.28	+0.07
B-19	3.06	3.01	+0.05
C-13	3.46	3.43	+0.03
C-20	2.86	2.72	+0.14
C-30	1.71	1.70	+0.01
C-33	1.60	1.58	+0.02
C-35	1.54	1.50	+0.04
C-37	1.53	1.34	+0.19
C res.	0.03[a]	0.04	−0.01

[a] k = 2.0 assumed.

TABLE VII. MEASUREMENTS ON OIL BLENDS, USED FOR COMPARING NOMOGRAPHIC AND WATERMAN ANALYSES

Sample	d^{20}_4	n^{20}_D	$(n_F - n_C) \times 10^4$ (Abbé)	% C	% H	Aniline Point[a], ° C.	M[b]	r^{20}_D	S	y
AAAA	0.9679	1.5451	166	87.60	10.54	8.0	263	0.3267	171	10.74
AAAB	0.9309	1.5214	140	87.27	11.50	41.6	264	0.3273	150	11.64
AABB	0.8969	1.4995	121	86.76	12.37	64.6	269	0.3277	135	12.48
ABBB	0.8651	1.4794	101	86.24	13.29	81.8	271	0.3280	117	13.35
BBBB	0.8356	1.4606	82	85.88	14.21	95.7	275	0.3281	98	14.20
BBBC	0.8801	1.4919	121	86.39	12.97	81.1	304	0.3296	137	13.05
BBCC	0.9304	1.5271	162	86.87	11.72	62.0	316	0.3305	174	11.89
BCCC	0.9842	1.5650	210	87.51	10.53	35.1	333	0.3309	213	10.74
CCCC	1.0460	1.6088	266	88.03	9.23	−15.4	346[c]	0.3309	254	9.49
CCCD	1.0012	1.5735	213	87.63	10.40	42.2	361[c]	0.3293	213	10.61
CCDD	0.9590	1.5407	168	87.27	11.53	70.0	380	0.3275	175	11.67
CDDD	0.9233	1.5129	128	86.86	12.51	88.9	386	0.3255	139	12.59
DDDD	0.8857	1.4835	88	86.19	13.63	105.5	392	0.3227	99	13.65
DDDA	0.9048	1.4976	106	86.86	12.93	90.4	356	0.3238	117	12.96
DDAA	0.9247	1.5126	124	87.08	12.12	71.4	317	0.3248	134	12.22
DAAA	0.9452	1.5279	144	86.98	11.31	48.0	295	0.3258	152	11.51

[a] Equal volumes of sample and aniline.
[b] Cryoscopic in benzene, except as noted. Trend of apparent M with changing concentration extrapolated to 0 concentration.
[c] Ebullioscopic in benzene.

TABLE VIII. COMPARISON OF RESULTS OF WATERMAN AND NOMOGRAPHIC ANALYSES OF OIL BLENDS

For uniformity, the Waterman analysis assumption that number of ring C atoms = $4R + 2$ was used for all nomographic analyses, although better assumptions might conceivably have been made.)

Blend	Nomographic h	Nomographic k	Rings per Molecule, R: Nomographic	Rings per Molecule, R: Waterman	Per Cent Carbon: Aromatic Rings: Nomographic	Aromatic Rings: Waterman	Naphthenic Rings: Nomographic	Naphthenic Rings: Waterman	Paraffinic Chains: Nomographic	Paraffinic Chains: Waterman
AAAA	2.70	2.12	3.02	3.25	36	55	36	23	28	22
AAAB	2.01	2.09	2.54	2.45	27	33	36	28	37	39
AABB	1.34	2.12	2.17	1.85	18	18	36	30	46	52
ABBB	0.73	2.00	1.63	1.47	10	7	33	34	57	59
BBBB	0.00	..	1.27	1.20	0	0	36	35	64	65
BBBC	0.97	2.37	1.87	1.47	13	13	30	23	57	64
BBCC	1.84	2.40	2.66	1.95	25	28	29	15	46	57
BCCC	2.70	2.40	3.55	2.87	36	49	29	6	35	45
CCCC	3.51	2.45	4.75	4.45	46	85	35	−7	19	22
CCCD	2.61	2.44	4.19	3.15	35	45	35	10	30	45
CCDD	1.70	2.46	3.75	2.75	23	26	38	20	39	54
CDDD	0.93	2.44	3.20	2.55	13	12	40	31	47	57
DDDD	0.00	..	2.63	2.70	0	0	44	44	56	56
DDDA	0.62	2.08	2.81	2.65	8	6	43	43	49	51
DDAA	1.25	2.16	2.99	2.65	17	16	43	37	40	47
DAAA	1.95	2.14	3.04	2.90	26	31	39	32	35	37

to be the proper number of significant figures for each quantity. This discussion of accuracy applies only to mixtures of hydrocarbons, substantially uncontaminated. The effects of non-hydrocarbon molecules have not been studied.

Self-Consistency of Nomographic and Waterman Analyses

If a series of blends is made from two component oils, certain constitutional properties of the blends vary in a linear manner with weight composition. This fact was utilized in testing the nomographic analysis, and the analysis of Waterman (*15*) in comparison with it. We are primarily interested in the accuracy with which the two analyses report unsaturation and ring content. Unsaturation may be represented by h, the number of grams of hydrogen which may be taken up by 100 grams of oil, and ring structure by $\frac{R}{M}$, the number of rings per unit molecular weight; in a two-component mixture these quantities are by definition linear functions of weight composition. The latter may readily be known without error; hence, plots of h and $\frac{R}{M}$ as obtained from the two analyses, against weight composition, afford a check on the self-consistency of each analysis. Percentages of aromatic, naphthenic, and alkyl carbon atoms are not linear functions of weight composition, and hence cannot be used for an accurate check.

difficult to check the relations directly with actual oil samples. The direct measurement of h by hydrogenation is laborious in the extreme, and if synthetic mixtures are used for a test, there is always the objection that they are not typical of naturally occurring oils. Recourse must be had to indirect methods for an estimation of the accuracy of the relationships.

The unsaturated extraction cuts of Mair and associates represented in Figure 2 were carefully hydrogenated by those authors, and values of y, M, etc., were measured (*11*) on the saturates obtained (represented in Figure 1); these measurements enable the authors to calculate values of h. In Table VI these calculated values are compared with values from the nomograph. Since these oils were considered in the construction of the mean line of Figure 1 (though not of the line of Figure 2), they do not afford a wholly independent test of error, nor can errors of measurement be separated from those inherent in the relations. Nevertheless, the comparison does suggest magnitudes likely to be encountered.

Summarizing the results of such indirect evidence so far available, it is concluded that for substantially uncontaminated hydrocarbon mixtures the absolute error in h, the unsaturation in grams of hydrogen per 100 grams of oil, is not likely to exceed 0.2 (2.5 per cent in aromaticity), whatever the value of h, and the absolute error in R, the number of rings per average molecule, is not likely to exceed 10 per cent. In control testing of related samples, differences smaller than these magnitudes may be significant, because of the systematic nature of some of the contributing errors. In the example of Table III, the authors have used what they consider

Four series of two-component blends were prepared, utilizing two highly aromatic (A and C) and two completely saturated (B and D) petroleum oils as components. All measurements necessary to conduct both analyses on each blend were made. A complete record of these measurements is presented in Table VII. In Table VIII are recorded the results of the two analyses based on these measurements. Throughout each series of blends the value of k obtained in the nomographic analysis remains substantially constant, as it should if k is a function of the kind of aromatics present only, and independent of the quantity.

In Figure 4 values of h are plotted against weight composition for all series. Since h is not stated directly in the Waterman analysis, it was calculated from

$$h = \frac{201.6}{M}\left[\frac{M - 2.016 + 2.016R}{\frac{2805}{A} - 2.016}\right]$$

The quantities M, R, and A are explicitly stated in the analysis. As may be seen in considering the plots of h against composition, the deviations from linearity of values from the nomographic analysis are extremely small. For each series of blends, a straight line can be drawn through the origin so as to agree with all points to less than 0.1 in h (corre-

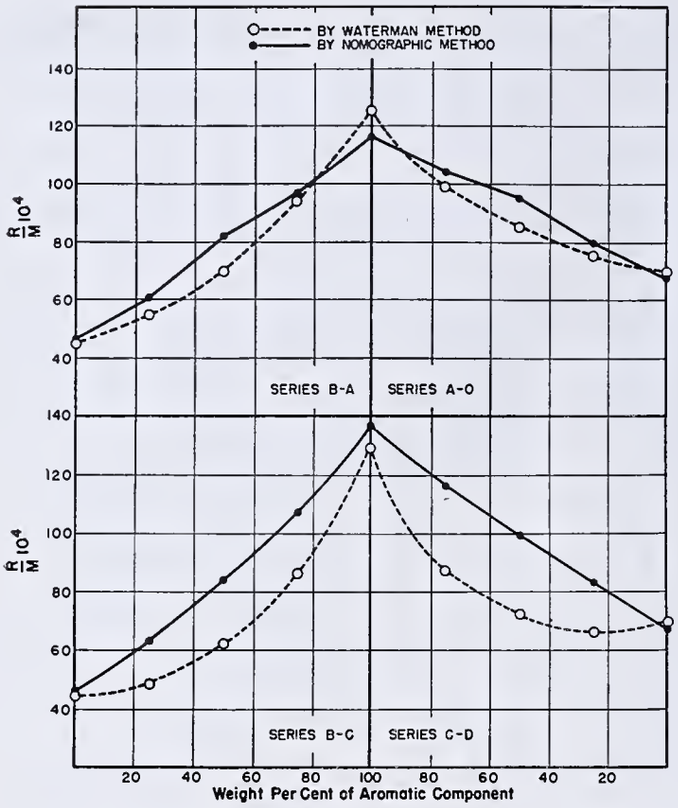

FIGURE 5. DEVIATIONS FROM LINEAR BLENDING OF $\frac{R}{M}$

Calculated by Waterman and nomographic methods

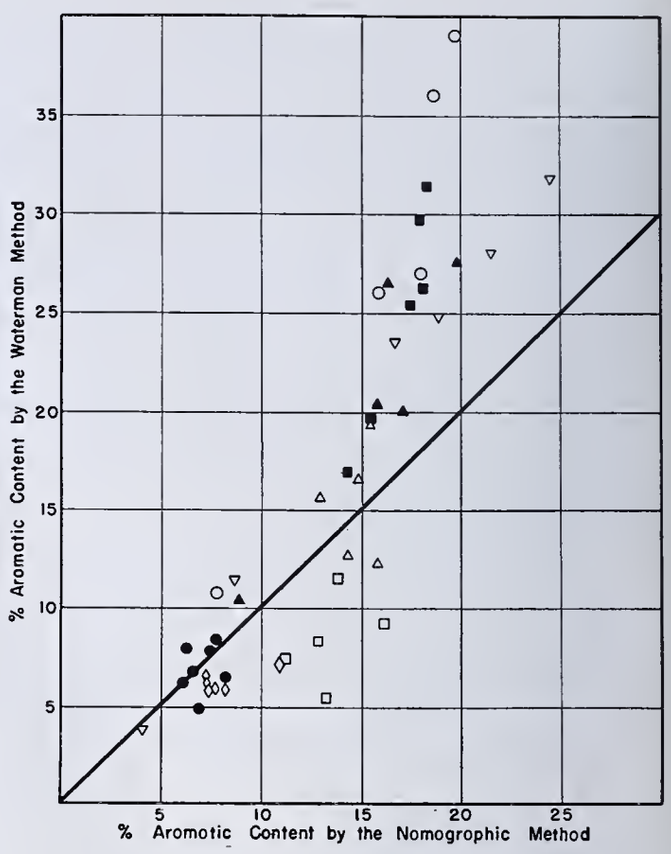

FIGURE 6. COMPARISON OF WATERMAN AND NOMOGRAPHIC AROMATICITIES

sponding to about 1 per cent aromatics). The deviations from linearity of values from the Waterman analysis are considerably larger. It is realized that the latter analysis is not intended for highly aromatic samples, and the agreement of the two methods for low aromaticities—i. e., $h < 1.5$—is fairly good. As aromaticity increases, however, the deviation becomes marked. In the extreme case, that of the pure component *C*, the Waterman analysis finds so high an aromatic content that a negative content of naphthenes (Table VIII) results. Considering next the plots of $\frac{R}{M}$ against composition, Figure 5, we see that, except for one series, there are only small consistent deviations from linearity in values calculated from the nomographic analysis, while all the values from the Waterman analysis yield lines with pronounced curvatures.

That the relatively higher aromaticities of the Waterman analysis are not due to peculiarities of the oils selected for blending, but to differences inherent in the two methods, is shown by Figure 6. This figure compares the results of analysis, by the two procedures, of distillation cuts from several petroleum crudes of widely different origin and character. The range of molecular weights represented in the various cuts is approximately 190 to 670. For every oil for which the nomographic analysis yields an aromaticity of 17 per cent or more, the Waterman analysis yields a higher aromaticity.

Acknowledgment

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Summary

A new type analysis of petroleum oils has been developed. In its development this method is consistent with the known data on both pure hydrocarbons and hydrocarbon oils, and appears to be equally applicable to straight-run and cracked products and to synthetic oils over all the range in which the requisite measurements are possible. An absolute check could not be made, but estimates indicated the errors to be relatively small and random. The analysis yielded self-consistent results, while that of Waterman, the only other generally used for the same purpose, did not.

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Evaluating Starches for Textile Purposes

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ALTHOUGH the starching of textile fabrics has long been a major operation in the textile and laundry industries, it is still a moot question as to how the efficiency of this process may be judged. Uniform methods for evaluating starched fabrics and starch mixtures have not been generally adopted. This has undoubtedly contributed to the tendency of the processors, when buying starching materials, to stress price considerations rather than quality of product. The result seems to be that the manufacturer of a new kind of starch finds himself in an unfavorable trade position. The way has not been cleared for him to prove with any degree of certainty and dispatch the superiority of his product over another for any particular use. Without doubt, the initial step to overcome this handicap would be to make available technical test methods by which it will be possible to measure certain of the desirable properties imparted to fabrics by the starching operation, and to measure certain properties of starch mixtures which affect the starching operation.

It is the purpose of this paper to describe test methods for appraising the properties of starched fabrics and starch mixtures, and to give data obtained by application of these methods.

Stickiness of Starched Fabrics during Drying

During either the ironing or drying over "dry cans" of starched fabrics, it is essential that these fabrics do not stick excessively to the heating surfaces. If sticking occurs, there is a tendency for the quality and the uniformity of the fabric to suffer—i. e., the number of surface imperfections may be increased and the fabric construction distorted. Then, too, the wear life of the fabric may be adversely affected by the pulling action required to remove the fabric from the heated surface. Lastly, excessive sticking may make it necessary to clean the drying surfaces so frequently that both the plant operating efficiency and capacity will be reduced.

To determine the relative stickiness which may be expected from a starch mixture, the apparatus developed in this laboratory and described in this paper is suggested. The method, in brief, consists in pressing a heated metallic surface against a starched sample prepared for ironing and subsequently determining the pull necessary to free the sample from that surface.

Method for Measuring Stickiness

The apparatus used consisted of a rotary electric ironer and a Jolly balance with a pulley setup so arranged that a horizontally exerted pull may be measured (Figure 1).

Fabric samples 20 × 7.5 cm. (8 by 3 inches) were starched according to a definite procedure. It may be preferable in some instances to simulate the particular practice of a given plant. In the work of this laboratory, a starch mixture was cooked with live steam, at a fixed steam valve setting, for exactly 30 minutes. The mixture was made up to volume with boiling water and thoroughly mixed.

The fabric samples were first immersed in water (50° C.) for 5 minutes, passed through a wringer having a given pressure setting, and then immersed for 3 minutes in the cooked starch mixture (90° C.). Each sample was separately "whizzed" in a small basket centrifuge for a given length of time—e. g., 10 seconds for samples which had been treated with thin-boiling starches and 30 seconds for those starched with thick-boiling starches. After centrifuging, the samples were placed in a small stoppered bottle to keep the moisture content fixed; they were then ready to be tested for stickiness.

It is suggested that the samples be starched and tested in sets of 10 and that after each set has been tested, but not until then, the heated shoe surface be thoroughly cleaned.

The sample to be tested was placed in a fixed position on or over the roll of the ironer, so that when the shoe was lowered it covered the center portion of the sample, leaving uncovered equal lengths on both sides of the shoe surface. When the shoe had been heated so that the thermometer, *T* (Figure 1), registered 150° C., the shoe was automatically lowered and allowed to rest under its own weight on the sample for 10 seconds, when the shoe carrying the sample with it was raised from the roll. During the drying interval, a hook was attached to the free end of the sample nearest to the balance; the hook in turn was attached to a cord, *K*, which had been brought under pulley *A* over movable pulley *B* under pulley *C* and fastened to reel *E*.

As soon as the shoe was raised, the reel was slowly turned, thus winding or shortening the length of the cord and, hence, exerting a horizontal pull on the sample. This pull was simultaneously transmitted to the Jolly balance and could be accurately determined from the extension of the balance spring. The reel was turned until the fabric sample had been pulled free from the shoe. At the moment of separation the elongation of the spring was read and considered as the final reading. The difference between the zero reading of the spring and the final reading,

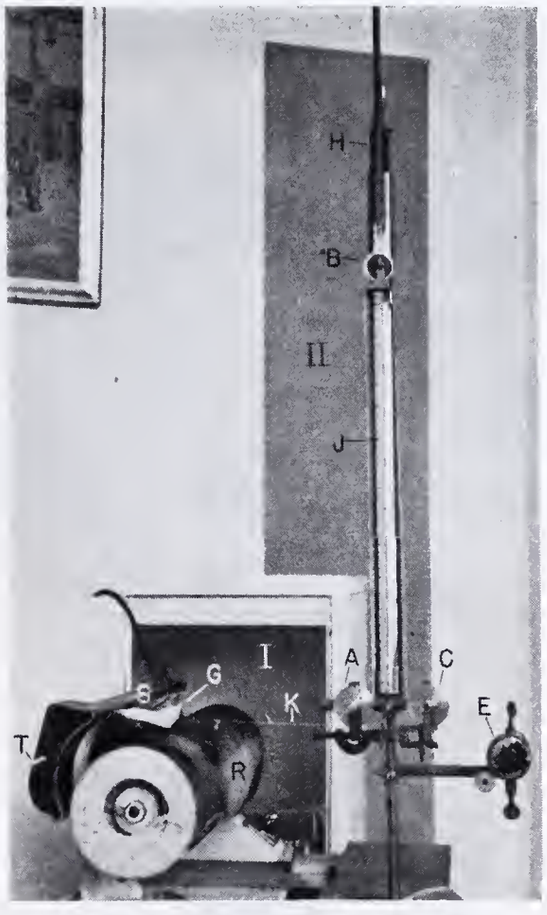

FIGURE 1. ROTARY ELECTRIC IRONER (I) AND JOLLY BALANCE SETUP (II)

A, C. Fixed-position low-fraction pulleys
B. Movable pulley
E. Reel
H. Spring
J. Jolly balance scale
K. Cord
R. Ironing roll
S. Heating shoe
T. Thermometer

Table I. Stickiness Data

Starch[a]	Stormer Viscosity	C. P. R. No.	Force Required to Release Sample[b]	Moisture Analysis[c]	Starched Fabrics Starch[d]
			Grams	%	%
A	75	2	598	205	8.4
B	170	1	940	231	8.7
C	38	45	231	156	5.2

[a] Starch concentration, 40 grams per liter of water.
[b] Mean deviation 10 sets of 10 determinations, 29 grams.
[c] Dried at 105° C. to constant weight.
[d] Desized with desizing agent, heated with water under reflux 1 hour, washed with hot water, and dried at 105° C.

after conversion into grams' pull from the calibration curve of the spring, was considered to be the stickiness factor for the starch mixture used or the force required to release the sample.

Table I gives data on stickiness.

Stormer Viscosity Method (used by the Laurel starch plant). Three grams of starch (dry basis) were wetted with 10 ml. of water in a 200-ml. Erlenmeyer flask, 100 ml. of boiling water were added with mixing, and the flask was fitted to an air condenser and immersed in a boiling water bath for 1 hour. Measurement was made at 90° C. in a Stormer viscometer. Values were expressed as the number of $^1/_5$ seconds required for spindle to make 100 revolutions when actuated by a 70-gram weight.

Corn Products Refining Company Fluidity Determination. Starch (4.5 grams dry weight) was wetted with 10 ml. of water (23.89° C., 75° F.), stirred for 3 minutes after the addition of 90 ml. of 1 per cent sodium hydroxide (75° F.), allowed to stand 27 minutes (75° F.), and transferred to a funnel having a standardized orifice, and the milliliters which passed through the orifice in 70 seconds were determined. This volume was considered to be the C. P. R. number. The orifice opening had been made so that 100 ml. water passed through that opening in 70 seconds.

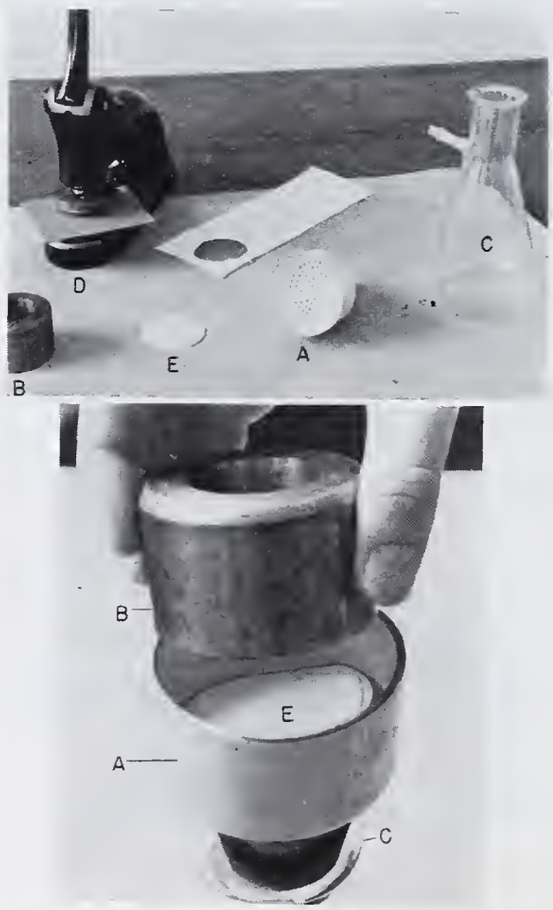

Figure 2. Apparatus for Disk Penetration Method

A. Büchner funnel
B. Weight
C. Flask
D. Die to cut samples
E. Sample

Penetration of Starch Mixtures

The speed with which a starch mixture will penetrate a fabric is of considerable importance to the starching operator.

Analysis of cloth used (desized with desizing agent)
Warp, 62 ends per inch; count, 20.4
Filling, 48 pick per inch; count, 19
Selvage, 80 ends
Ends in body, 4530
Body of cloth reeded, 2 per cent
No. of ends per beam, 2305
Width of each beam 36.75 inches
Total possible number of ends, 4646
Width of reed 73.25 inches (30 dents per inch)

Plant production capacity is more or less dependent upon how quickly a starch mixture will be taken up by and pass through a fabric. As an aid in evaluating this factor the two methods described below are suggested. The first or disk method is a modification of the procedure described by the Laundry Owners' National Association (*2*).

Disk Method (for measuring penetration through interstices of fabric and by capillary flow). This method consists in determining under fixed conditions the number of wet fabric disks through which a given volume of starch will penetrate in a specified time (Table II).

Apparatus (Figure 2). *A*, Büchner funnel, 66 mm. in outside diameter, 56 mm. in inside diameter. *B*, weight, 510 grams. Outside diameter 56 mm., diameter of center opening 38 mm.

Procedure. Twelve desized fabric disks, *E*, 5 cm. (2 inches) in diameter, were placed in funnel *A*, and weight *B* was placed over the disks. Fifty milliliters of boiling water were poured through the opening in the weight onto the disks. After 3 minutes were allowed for the water to drain, 10 drops of the starch mixture (82.22° C., 180° F.) were added through the opening. At the end of another 3 minutes, the weight was removed, the disks were separated, and each was tested for starch with an iodine solution. The number of disks which gave a test for starch was considered to be the penetration number for that particular starch.

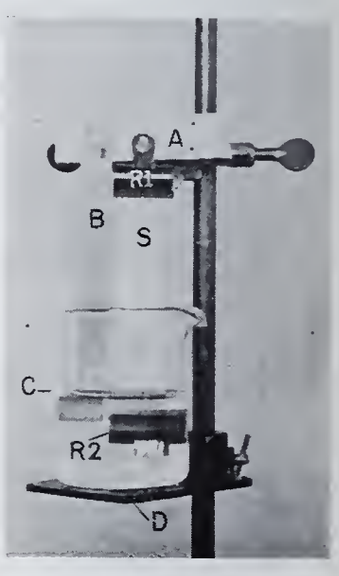

Figure 3. Capillary Penetration Apparatus

A. Rack to hold sample and ruler
B. Measuring ruler
C. Starch mixture level
D. Adjustable platform
S. Fabric sample
R_1, R_2. Clamps

Method for Determining Penetration of Starch Mixtures Caused by Capillary Pull. *Apparatus* (Figure 3). *A*, rack to hold sample, *B*, measuring scale, and *D*, adjustable platform.

Procedure. A strip of fabric 2.5 × 11.25 cm. (1 by 4.5 inches), which had been previously desized and then humidified at 65 per cent relative humidity and 21.1° C. (70° F.), was used as the test sample. Two small paper clamps were fastened to the strip, one at each end, and the sample was hung vertically from rack *A* parallel to ruler *B*.

A beaker containing a hot starch mixture (88.22° C., 180° F.) was raised under the free-hanging strip until the starch mixture reached a definite level—i. e., until the lower end of the strip had become immersed to about 1.25 cm. (0.5 inch) above the lower clamp. The platform, *D*, on which the beaker rested was made fast in this position and an initial reading of the liquid level on the fabric was taken immediately. At the end of 3 minutes the platform was lowered and several drops of an iodine solution were applied to the test sample. The iodine solution made possible an accurate reading of the vertical creep or rise of the starch mixture during a 3-minute period. The difference between the initial and the final reading was considered to be a measure of the penetration.

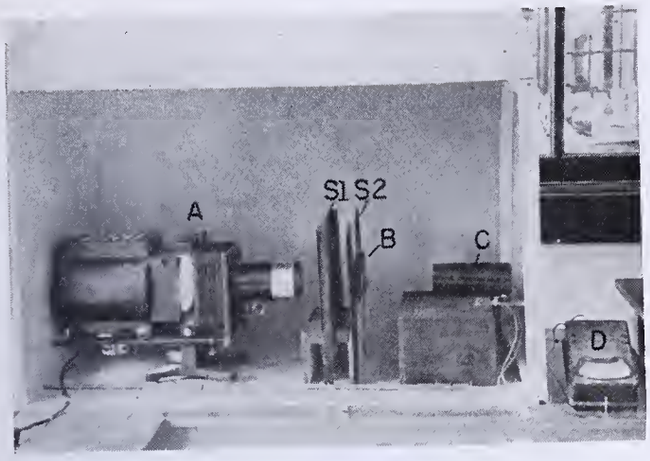

FIGURE 4. TRANSPARENCY DEVICE
A. Projector
B. Rack for holding sample slide
C. Photoelectric cell
D. Galvanometer
S_1, S_2. Cardboard shelf with slide

Transparency of Starch Films

A more or less continuous starch film is acquired on the surface of a fabric during the starching or finishing operation. In some types of fabrics it is almost imperative that this film be clear and transparent. A nontransparent film covering on a dyed fabric may change the tone of a previously matched shade, may detract from the brightness of a shade, or may partially hide the color of a delicate shade.

METHOD FOR DETERMINING TRANSPARENCY OF STARCH FILMS. The method suggested for measuring this property is based on a determination of the loss in light intensity caused by placing a starch film between a light of constant and known intensity, and a light intensity–measuring device. For convenience the starch film may be mounted on a microscope slide glass. The method makes use of a photoelectric cell, a galvanometer, and a 40-watt light bulb enclosed in a projector (Figure 4). The entire setup, with the exception of the galvanometer, is enclosed in a light-tight box. A thickness gage, calibrated in units of 0.0001 inch, is used to measure the thickness of the starch film.

Procedure. The thickness of a blank slide glass, at its center, was measured.

A galvanometer reading was taken when the blank slide had been placed in the rack between the light source and the photoelectric cell and considered to be the initial reading. [All but a 1.25-cm. (0.5-inch) portion at the center of the slide was screened from the light by the rack.]

The slide glass was coated by dipping in a hot starch mixture and the films on the slide were dried overnight at 65 per cent relative humidity and 21.1° C. (70° F.). (It may be advantageous to use a drying temperature corresponding to that used in the particular plant in which one may be interested.)

The coated slide was returned to the rack between the light and the cell and a final galvanometer reading was taken. [It is suggested that all determinations be made at fixed conditions of humidity and temperature, preferably at 65 per cent relative humidity and 21.1° C. (70° F.).]

The combined thickness of the two starch films at the center of the slide was determined by measuring total thickness and subtracting that of the glass.

TABLE II. PENETRATION OF STARCH MIXTURES THROUGH FABRIC INTERSTICES AND BY CAPILLARY FLOW

Starch[a]	Stormer Viscosity	C. P. R. No.	No. of Disks[b]
A	75	2	5
B	170	1	4
C	38	45	8

[a] 40 grams of starch per liter of water.
[b] Mean deviation was less than 0.3 disk.

TABLE III. PENETRATION CAUSED BY CAPILLARY PULL

Starch[a]	Stormer Viscosity	C. P. R. No.	Capillary Rise in 3 Minutes	Mean Deviation
			Mm.	*Mm.*
A	75	2	16.3	1.3
B	178	1	14.0	1.3
C	38	45	22.5	1.8

[a] 40 grams of starch per liter of water.

TABLE IV. TRANSPARENCY OF STARCH FILMS

Starch	Stormer Viscosity	C. P. R. No.	Light Intensity Loss	Film Thickness
			%	*Inch*
A	75	2	10.4	0.0040
B	170	1	26.3	0.0040
C	38	45	11.0	0.0040

Effect of Crushing Starched Fabrics

Of several fabrics, the one which is affected least by a given crushing or crumpling action will probably remain "fresh" for the longest time when worn in the form of a garment. In short, the method suggested consists in crushing a folded sample and then measuring the effect of that crushing action by determining the increase in the angle of bend from the normal caused thereby.

EXPERIMENTAL METHOD FOR MEASURING EFFECT OF CRUSHING. The apparatus was made up of a crushing device and a means for measuring the angle with the horizontal made by a freely hanging portion of a sample. More specifically, the former consisted of a modified dissecting microscope chassis, having as a fixed stage a piece of pressed board and as the adjustable stage a metal plate. A mirror graduated in millimeter squares was mounted directly in back of the device. By the use of this mirror it was possible accurately to adjust the height of the movable stage. The means for measuring the angle consisted of a smooth metal plate under the sample, a glass plate partially to cover the sample, and a mirror mounted vertically which was calibrated in degrees from 0° to 90° C.

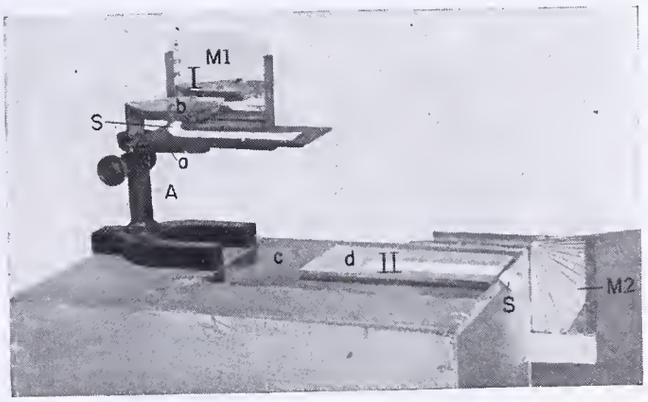

FIGURE 5. CRUSHING DEVICE (I) AND ANGLE-MEASURING DEVICE (II)
A. Modified dissecting microscope
a. Fixed stage
b. Adjustable stage
c. Metal plate under sample
d. Glass plate, partially covers sample
M1. Mirror
M2. Mirror calibrated in degrees
S. Sample

The test sample consisted of a regular piece of cloth cut 2.5 × 15 cm. (1 by 6 inches), one end of which had been trimmed to a point by cutting back from the center of the end to points 2.5 cm. (1 inch) along the sides of the strip. It was placed flat on the stage (*a*, Figure 5) with the marked side up and the pointed end towards *A*. The movable plate, *b*, was brought down to a position 1 mm. distant from *a*. The sample was allowed to remain in this position for 1 minute, after which the plate was lifted. The sample strip was moved to the metal plate, *c*, with the marked side down and the pointed end toward the end of the box having the extending mirror. The edge of the square glass plate, *d*, was placed along the 2.5-cm. (1-inch) line on the sample and thus all but the 2.5-cm. (1-inch) length, including the pointed end, was covered.

TABLE V. EFFECT OF CRUSHING STARCHED FABRICS

Starch[a]	Stormer Viscosity	C. P. R. No.	Angle of Bend	Mean Deviation
A	75	2	29.1	1.3
B	170	1	29.4	1.3
C	38	45	24.7	2.0

[a] 40 grams of starch per liter of water.

TABLE VI. STIFFNESS OF STARCHED FABRICS[a]

Starch[b]	Stormer Viscosity	C. P. R. No.	Stiffness Factor	Mean Deviation
A	75	2	33.11	2.4
B	170	1	31.39	1.7
C	38	45	30.10	1.3

[a] Fabrics were conditioned and tested at 65 per cent relative humidity and 70° C.
[b] 40 grams of starch per liter of water.

The sample and the glass plate were slid along the metal plate until the edge of the glass and, hence, the 2.5-cm. (1-inch) line coincided with the edge of the metal plate. In this position 2.5 cm. (1 inch) of the sample extended freely beyond the end of the box. After 10 seconds the angle of bend which the extending fabric made with horizontal was read directly from the mirror, M_2, mounted in back of the box. This angle was considered to be the zero or normal reading of the strip.

Again the sample was placed on stage *a*. The pointed end was lightly folded up and over, forming a U-shaped bend along the line 2.5 cm. (1 inch) from the tip. Plate *b* was then lowered to a position 1 mm. from *a*, creasing the sample along the 2.5-cm. (1-inch) line, and held in this position for one minute. The sample was then laid flat and pressed by lowering *b* to within 1 mm. of *a* and holding it there for one minute. Again the angle of bend made by the sample with the horizontal was determined as described above. The difference between the two readings, before and after the sample had been exposed to the crushing action, was considered to be the angle of bend caused by that action.

FIGURE 6. STIFFNESS TESTER
A. Set of rubber rolls
B. Shelf, set at 45° angle with vertical

The angle of bend here given represents the angle of deformation from the normal caused by a given crushing action on the fabric—i. e., when the adjustable stage was lowered to point 1 mm. from the fixed stage. It follows that the smaller this angle the less it has been affected by this action.

Stiffness of Fabrics

One of the factors which, to a large extent, determines the so-called "body" and "handle" of a fabric is stiffness. This property may be judged by the results obtained by the use of the method of Peterson and Dantzig (*1*, *4*).

EXPERIMENTAL METHOD FOR MEASURING STIFFNESS. This method (*4*) depends upon the deformation of a supported strip bent under its own weight. The apparatus consisted of a set of rubber-covered rolls through which samples passed in a horizontal plane and a shelf fastened at a 45-degree angle with the vertical at the zero reading of the scale (Figure 6).

A fabric sample 5 × 10 cm. (2 by 4 inches) was inserted lengthwise between the rollers and slowly fed through those rollers in the direction of the scale until the end of the sample barely touched the metal shelf. The distance in millimeters from the nip of the rolls to the point on the shelf where the strip made contact multiplied by 0.43 was considered to be the stiffness of the fabric tested. It follows that the larger the reading the greater will be the stiffness.

FIGURE 7. SMOOTHNESS TESTER
A. Base board
B. Sliding block, covered with sample
C. Adjustable board, covered with sample
D. Crank to adjust angle between *A* and *C*
S. Scale measuring between *A* and *C*
W. Weight to hold sample under tension

TABLE VII. SMOOTHNESS OF STARCHED FABRICS

Starch[a]	Stormer Viscosity	C. P. R. No.	Smoothness Factor	Mean Deviation
A	75	2	0.6395	0.0209
B	170	1	0.5459	0.0152
C	38	45	1.0724	0.0262

[a] 40 grams of starch per liter of water.

Smoothness of Fabrics

The surface characteristics of a fabric are undoubtedly made up of a number of contributing properties, which, in turn, have a bearing upon the "feel", appearance, and, in some instances, the utility of the fabric. One of these properties is smoothness. The method of Mercier (*3*) for measuring the coefficient of friction of a fabric may be used to evaluate the smoothness of a fabric.

METHOD FOR MEASURING SMOOTHNESS OF STARCHED FABRICS (*3*). Two boards, each 55 cm. (22 inches) long and 15 cm. (6 inches) wide, are hinged together at one end. One of the boards rests on three small supports which can be adjusted to bring the board to a horizontal position. The angle between the boards can be changed by a screw arrangement, and this angle or its tangent read on a scale on the upper surface of the horizontal board. The block of wood 20 cm. (8 inches) long and 15 cm. (6 inches) wide weighs about 560 grams (1.25 pounds). This block of wood and the inclined plane are covered with the fabric to be tested. Clamps for holding the fabric on the block and on the inclined plane are shown in Figure 7. A spring keeps the fabric under tension on the block and a small weight clamped to the lower end of the fabric keeps the fabric taut on the inclined plane during the test.

The wooden block, covered with sample of fabric to be tested, was placed on the inclined plane which had also been covered with another piece of the same fabric. The angle between the inclined plane and the horizontal was then increased until, with the assistance of light tapping on the inclined board, the block began to slide. A reading of the tangent of the angle thus made by the inclined plane was considered to be the smoothness factor of the fabric tested. The smaller the angle, and, hence, the smaller the tangent, the greater will be the smoothness.

Summary

Methods are described for evaluating starches for use on fabrics; these include methods for judging the starch mixtures from a processing or plant operating viewpoint and methods for evaluating the quality of starched fabrics. The former may be used for measuring the stickiness of starch mixtures during ironing, and the penetration of such mixtures; the latter may be used to measure the transparency of starch films, and to determine the smoothness, stiffness, and resistance to crushing of starched fabrics.

These methods may also be used to advantage in evaluating other sizing or finishing agents.

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This work was carried out by the Carbohydrate Unit, Agricultural Chemical Research Division, Bureau of Agricultural Chemistry and Engineering, in cooperation with, and in the laboratories of, the Engineering Experiment Station of the Alabama Polytechnic Institute. Contribution No. 157 from the Carbohydrate Unit, Agricultural Chemical Research Division, Bureau of Agricultural Chemistry and Engineering, U. S. Department of Agriculture.

Use of Enzymes in the Refractometric Method for Egg Solids

A Production Control Method

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IN ORDER to prepare eggs of definite solids content, the frozen egg industry requires a rapid method of determining egg solids. Ordinary drying methods, such as the official A. O. A. C. vacuum method (*3*), require too long a time to serve as production control methods. Among the shorter procedures developed, the refractometric method (*2*, *4*) has become important. In applying this method, the refractive index of the egg is determined and, from suitable charts previously prepared on eggs of known solids content, the refractive index is converted into per cent solids. Egg white solids can be determined by this method, but when whole eggs or yolks are examined, the line of demarcation between the light and dark fields of the refractometer is indistinct, and accurate determinations cannot be made (*4*).

Recently, Cahn and Epstein (*6*) reported that the addition of an electrolyte renders the egg optically homogeneous, with the result that sharp contrast between the light and dark fields is obtained, permitting accurate determinations. The use of an electrolyte for this analytical purpose is covered by a patent to Cahn and Epstein (*6*).

The difficulty of determining solids of whole eggs and yolks with a refractometer is occasioned by the turbidity of these products. This turbidity causes a reduction in the amount of light passing through the sample and, in addition, causes scattering of the light into the dark portion of the refractometer field, thereby reducing the contrast between the two portions of the field and rendering the line of demarcation indistinct. Any suitable agent which will remove this turbidity should make it possible to obtain accurate readings.

The turbidity of eggs resides chiefly in the yolk. To some extent it is due to an inhomogeneity resulting from the presence of globules of yellow and white yolk which are probably associated with the laminar structure of the yolk (*7*). In addition to this, the chief protein of the yolk lecithovitellin, is probably not completely in solution in the yolk, since it is not a readily soluble protein (*5*). It is well known, however, that lecithovitellin is soluble in the presence of electrolytes (*1*, *5*), and this probably accounts for the clarifying action of electrolytes in the method of Cahn and Epstein (*6*). Since solution of protein material is involved, it seems obvious that a proteolytic enzyme would be capable of accomplishing the desired clarification by a process of enzymatic digestion.

The choice of a particular enzyme for this purpose is governed by availability, ranges of pH and temperature in which it exerts its action, and speed of reaction. The purpose of this paper is to present refractometric methods of determining solids of whole eggs and yolks in which essential optical homogeneity of sample is obtained by digestive action of the enzymes trypsin and papain.

Materials and Apparatus

The trypsin was a dry preparation of the Difco Laboratories, Detroit, Mich., designated as Trypsin 1:250. The papain was a dry preparation obtained from Parke, Davis and Company, Detroit, and was the dried fruit juice of the papaw tree (*carica papaya* Lin).

Eggs were obtained in the local markets as shell eggs and broken out as needed. Varying solids contents were obtained by changing the ratio of yolks to white.

A Zeiss sugar refractometer having the range 1.33 to 1.54 was employed. A constant-temperature water bath, operating either at 30° or 50° C. ± 0.1°, supplied water to the instrument for uniform temperature. A 25-watt incandescent lamp provided the illumination for the refractometer.

Trypsin Methods

WHOLE EGGS. The dry enzyme was dissolved in water containing sodium hydroxide according to the following formula:

Trypsin Solution A

Trypsin	500 grams
Water	770 cc.
0.25 *N* NaOH	800 cc.

This solution had a refractive index of about 1.377 at 30° C., approximately that for whole eggs. It was found that 1.8 cc. of this solution, added to 10 grams of whole eggs, gave a sharp line on the refractometer field. A reasonable quantity of solution in excess of this does not affect the refractive index reading; less than this quantity will yield a distinct field with time, but since in practice the minimum possible time is desirable, an amount was chosen (1.8 cc. per 10 grams of egg) which would yield a clear field in about 15 seconds. The incorporation of sodium hydroxide in trypsin solution A rendered the mixture of enzyme and egg more alkaline—(i. e., brought it closer to the range of optimum activity for trypsin)—and in this way materially accelerated the digestion. The amount of sodium hydroxide used in this way was not sufficient by itself to render the egg optically homogeneous.

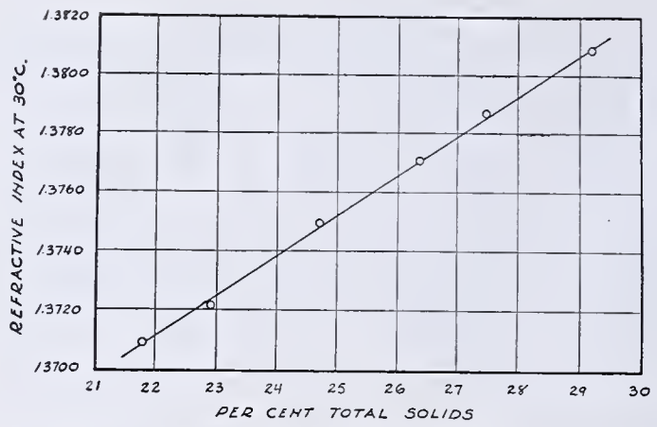

FIGURE 1. CHANGE OF REFRACTIVE INDEX WITH TOTAL SOLIDS FOR WHOLE EGGS WITH TRYPSIN SOLUTION A

That a true enzymatic digestion occurred in as short a period as one minute was demonstrated as follows:

It was assumed that enzymatic digestion of the egg would increase the water-soluble nitrogen. To 25 grams of whole egg 4.5 cc. of trypsin solution A were added. The mixture was stirred for one minute at 27° C. and then diluted with 100 cc. of water. To this were added 100 cc. of a 5 per cent solution of trichloroacetic acid. After one-half hour, the precipitated proteins were filtered off and a Kjeldahl nitrogen determination was made on the filtrate. The same procedure was repeated with heat-inactivated trypsin solution, with water in place of the trypsin solution, and with sodium chloride instead of the trypsin solution. In addition, in place of the egg, 25 grams of water were used with 4.5 cc. of active trypsin solution A and with 4.5 cc. of heat-activated trypsin solution A.

TABLE I. WATER-SOLUBLE NITROGEN DATA ON WHOLE EGG MIXTURES

Mixture	Soluble N in Filtrate	
	%	%
1. 4.5 cc. of trypsin solution A, 25 grams of egg	8.24	7.98
2. 4.5 cc. of trypsin solution A, 25 grams of water	5.81	5.68
3. 4.5 cc. of water, 25 grams of egg	1.52	1.44
4. 4.5 cc. of water, 0.6 gram of NaCl, 25 grams of egg	1.52	1.46
5. 4.5 cc. of heat-inactivated trypsin solution A, 25 grams of egg	8.14	8.40
6. 4.5 cc. of heat-inactivated trypsin solution A, 25 grams of water	6.58	7.00
Increase in soluble N due to active trypsin (mixture 1 minus sum of 2 and 3)	0.91	0.86
Increase in soluble N due to inactive trypsin (mixture 5 minus sum of 3 and 6)	+0.04	−0.04

The data of Table I show that a true increase in water-soluble nitrogen occurs only when active trypsin is used. In arriving at the amount of the increase, it is necessary to deduct the contributions of soluble nitrogen made by the enzyme and by the egg, which are not a result of enzymatic digestion of the egg protein. It is noteworthy that sodium chloride, which may be taken as a typical electrolyte, does not increase the water-soluble nitrogen of the egg protein under the conditions of this test. While the electrolytes may cause solution of proteins, such as lecithovitellin, this action does not alter the proteins and, upon dilution, they are precipitated intact. The action of enzymes, however, is to dissolve the proteins by hydrolyzing them to smaller units, which are soluble.

Heat-inactivated trypsin solution A does not act to yield a distinct field in the refractometer.

The curve shown in Figure 1 gives the variation of the refractive index at 30° C. with thatof the total solids for whole eggs treated with 1.8 cc. of trypsin solution A per 10 grams of egg. Oven moisture solids were obtained by vacuum-drying the samples for 2 to 3 hours at 100° C. (Approximately two dozen eggs were used in obtaining the curve shown in Figure 1.)

The data of Table II show the temperature variation of the refractive index of a sample of whole eggs. From these data, a temperature coefficient of 0.00016 unit per degree centigrade is computed. To obtain an accuracy of 0.15 per cent solids, the temperature of the refractometer must, therefore, be kept within 1.3° of 30° C. (0.00021 refractive index unit equivalent to 0.15 per cent whole egg solids).

TABLE II. CHANGE OF REFRACTIVE INDEX OF A WHOLE EGG SAMPLE WITH TEMPERATURE

Temperature, ° C.	Refractive Index	Temperature, ° C.	Refractive Index
26.2	1.3784	31.0	1.3775
27.0	1.3782	33.0	1.3772
29.0	1.3778	35.0	1.3770

The procedure in making a solids determination on whole eggs is as follows:

Trypsin solution A (1.8 cc.) is added to 10 cc. (roughly equivalent to 10 grams) of whole egg, the two are intimately mixed, and several drops of the mixture are placed on the prisms of the refractometer. The prisms are immediately closed and after 30 seconds (to allow for temperature adjustment), the refractive index is read to the nearest 0.0001 unit. The solids figure is obtained by reference to Figure 1. In practice the refractometric determination yields results agreeing with vacuum-oven method within 0.15 per cent solids.

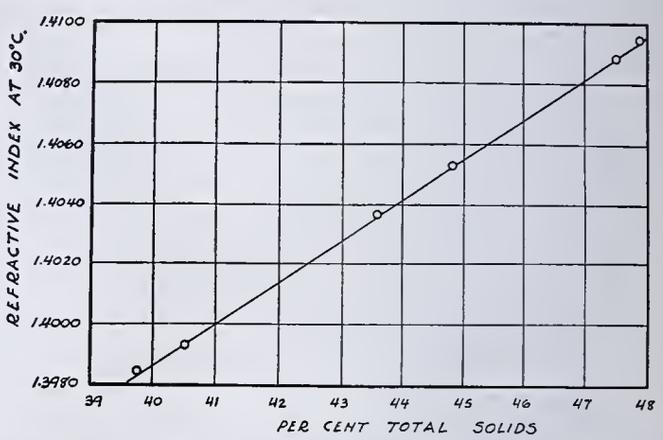

FIGURE 2. CHANGE OF REFRACTIVE INDEX WITH TOTAL SOLIDS FOR YOLKS TREATED WITH TRYPSIN SOLUTION B

YOLKS. The reagent for yolks differs from the whole egg reagent chiefly in its refractive index which has been adjusted to a higher value in order to be approximately the same as that of the yolks. The following approximate formula has been devised:

Trypsin Solution B

Trypsin	500 grams	0.25 *N* NaOH	800 cc.
Water	700 cc.	Dextrose	1100 grams

The reagent should have a refractive index of about 1.403 at 30° C. It may be necessary to vary the amount of dextrose to obtain this value. Treatment of 10 grams of egg with 1.5 cc. of this solution yields a refractometer field with a sharp line of demarcation in about 15 seconds.

Table III lists data for yolks corresponding to those of Table I for whole eggs, demonstrating that true enzymatic digestion occurs with the active enzyme.

TABLE III. WATER-SOLUBLE NITROGEN DATA ON YOLK MIXTURES

Mixture	Soluble N in Filtrate	
	%	%
1. 3.6 cc. of trypsin solution B, 25 grams of egg	6.72	6.08
2. 3.6 cc. of trypsin solution B, 25 grams of water	4.14	4.00
3. 3.6 cc. water, 25 grams of egg	1.40	1.30
4. 3.6 cc. of water, 0.6 gram of NaCl, 25 grams of egg	1.40	1.26
5. 3.6 cc. of heat-inactivated trypsin solution B, 25 grams of egg	6.24	6.08
6. 3.6 cc. of heat-inactivated trypsin solution B, 25 grams of water	4.80	4.84
Increase in soluble N due to active trypsin (mixture 1 minus sum of 2 and 3)	1.18	0.78
Increase in soluble N due to inactive trypsin (mixture 5 minus sum of 3 and 6)	+0.04	−0.06

The curve shown in Figure 2 gives the variation of the refractive index at 30° C. with that of the total solids for yolks treated with 1.5 cc. of trypsin solution B per 10 grams of egg. Each experimental point represents the determination upon approximately 120 grams of mixed yolks.

The temperature variation of the refractive index of yolks is shown in Table IV. From these data, a temperature coefficient of 0.00019 unit per degree can be computed. To obtain an accuracy of 0.15 per cent solids, the temperature of the refractometer must, therefore, be kept within 1.1° of 30° C. (0.00021 refractive index unit equivalent to 0.15 per cent yolk solids).

The procedure of making a solids determination on a yolk sample is identical with that given for whole eggs, except that 1.5 cc. of trypsin solution B per 10 cc. of yolk are required.

Papain Methods

Papain differs from trypsin in several respects. The commercially available papain is not so active as trypsin 1:250; its optimum activity lies in a more acid range; and the temperature of optimum activity is considerably higher. These facts alter the procedure in using papain.

Whole Eggs. A suitable reagent for whole egg determinations may be prepared according to the following formula:

Papain Solution A		Papain Solution A	
Papain	40 grams	2 N H_2SO_4	23.4 cc.
Water	100 cc.	Dextrose	15.6 grams

Acid is added to this reagent to lower the pH of the reagent-egg mixture more nearly to approximate the pH range for optimum activity of papain. Commercial papain contains appreciable insoluble material which it is advantageous to remove by centrifuging before using this reagent.

Table IV. Change of Refractive Index of Yolks with Temperature

Temperature, ° C.	Refractive Index	Temperature, ° C.	Refractive Index
27.0	1.4072	33.0	1.4061
29.0	1.4070	35.0	1.4057
31.0	1.4065		

In order to obtain reasonably rapid digestion with papain it is necessary to raise the temperature to about 50° C. A simple way to accomplish this is to raise the temperature of the refractometer to 50° C. and permit the digestion of the sample to occur on the prisms. Treatment of 10 grams of whole egg with 1 cc. of papain solution A will yield a distinct refractometer field in 3 to 4 minutes at 50° C. When the temperature of the refractometer is as high as 50° C., extreme care must be exercised to avoid loss of moisture from the sample on the prisms.

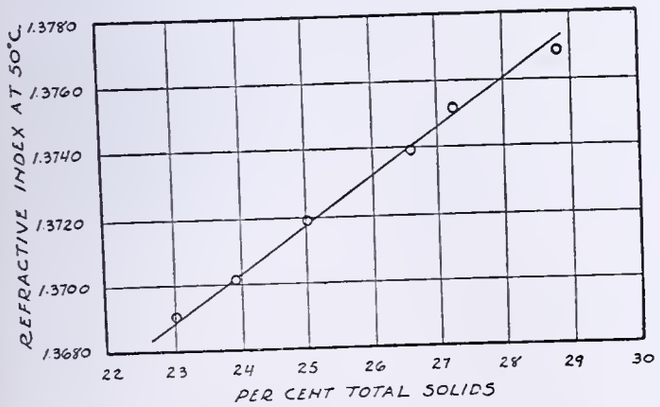

Figure 3. Change of Refractive Index with Total Solids for Whole Eggs Treated with Papain Solution A

Digestion data, similar to those given for trypsin, have demonstrated that the removal of the turbidity of whole eggs involves a true enzymatic digestion.

The curve shown in Figure 3 gives the variation of the refractive index at 50° C. with total solids for whole eggs treated with 1 cc. of papain solution A per 10 grams of whole egg. By controlling the temperature of the refractometer within the limits given for whole eggs under trypsin methods, solids determinations can be made with an accuracy of 0.15 per cent solids by using papain solution A and the curve of Figure 3.

Yolks. A suitable reagent for yolks may be prepared according to the following formula:

Papain Solution B	
Papain	40 grams
Water	100 cc.
Dextrose	Sufficient to yield a refractive index for the solution at 50° C. of 1.403

Treatment of 10 grams of yolk with 1 cc. of this reagent will yield a distinct refractometer field in 3 or 4 minutes at 50° C. Because of the more acidic nature of the yolks, no added acid is required. Digestion data here, too, support the belief that the process is dependent upon enzymatic digestion.

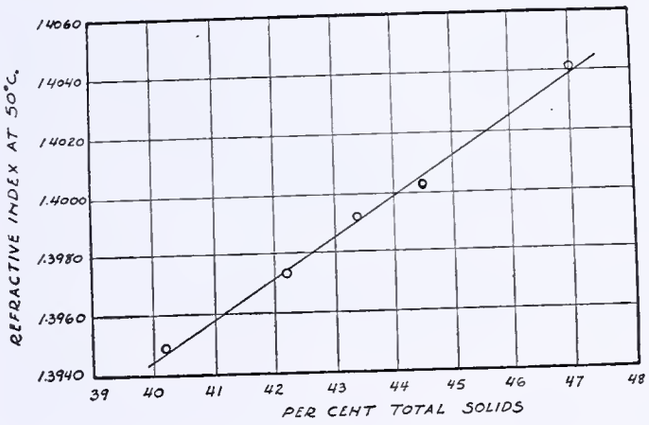

Figure 4. Change of Refractive Index with Total Solids for Yolks Treated with Papain Solution B

Figure 4 shows the solids–refractive index curve for yolks at 50° C. using this reagent. With proper temperature control, an accuracy of 0.15 per cent solids can be obtained.

Summary

A refractometric method of determining solids of whole eggs and yolks is described in which these products are rendered essentially optically homogeneous by enzymatic digestion, so that their refractive indices can be determined with ease and accuracy.

Specific procedures are given employing either trypsin or papain.

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Direct Determination of Potassium in Silicate Rock

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THE advent of the triple acetate methods for the determination of sodium has made possible the direct determination of sodium in silicate materials. The silicate may be decomposed by treatment with hydrofluoric acid, the fluoride removed by evaporation with sulfuric acid, and the sodium precipitated immediately as magnesium or zinc sodium uranyl acetate, in which form it is weighed. Unfortunately, sulfate cannot be present in the determination of potassium when it is weighed as perchlorate. The Berzelius method, in which the sulfate is removed by precipitation as barium sulfate and the excess barium and other heavy metals are removed by precipitation with ammonia and ammonium carbonate, is long, and the coprecipitation of potassium with the barium sulfate makes the results somewhat uncertain. The J. Lawrence Smith method, in which the decomposition is accomplished by a fusion with ammonium chloride and calcium carbonate, is extremely time-consuming but yields results beyond reproach.

Of the alternative ways of eliminating fluoride following the decomposition of the silicate by hydrofluoric acid, evaporation with perchloric acid would appear feasible; actually, however, all fluoride cannot be removed even by repeated evaporations with perchloric acid, probably because of the great stability of the fluoaluminate ion, AlF_6^{---}. Removal of fluoride by precipitation as calcium fluoride was suggested by Koenig (*1*) in 1933, but this method in the authors' hands has not been satisfactory. Another method of eliminating fluoride is now proposed in which the fluoride is removed by volatilization as hydrofluosilicic acid, H_2SiF_6, as in the method of Willard and Winter (*5*) for the determination of fluoride.

Unfortunate results with the Koenig method led the authors to perform a number of determinations with the view of locating the sources of error in the procedure.

In a series of determinations of the sodium plus potassium in Bureau of Standards Sample No. 70, a high-potassium feldspar, the results obtained were highly erratic and invariably low, sometimes by as much as 50 mg. in the 0.2440 gram of sodium plus potassium chlorides which should have been derived from a 1-gram sample. The mixed chlorides obtained were also found to contain appreciable quantities of fluoride as measured by the bleaching effect on pertitanic acid. Treatment with calcium hydroxide does not remove fluoride completely in the analysis of a silicate, again probably because of the stability of the fluoaluminate ion. The calcium oxide used was prepared by ignition of the special grade of calcium carbonate usually used for J. Lawrence Smith fusions. In some experiments the calcium oxide was slaked slowly by treatment with steam prior to use, with the object of improving the physical character of the calcium hydroxide. This had no beneficial effects on the results, nor did more extensive washing during the filtrations. Finally, weighed portions of pure potassium chloride and mixtures of pure potassium chloride and pure sodium chloride were treated with hydrofluoric acid, the solutions evaporated to dryness, dissolved, added to the calcium oxide, and carried through the procedure exactly as in the silicate analysis. The results were erratic and low by as much as 30 mg., samples of about 1 gram having been taken. No fluoride was found in the chlorides obtained. It thus appears that the alkalies are retained somewhere in the process, undoubtedly with the calcium hydroxide. In view of these results the authors considered the problem as still lacking an adequate solution.

The separation of fluoride by distillation as hydrofluosilicic acid has received considerable study since it was originally proposed and the conditions and completeness of the separation have been confirmed. As the distillation may be effected with either sulfuric or perchloric acid to elevate the temperature of distillation, the obvious choice is perchloric acid, which yields directly the solution of perchlorates needed for the determination of potassium by the perchlorate method and also furnishes a method of dehydrating any residual silica prior to the determination of the potassium. As no ammonium salts are introduced during the analysis their removal is obviated.

Since in this case it is immaterial whether some perchloric acid is distilled with the hydrofluoric acid, the distillation was carried out at temperatures of 140–150° C.—that is, somewhat above the 135° recommended by Willard and Winter—and the time required for the distillation was thus shortened. The distillation is best effected by injecting steam into the distillation flask and regulating the flame below the flask to give the desired temperature.

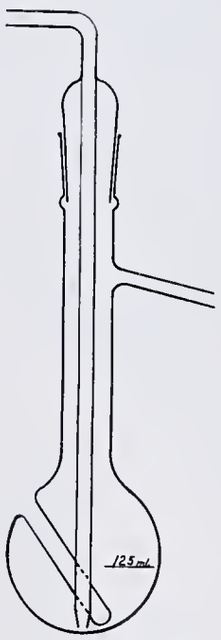

FIGURE 1

In final form the all-Pyrex distillation vessel shown in Figure 1 was employed. If the bulb were attached to the stem by a ground joint, the removal of the contents would be somewhat facilitated. A volume of distillate of 400 to 500 ml. is sufficient to remove all fluoride. As is customary in the analysis of the alkali metals, blanks must be run concurrently. Since the apparatus used is platinum and Pyrex and the time required for the determination is not great, the blank is very small and consistent. Twenty blank determinations run during the course of the work gave an average value of 0.0005 gram of potassium perchlorate, the minimum and maximum values being 0.0003 and 0.0009 gram.

Table I indicates that the method tends to give slightly high results. This was thought to be due possibly to the fact that fluoride was taken into the distilling flask in the samples but not in the blank, in which no aluminum was present; this fluoride might then attack the distilling flask, introducing potassium into the sample but not into the blank. This would account for high results. In order to make the blank duplicate the determination as closely as possible, a special grade of cryolite, Na_3AlF_6, known to contain a negligible amount of potassium, was added to the blank in a series of determinations. The values obtained in this way were 0.0005, 0.0004, and 0.0008 gram of potassium perchlorate, identical in size and variation with the blanks run in the normal manner. It thus appears that the slightly higher results cannot be explained in this manner.

Incomplete dehydration of silica picked up during the distillation might also account for the higher results. Following the distillation, the silica was dehydrated by evaporation with an additional amount of perchloric acid and the usual boiling after the appearance of perchloric acid fumes. It was improbable that any silica contaminated the potassium salt, but as a check the potassium perchlorate finally obtained and

Table I. Determination of Potassium in Bureau of Standards Samples

Analysis No.	Weight of Sample *Grams*	Weight of $KClO_4$ *Gram*	K_2O Present Found %	K_2O Present B. of S. %
	B. of S. No. 70 Feldspar			
1	1.0000	0.3707	12.59	12.58
2	1.0004	0.3708	12.59	
3	1.0000	0.3730	12.66	
4	1.0011	0.3741	12.70	
5	1.0000	0.3676	12.48	
6	1.0000	0.3680	12.49	
7	1.0000	0.3680	12.49	
8	1.0010	0.3676	12.48	
	B. of S. No. 99 Soda Feldspar			
1	3.000	0.0396	0.44	0.41
2	3.000	0.0412	0.46	
3	3.000	0.0400	0.45	
4	3.000	0.0336	0.38	
	B. of S. No. 91 Opal Glass			
1	2.000	0.1959	3.32	3.25
2	2.000	0.1936	3.28	
3	2.000	0.1952	3.31	
4	1.0028	0.0988	3.34	
5	1.0006	0.0955	3.23	
6	1.0010	0.0948	3.21	
	B. of S. No. 89 Lead-Barium Glass			
1	2.000	0.4908	8.33	8.40
2	2.000	0.4965	8.43	
3	2.000	0.4915	8.34	
	B. of S. No. 97 Flint Clay			
1	1.0015	0.0176	0.58	0.54
2	1.0008	0.0174	0.57	
3	1.0016	0.0178	0.59	
	B. of S. No. 98 Plastic Clay			
1	2.0000	0.1896	3.22	3.17
2	2.0010	0.1891	3.21	
3	1.5000	0.1411	3.19	
4	1.5015	0.1439	3.25	

weighed was dissolved in water and the crucible dried and weighed. In a few cases in earlier work some silica was found but none if the dehydration was properly performed.

Results by students on the silicate samples used as unknowns at the University of Michigan and at Iowa State College have also shown that the method tends to give slightly higher values for potassium than the J. Lawrence Smith fusion followed by the perchloric acid method. The method is more rapid than the Smith method and requires less applied time. Sulfates must, of course, be absent.

Procedure

Weigh 1 to 2 grams of the sample, depending on the potassium content, into a 20-ml. platinum crucible, moisten with water, and add 3 ml. of 70 per cent perchloric acid and 10 ml. of hydrofluoric acid. Stir well with a platinum wire or the rubber end of a policeman, taking care not to let the acid touch the glass; rinse, remove the stirring rod, place in a Hillebrand evaporator in a good hood, and allow to evaporate to dryness. Moisten with water, add 2 to 5 ml. more of hydrofluoric acid, depending on the size of the sample taken, and 3 ml. of perchloric acid, stir well, and again evaporate to dryness. Transfer the salts to a 125-ml. Pyrex distilling flask, using a long-stemmed funnel and making sure that all insoluble material is transferred to the flask. Blanks should be started with the samples and carried through the entire procedure.

The use of a two-hole rubber stopper carrying the thermometer and steam inlet tube is permissible only if care is taken to avoid concentration of the acid, which would then attack the rubber, perhaps violently. With this precaution no trouble has been experienced from this source. The all-glass distilling apparatus previously described is convenient, and eliminates the possibility of danger of the hot perchloric acid coming into contact with the rubber stopper. Add a few pieces of broken quartz or Pyrex to the flask and connect the flask to a steam generator and to a water-cooled condenser. Four of these may be set up and run simultaneously by one person.

With the steam generator disconnected and the inlet tube closed with a piece of rubber tubing and a pinch clamp, concentrate the liquid in the flask to a volume of about 12 ml. by boiling gently. Cool somewhat, add 8 ml. of 70 per cent perchloric acid, heat to 140–150°, and pass steam through the mixture at a moderate rate until 300 to 500 ml. of distillate have been collected. The larger volume is necessary only in case a large sample is employed. Shake the flask occasionally to wash down material spattered on the walls.

Transfer the contents of the flask to a 250-ml. beaker and wash out well with hot water, leaving behind the pieces of glass or quartz. Evaporate on a hot plate to strong fumes of perchloric acid, adding 2 ml. or enough perchloric acid so that the mixture may be boiled without spattering. Continue the evaporation at a somewhat higher temperature to dehydrate completely the silica and to expel the excess of perchloric acid. Evaporate until the residue is just moist but not to dryness; otherwise insoluble basic salts of aluminum will be formed.

A little silica will separate with the potassium perchlorate. Cool the residue by immersing the beaker in cold water. Add 20 to 30 ml. of anhydrous ethyl acetate (*2*, *4*), and stir until the calcium and aluminum perchlorates dissolve and the potassium perchlorate remains. Cool in cold water and filter through a small, dry, fine filter paper, transferring most of the precipitate with ethyl acetate from a wash bottle. Wash three or four times with 2-ml. portions of ethyl acetate and discard the filtrate. Dissolve the potassium perchlorate remaining in the beaker with hot water, and pour it through the filter, collecting the filtrate in a 150-ml. beaker. Wash with hot water until all the potassium perchlorate has dissolved and at least ten times more, using small portions. Any silica will remain on the filter. Evaporate to gentle dryness. Cool, add 15 ml. of anhydrous ethyl acetate, warm slightly, and stir to extract the small amount of soluble perchlorates remaining. Cool, transfer to a weighed filtering crucible, and wash with five or six portions of ethyl acetate of about 1 ml. each. Pyrex sintered-glass crucibles of medium porosity are very satisfactory. Dry the beaker and scrape loose any adhering salt crystals with a bright metal spatula and brush into the crucible. Dry in an oven at 110° for 20 to 30 minutes and then heat the covered crucible to 310° (*3*) for 20 minutes, using a muffle or oven. Cool and weigh. Reheat at 310°, cool, and weigh to ensure constant weight. The crystals burst during the heating and leave a fine powder. Subtract the weight of the potassium perchlorate in the blanks, usually 1 mg. or less.

Summary

The metals in insoluble silicates are completely converted into perchlorates by evaporation of the silicate with hydrofluoric and perchloric acids, followed by a steam-distillation at 140° to 150° C. to remove fluoride as hydrofluosilicic acid.

After dehydrating the small amount of silica, the solution is evaporated nearly to dryness and the potassium separated twice as perchlorate by extracting the soluble perchlorates with ethyl acetate.

Acknowledgment

The authors wish to acknowledge the contribution of James B. Montgomery of Purdue University, who carried out some of the preliminary work of this investigation, and to thank H. V. Churchill of the Aluminum Company of America for providing the special grade of cryolite used in the work.

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Determination of Copper and Nickel in Steels

Determination of Copper by Electrodeposition and Nickel by Cyanide Titration

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IN A RECENT paper Frediani and Hale (*1*) presented a method for the electrolytic determination of copper in ferroalloys, wherein the interference of the iron–nitric acid combination is eliminated by temperature control. Unfortunately, a special cooling cell is required. The twofold purpose of the present paper is to electroplate copper in steel without the use of special apparatus and to titrate nickel without previous separation by dimethylglyoxime.

When a large number of determinations are to be made simultaneously, with no special accommodations other than the usual electrolytic board of ten to thirty electrode units, the procedure outlined here may be used for both copper and nickel in the same steel sample.

Experimental

Experiments were performed with a steel comparable to Bureau of Standards No. 101a (18 per cent chromium, 9 per cent nickel), a steel containing small amounts of chromium and nickel, and finally a steel containing 1 per cent chromium, 1 per cent copper, and 1 per cent nickel. Steel samples varying from 0.1 to 2.0 grams were used with 0.020 gram of added copper, with and without added glacial phosphoric acid.

For the nickel determinations a special steel of the Ni-Resist type (6 per cent copper, 14 per cent nickel, and 3 per cent chromium) was used. The nickel was determined in the copper-free electrolyte, after oxidation of ferrous iron, by cyanide titration.

TABLE I. DETERMINATION OF COPPER IN SYNTHETIC SOLUTIONS
(0.020 gram of copper taken)

	A		B		C	
Steel Taken	Copper found[a]	Time for appearance of copper	Copper found[a]	Time for appearance of copper	Copper found[a]	Time for appearance of copper
Grams	*Gram*	*Min.*	*Gram*	*Min.*	*Gram*	*Min.*
0.1	0.020	0	0.020	0	0.020	0
0.2	0.020	0	0.020	0	0.020	0
0.3	0.020	0	0.020	0	0.020	0
0.4	0.020	0	0.020	0	0.020	0
0.5	0.020	5	0.020	0	0.020	15
0.6	0.020	5	0.020	0	0.020	20
0.7	0.020	10	0.020	0	0.020	25
0.8	0.020	10	0.020	0	0.020	25
0.9	0.020	20	0.020	5[b]	0.020	25
1.0	0.020	20	Dark plate	10[b, c]	0.020	25
2.0	[c]	[c]	Dark plate	30[b, c]	0.019	[d]

A. Plain steel plus 0.020 gram of copper. Dissolve in aqua regia, fume with perchloric acid, neutralize with ammonium hydroxide, acidify, and add 4 cc. of (1 to 1) sulfuric acid excess.
B. Same as A, but 2 cc. of glacial phosphoric acid added.
C. 18 Cr–8 Ni steel, treated as in A.
[a] 0.020 ± 0.0002 gram.
[b] 5 cc. of glacial phosphoric acid added after 5 minutes.
[c] Stirred, incomplete.
[d] Intermittent stirring over 4 hours.

Results

Table I shows that 20 mg. of copper may be deposited from steel solutions under the conditions noted. Copper appears sooner from electrolytes that contain phosphoric acid than from those that contain only sulfuric acid. Copper separates more slowly from chromic acid electrolytes.

Table II shows that the method may be used to separate 6 per cent copper and 14 per cent nickel in steel.

Table III shows that the proposed method may be used with steels containing 1 per cent copper and 1 per cent nickel, and with steels containing as little as 0.25 per cent copper.

If after the appearance of copper the electrolyte is shaken until the plate dissolves and after another hour of electrolysis the electrolyte is removed, the percentage of ferrous iron in the electrolyte is in the neighborhood of 95. Thus, copper is deposited in a solution containing a preponderance of ferrous ion.

Volatilization of chromium by hydrochloric acid (*2*) has no important effect.

TABLE II. ANALYSIS OF NI-RESIST STEEL

	Thiosulfate Method (*3*)		Proposed Method	
	Sample 1	Sample 2	Sample 1	Sample 2
	%	%	%	%
Carbon	..	..	2.54	2.68, 272
Silicon	..	..	2.25, 228	1.81, 182
Manganese	..	..	1.36	1.15
Phosphorus	..	..	0.110	0.12
Sulfur	..	..	0.065	0.083
Nickel	..	..	13.5	13.4
Copper	6.14	6.08	6.13 6.15	6.06

Procedure

NI-RESIST STEELS. Weigh 2.500 grams of steel, transfer to a tall-form 300-cc. beaker, add 25 cc. of mixed acid (3 parts of hydrochloric acid, 1 of nitric acid, and 4 of water), and set on a warm plate. After solution is complete, add 30 cc. of technical (70 per cent) perchloric acid and heat to fumes of perchloric acid oxidizing chromium to chromic acid and graphitic carbon to carbon dioxide. Filter into a 250-cc. volumetric flask, wash the silica with hot (1 to 99) sulfuric acid, then with water, ignite, and weigh as silica.

Make the filtrate up to the mark and shake. Transfer 20 cc. (0.20 gram) to a 400-cc. beaker and determine manganese and chromium (*4*). Transfer 100 cc. (1.00 gram) to a tall-form 300-cc. beaker and determine phosphorus.

Transfer 100 cc. (1.00 gram) to a tall-form 300-cc. beaker, add (1 to 1) ammonium hydroxide until a permanent precipitate forms, and add (1 to 1) sulfuric acid to dissolve the precipitate and then 4 cc. in excess. Add 5 cc. of glacial phosphoric acid, and electrolyze without stirring. Use an impressed voltage of about 6 volts and 0.6 ampere for about 2 hours on platinum electrodes. Remove the electrolyte, wash the copper plating with water, dry, and weigh.

Transfer the electrolyte to a 250-cc. volumetric flask, dilute to the mark, and shake. Transfer 50 cc. (0.20 gram) to a 400-cc. beaker, add 10 cc. of (1 to 1) sulfuric acid and 20 cc. of 6 per cent ammonium persulfate solution, stir, and heat to decompose excess persulfate. Cool, and determine nickel by cyanide titration (*4*).

COPPER AND NICKEL IN STEELS. Dissolve 1.00 gram of steel in 20 cc. of mixed acid, add 15 cc. of technical (70 per cent) perchloric acid, and heat to boil out the nitric and hydrochloric acids. Continue until the perchloric acid condenses on the underside of the cover glass. Cool, add 50 cc. of water, shake, boil out free chlorine, and cool. Add (1 to 1) ammonium hydroxide until a permanent precipitate forms, acidify with (1 to 1) sulfuric acid, and add 4 cc. in excess. Add 5 cc. of glacial phosphoric acid, and electrolyze without stirring. Use an impressed voltage of 4 volts maximum for copper less than 0.01 gram, 4 to 6 volts for copper above 0.01 gram, and 0.6 ampere. After 2 hours remove the electrolyte, wash the copper plating, dry, and weigh.

To the electrolyte add 10 cc. of (1 to 1) sulfuric acid and about 50 cc. of 6 per cent ammonium persulfate solution, stir, and heat to destroy any excess persulfate. Cool. Test with permanganate, and oxidize any ferrous ion that may be present. Determine nickel by cyanide titration (*4*).

Discussion

The Frediani and Hale method for the determination of copper features low temperature in the presence of large

Table III. Analysis of Copper-Nickel Steel

Sample	Thiosulfate Method (3) Cu %	Thiosulfate Method (3) Ni %	Proposed Method Cu %	Proposed Method Ni %	Manufacturers Cu %	Manufacturers Ni %
1 Bureau of Standards No. 106	..	..	0.15	..	0.14	..
2 Low chromium	0.20	..	0.20	..	..	..
3 Low chromium	..	..	0.23	..	0.26	..
4	1.08	..	1.10	1.70	1.07	1.73
			1.08			
5	1.12	..	1.10	..	..	..
			1.10			
6	1.16	..	1.14	1.62	1.12	1.56
7 18-8	..	..	0.15	8.50	..	8.56

amounts of ferric ion complex and ammonium ions, whereas in the proposed method room temperature prevails and the order of the reaction must be: (1) reduction of all hexavalent to trivalent chromium, probably by the electrolytic production of ferrous ion (which would reduce dichromic acid), (2) complete reduction of ferric to ferrous ions in the immediate vicinity of the cathode, and (3) complete deposition of the copper.

Stirring the liquid will prevent formation of the copper plate, or will redissolve it after it has formed. Using a potential source of 4 volts, the actual potential across the electrodes of an agitated solution is about 1.6 volts, while the copper deposits in an unagitated solution only when the voltage drop is greater than 2 volts. Copper is deposited from electrolytes that show between 0.6 and 0.95 gram of ferrous ion. The assumption is that, in an unstirred solution a protective coating of hydrogen gas forms and serves as a cause for overvoltage to permit deposition of copper and as a blanket to prevent resolution by the electrolyte.

When phosphoric acid is present, copper begins to deposit sooner than in its absence. Manganese dioxide does not appear at the anode. In order to procure bright adherent plates, if the copper is below 0.010 gram the voltage source (impressed voltage) must not be greater than 4 volts. Ni-Resist steels (6 per cent copper) give bright copper plates, but the results are about 0.02 per cent higher, on the general run of samples, than those obtained by the thiosulfate method (3). Neither manganese nor nickel has been detected in the copper plates.

Nickel is easily determined in the electrolyte after oxidation of ferrous ion by persulfate or permanganate. Excess persulfate is destroyed by boiling, and even excess permanganate is avoided by ordinary precautions or additon of a sulfite.

The paper neither affirms nor disaffirms the work of Frediani and Hale, since there are no points of common interest.

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Determination of Alcohol by Volume in Distilled Liquors

Sources of Error

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PERSISTENT recurrence of relatively small but significant discrepancies between the results obtained in different laboratories in the determination of alcohol by volume on duplicate samples of distilled liquors led to an investigation of the A. O. A. C. official method for alcohol by volume (*1*, p. 172). This investigation disclosed a source of error inherent in the method, exclusive of any experimental errors.

The A. O. A. C. official method provides that the specific gravity of a distilled liquor shall be determined in air at 20/20° C. Alcohol by weight is determined by weighing 20 to 25 grams of sample, diluting with 100 ml. of water, distilling nearly 100 ml., weighing the distillate, determining the specific gravity of distillate, at room temperature if desired, obtaining the corresponding percentage of alcohol by weight from the proper A. O. A. C. tables, multiplying this figure by weight of distillate, and dividing by weight of sample taken.

Alcohol by volume is determined by either of two methods. In Method I, sample and distillate are treated as described above, the percentage of alcohol by volume corresponding to the specific gravity of the distillate is obtained from the proper A. O. A. C. table, the volumes of the sample and distillate are calculated from their respective weights and specific gravities, and the percentage of alcohol by volume in the distillate is multiplied by the volume of the distillate and divided by the volume of the sample to obtain the percentage of alcohol by volume in the original sample. In Method II, a 25-ml. sample is measured at room temperature and the distillate is made to a volume of 100 ml. at the same temperature. The specific gravity of the distillate is determined at room temperature and the corresponding percentage of alcohol by volume is multiplied by 4 to obtain the percentage of alcohol by volume in the original sample.

The percentage of alcohol by volume in any mixture of ethyl alcohol and water varies with the temperature; therefore the temperature at which a given mixture will have a stated percentage of alcohol by volume must be specified. The generally accepted standard of temperature for alcoholic concentration by volume is 60° F. or 15.56° C., and the A. O. A. C. tables give percentages of alcohol by volume at this temperature. However, the ratio of the volumes of definite weights of any two aqueous solutions of ethyl alcohol of different alcoholic concentrations varies with temperature, and herein lies the inherent source of error in the A. O. A. C. official method for alcohol by volume. In both Methods I and II the volumes of the sample and of the distillate may be determined at temperatures other than 15.56° C. Yet the true percentage by volume at 60° F. of ethyl alcohol in the distillate is multiplied by the inverse ratio of these volumes to obtain the percentage by volume at 60° F. of ethyl alcohol in the sample. The result is in error by an amount depending upon the alcoholic concentrations of the sample and the distillate and the temperature at which the volumes of the sample and the distillate are determined. The calculated data presented in Tables II and III show that this inherent error is very substantial under conditions often prevailing in the determination of alcohol by volume by the A. O. A. C. official method.

In order to show the magnitude of the inherent error over a wide range of conditions calculations were made for a series of

TABLE I. APPARENT SPECIFIC GRAVITIES CORRESPONDING TO PERCENTAGES OF ETHYL ALCOHOL

Percentage of Ethyl Alcohol By weight	By volume at 60° F.	Apparent Specific Gravities at Various Temperatures 15.56° C./15.56° C.	20° C./20° C.	25° C./25° C.	30° C./30° C.	35° C./35° C.
10.000	12.392	0.98384	0.98359	0.98330	0.98298	0.98267
20.000	24.472	0.97136	0.97032	0.96920	0.96810	0.96704
30.000	36.181	0.95740	0.95546	0.95340	0.95147	0.94962
40.000	47.326	0.93925	0.93677	0.93413	0.93165	0.92929
50.000	57.830	0.91810	0.91535	0.91240	0.90963	0.90696
60.000	67.689	0.89550	0.89258	0.88945	0.88648	0.88363
70.000	76.909	0.87208	0.86904	0.86578	0.86265	0.85965
80.000	85.458	0.84787	0.84476	0.84138	0.83817	0.83506

alcoholic concentrations of original sample ranging from 10 to 80 per cent by weight. The A. O. A. C. tables used were Nos. 19 and 21 (*1*, XLIII).

The values in the first column of Table I of the present paper, representing percentages of ethyl alcohol by weight in original samples, were chosen arbitrarily as a basis of calculation. The corresponding percentages by volume at 60° F. in the second column of Table I were obtained by interpolation from (*1*, Table 21, XLIII), and the corresponding apparent specific gravities in the remaining columns of Table I were obtained by interpolation from (*1*, Table 19, XLIII).

The values in the second column of Table II for weight of sample distilled were chosen arbitrarily. However, those for each alcoholic concentration of original sample include the values of 20 and 25 grams, the limits of sample weight given in the A. O. A. C. official method, and extend upward to yield alcoholic concentrations of distillate ranging up to 25 per cent by weight and downward in the case of higher original alcoholic concentrations.

The values in the third column of Table II represent the calculated alcoholic concentration by weight in 100 grams of distillate and were obtained by multiplying the values in the second column by the values in the first column and dividing by 100. The corresponding percentages by volume at 60° F. in the fourth column were obtained by interpolation from (*1*, Table 21, XLIII). The corresponding apparent specific gravities in columns 5 to 9, inclusive, were obtained by interpolation from (*1*, Table 19, XLIII). The values in columns 10 to 14, inclusive, were calculated, as directed (*1*, XVI, 4) by calculating the volumes of sample and distillate at each temperature from the corresponding apparent specific gravities, multiplying the values in column 4 by the volume of distillate at each temperature, and dividing by the volume of sample at the same temperature. The amounts by which these calculated values differ from the true percentages by volume at 60° F. are given in columns 6 to 10, inclusive, of Table III.

All the values in column 6 of Table III should, of course, be zero. The amounts by which these values differ from zero merely indicate the limits of accuracy of interpolations and logarithmic calculations involved. The largest of these differences is only 0.018 per cent by volume at 60° F., or 0.036 proof, and most of them are much smaller. Errors of such magnitude would not ordinarily be of any significance. However, as soon as the working temperature is raised to 20° or 25° C. or higher, with original alcoholic concentrations of 20 per cent by weight or higher and sample weights of 20 to 25 grams, the inherent error begins to be significant. For example, with 20 per cent alcohol by weight in the original sample, a 20-gram sample and 25° C. working temperature, the error is 0.055 per cent by volume, or 0.11 proof; with 40 per cent alcohol by weight in the original, a 20-gram sample and 25° C. temperature, the error is 0.248 per cent by volume, or 0.496 proof; and with 80 per cent alcohol by weight in the original, a 15-gram sample and 35° C. temperature, the error reaches a value of 1.16 per cent by volume, or 2.32 proof.

The most obvious, and, whenever feasible, the easiest way to avoid this error is to work at a temperature of 15.56° C. However, when the dew point is above 15.56° C., it is difficult to determine specific gravities accurately by pycnometer at that temperature.

TABLE II. CALCULATED DATA ON DISTILLATE FROM SAMPLES OF ORIGINAL AQUEOUS SOLUTIONS CONTAINING ETHYL ALCOHOL

Ethyl Alcohol in Original, % by Wt.	Wt. of Sample Taken, Grams	Distillate Bulked with Water to 100 Grams: Percentage of Ethyl Alcohol By wt.	By volume at 60° F.	Apparent Specific Gravities 15.56° C./15.56° C.	20° C./20° C.	25° C./25° C.	30° C./30° C.	35° C./35° C.	Percentage by Volume at 60° F. of Ethyl Alcohol in Original, Calculated from Apparent Specific Gravities of Original and Distillate 15.56° C./15.56° C.	20° C./20° C.	25° C./25° C.	30° C./30° C.	35° C./35° C.
10	20	2.0	2.509	0.99629	0.99629	0.99627	0.99625	0.99623	12.388	12.385	12.382	12.378	12.374
	25	2.5	3.135	0.99539	0.99538	0.99536	0.99534	0.99531	12.395	12.392	12.388	12.384	12.381
	50	5.0	6.243	0.99118	0.99112	0.99106	0.99098	0.99090	12.394	12.391	12.388	12.385	12.382
	75	7.5	9.327	0.98739	0.98726	0.98710	0.98693	0.98675	12.391	12.390	12.388	12.386	12.385
	100	10.0	12.392	0.98384	0.98359	0.98330	0.98298	0.98267	12.392	12.392	12.392	12.392	12.392
	125	12.5	15.440	0.98056	0.98012	0.97966	0.97919	0.97871	12.393	12.396	12.398	12.400	12.402
20	20	4.0	5.002	0.99281	0.99277	0.99273	0.99267	0.99261	24.470	24.444	24.417	24.391	24.366
	25	5.0	6.243	0.99118	0.99112	0.99106	0.99098	0.99090	24.473	24.448	24.421	24.396	24.371
	40	8.0	9.942	0.98666	0.98651	0.98634	0.98615	0.98593	24.470	24.447	24.423	24.400	24.379
	60	12.0	14.832	0.98120	0.98081	0.98037	0.97994	0.97950	24.472	24.456	24.439	24.421	24.406
	80	16.0	19.676	0.97624	0.97556	0.97480	0.97408	0.97335	24.472	24.463	24.453	24.444	24.436
	100	20.0	24.472	0.97136	0.97032	0.96920	0.96810	0.96704	24.472	24.472	24.472	24.472	24.472
30	20	6.0	7.479	0.98962	0.98954	0.98945	0.98934	0.98922	36.177	36.107	36.033	35.963	35.899
	25	7.5	9.327	0.98739	0.98726	0.98710	0.98963	0.98675	36.175	36.106	36.036	35.967	35.904
	30	9.0	11.169	0.98523	0.98503	0.98479	0.98455	0.98429	36.178	36.112	36.043	35.978	35.919
	40	12.0	14.832	0.98120	0.98081	0.98037	0.97994	0.97950	36.180	36.122	36.062	36.002	35.949
	60	18.0	22.080	0.97384	0.97297	0.97204	0.97114	0.97024	36.179	36.138	36.095	36.054	36.018
	80	24.0	29.212	0.96620	0.96477	0.96325	0.96181	0.96041	36.182	36.162	36.142	36.122	36.105
40	20	8.0	9.942	0.98666	0.98651	0.98634	0.98615	0.98593	47.321	47.203	47.078	46.963	46.854
	25	10.0	12.392	0.98384	0.98359	0.98330	0.98298	0.98267	47.322	47.209	47.089	46.980	46.876
	30	12.0	14.832	0.98120	0.98081	0.98037	0.97994	0.97950	47.326	47.220	47.109	47.004	46.906
	40	16.0	19.676	0.97624	0.97556	0.97480	0.97408	0.97335	47.326	47.235	47.137	47.048	46.964
	50	20.0	24.472	0.97136	0.97032	0.96920	0.96810	0.96704	47.326	47.251	47.172	47.101	47.033
	60	24.0	29.212	0.96620	0.96477	0.96325	0.96181	0.96041	47.328	47.273	47.215	47.161	47.110
50	15	7.5	9.327	0.98739	0.98726	0.98710	0.98693	0.98675	57.817	57.650	57.475	57.310	57.151
	20	10.0	12.392	0.98384	0.98359	0.98330	0.98298	0.98267	57.819	57.661	57.493	57.336	57.187
	25	12.5	15.440	0.98056	0.98012	0.97966	0.97919	0.97871	57.827	57.678	57.521	57.374	57.233
	30	15.0	18.469	0.97744	0.97683	0.97617	0.97551	0.97488	57.826	57.688	57.542	57.406	57.274
	40	20.0	24.472	0.97136	0.97032	0.96920	0.96810	0.96704	57.826	57.713	57.596	57.485	57.379
	50	25.0	30.386	0.96485	0.96334	0.96172	0.96018	0.95871	67.828	57.744	57.657	57.572	57.492
60	15	9.0	11.169	0.98523	0.98503	0.98479	0.98455	0.98429	67.679	67.471	67.251	67.043	66.845
	20	12.0	14.832	0.98120	0.98081	0.98037	0.97994	0.97950	67.683	67.489	67.283	67.086	66.902
	25	15.0	18.469	0.97744	0.97683	0.97617	0.97551	0.97488	67.684	67.505	67.314	67.134	66.961
	30	18.0	22.080	0.97384	0.96297	0.97204	0.97114	0.97024	67.681	67.520	67.347	67.185	67.030
	35	21.0	25.662	0.97010	0.96895	0.96774	0.96655	0.96543	67.682	67.541	67.388	67.247	67.107
	40	24.0	29.212	0.96620	0.96477	0.96325	0.96181	0.96041	67.688	67.566	67.435	67.311	67.192
70	16	11.2	13.857	0.98224	0.98190	0.98153	0.98116	0.98076	76.891	76.648	76.390	76.143	75.909
	20	14.0	17.259	0.97866	0.97813	0.97755	0.97698	0.97641	76.897	76.670	76.429	76.198	75.976
	24	16.8	20.639	0.97529	0.97453	0.97370	0.97290	0.97213	76.896	76.689	76.465	76.251	76.047
	28	19.6	23.995	0.97186	0.97085	0.96977	0.96871	0.96769	76.898	76.710	76.507	76.314	76.128
	32	22.4	27.323	0.96831	0.96704	0.96566	0.96437	0.96310	76.899	76.731	76.553	76.378	76.214
	36	25.2	30.621	0.96458	0.96305	0.96140	0.95984	0.95836	76.902	76.756	76.600	76.446	76.299
80	15	12.0	14.832	0.98120	0.98081	0.98037	0.97994	0.97950	85.443	85.163	84.862	84.575	84.300
	18	14.4	17.743	0.97817	0.97761	0.97700	0.97640	0.97580	85.440	85.175	84.888	84.617	84.354
	21	16.8	20.639	0.97529	0.97453	0.97370	0.97290	0.97213	85.441	85.194	84.925	84.672	84.424
	24	19.2	23.517	0.97236	0.97139	0.97034	0.96932	0.96833	85.442	85.213	84.964	84.733	84.502
	27	21.6	26.374	0.96934	0.96814	0.96686	0.96562	0.96444	85.441	85.233	85.002	84.791	84.578
	30	24.0	29.212	0.96620	0.96477	0.96325	0.96181	0.96041	85.448	85.260	85.054	85.859	84.666

With relatively low alcoholic concentration of the original sample, it is feasible to use much larger sample weights than 20 to 25 grams, thus minimizing the error. This method is limited in its application, however, by the danger of reaching a point where not all of the alcohol will be distilled over in 100 grams of distillate. The author has not investigated the maximum alcoholic concentration of distillate that will permit substantially complete recovery of alcohol from the original sample, but it seems probable that this should not exceed 20 per cent by weight.

The methods of calculation used in this paper permit calculation of and correction for the error due to different temperature coefficients of expansion of sample and distillate for any specific conditions of alcoholic concentrations of sample and distillate and working temperature, assuming only that the temperature coefficient of expansion of the original sample does not differ significantly from that of a solution of pure ethyl alcohol in water of the same alcoholic concentration by weight. For distilled liquors with relatively low solids content this will be substantially true, but the method will not give accurate results on cordials or sweet wines, because of the effect of the dissolved sugar on the temperature coefficient of expansion. In such cases it is best to determine specific gravities of sample and distillate at 15.56° C., or to use the generally less accurate method of measuring the volume of sample and distillate directly at 15.56° C. In the latter case, the specific gravity of the distillate may then be determined at room temperature and its percentage of alcohol by volume at 60° F. (*1*, Table 19, XLIII).

Instead of actually calculating the error for each specific set of conditions, the value of the correction may be obtained by interpolation from Table III. This table may be expanded, if desired, by similar calculations to include intermediate values of original alcoholic concentrations, sample weights, and working temperatures, thus reducing the error of interpolation. However, in its present form it will serve to yield substantially accurate values for correcting determined results, permitting reduction of total error in the corrected results approximately to the magnitude of experimental error alone.

The corrections in Table III are equally applicable whether the volumes of sample and distillate are calculated from apparent specific gravities or are measured directly.

If the volumes of sample and distillate are measured directly, the experimental error of measurement is likely to be significant unless considerable care is taken. With an original alcoholic concentration of 40 per cent by weight, an error of 0.01 ml. in measuring the volume of a 25-ml. sample will contribute an experimental error of only 0.02 per cent by volume to the determined result, but a not improbable error of 0.05 ml. in measuring the volume of a 25-ml. sample will contribute an experimental error of 0.1 per cent by volume, or 0.2 proof, to the determined result. An error of 0.05 ml. in measuring the volume of the 100 ml. of distillate will contribute an experimental error of 0.024 per cent by volume, or 0.048 proof, to the determined result. An error of 1° C. in measuring the temperature of the 25-ml. sample described above will contribute an experimental error of 0.04 per cent by volume, or 0.08 proof, to the determined result, while an error of 1° C. in measuring the temperature of the 100 ml. of distillate will contribute an experimental error of 0.025 proof to the determined result. While the above experimental errors are not very large, they may be significant when accurate results are desired, and they can be greatly minimized by weighing the sample and distillate and calculating their volumes from their specific gravities.

When volumes of sample and distillate are calculated from weights and specific gravities, the most probably significant experimental errors are likely to occur in the determination of the specific gravity of the distillate, since this value is used to obtain the alcoholic concentration of the distillate, and any error in this value is multiplied by 4 or 5 in calculating the alcoholic concentration of the original. With an alcoholic concentration of 10 per cent by volume at 60° F. in the distillate, and a 25-ml. pycnometer, an error of 0.001 ml. in filling the pycnometer, or an error of 0.001 gram in weighing it, or an error of 0.15° C. in measuring the temperature of its contents, will contribute an error of 0.00004 to the calculated specific gravity, or an error of 0.032 to the percentage of alcohol by volume in the distillate, or an error of 0.13 to 0.16 per cent by volume, or 0.26 to 0.32 proof, to the determined result. This is a significant error when very accurate results are desired, yet it requires very careful technique to keep the experimental error down even to this magnitude.

Table III. Table of Corrections

(To be added to percentages by volume at 60° F. of ethyl alcohol in original samples as determined by distillation of various weights of samples to give 100 grams of distillate and calculation from apparent specific gravities of originals and distillates at various temperatures)

Percentage of Ethyl Alcohol in Original		Weight of Sample Taken, Grams	Percentage of Ethyl Alcohol in Distillate		Correction to Be Added to Percentage by Volume at 60° F. of Ethyl Alcohol in Original as Determined at				
By wt.	By volume at 60° F.		By wt.	By volume at 60° F.	15.56/15.56° C.	20/20° C.	25/25° C.	30/30° C.	35/35° C.
10	12.392	20	2.0	2.509	0.004	0.007	0.010	0.014	0.018
		25	2.5	3.135	−0.003	0.000	0.004	0.008	0.011
		50	5.0	6.243	−0.002	0.001	0.004	0.007	0.010
		75	7.5	9.327	0.001	0.002	0.004	0.006	0.007
		100	10.0	12.392	0.000	0.000	0.000	0.000	0.000
		125	12.5	15.440	−0.001	−0.004	−0.006	−0.008	−0.010
20	24.472	20	4.0	5.002	0.002	0.028	0.055	0.081	0.106
		25	5.0	6.243	−0.001	0.024	0.051	0.076	0.101
		40	8.0	9.942	0.002	0.025	0.049	0.072	0.093
		60	12.0	14.832	0.000	0.016	0.033	0.051	0.066
		80	16.0	19.676	0.000	0.009	0.019	0.028	0.036
		100	20.0	24.472	0.000	0.000	0.000	0.000	0.000
30	36.181	20	6.0	7.479	0.004	0.074	0.148	0.218	0.282
		25	7.5	9.327	0.006	0.075	0.145	0.214	0.277
		30	9.0	11.169	0.003	0.069	0.138	0.203	0.262
		40	12.0	14.832	0.001	0.059	0.119	0.179	0.232
		60	18.0	22.080	0.002	0.043	0.086	0.127	0.163
		80	24.0	29.212	−0.001	0.019	0.039	0.059	0.076
40	47.326	20	8.0	9.942	0.005	0.123	0.248	0.363	0.472
		25	10.0	12.392	0.004	0.117	0.237	0.346	0.450
		30	12.0	14.832	0.000	0.106	0.217	0.322	0.420
		40	16.0	19.676	0.000	0.091	0.189	0.278	0.362
		50	20.0	24.472	0.000	0.075	0.154	0.225	0.293
		60	24.0	29.212	−0.002	0.053	0.111	0.165	0.216
50	57.830	15	7.5	9.327	0.013	0.180	0.355	0.520	0.679
		20	10.0	12.392	0.011	0.169	0.337	0.494	0.643
		25	12.5	15.440	0.003	0.152	0.309	0.456	0.597
		30	15.0	18.469	0.004	0.142	0.288	0.424	0.556
		40	20.0	24.472	0.004	0.117	0.234	0.345	0.451
		50	25.0	30.386	0.002	0.086	0.173	0.258	0.338
60	67.689	15	9.0	11.169	0.010	0.218	0.438	0.646	0.844
		20	12.0	14.832	0.006	0.200	0.406	0.603	0.787
		25	15.0	18.469	0.005	0.184	0.375	0.555	0.728
		30	18.0	22.080	0.008	0.169	0.342	0.504	0.659
		35	21.0	25.662	0.007	0.148	0.301	0.442	0.582
		40	24.0	29.212	0.001	0.123	0.254	0.378	0.497
70	76.909	16	11.2	13.857	0.018	0.261	0.519	0.766	1.000
		20	14.0	17.259	0.012	0.239	0.480	0.711	0.933
		24	16.8	20.639	0.013	0.220	0.444	0.658	0.862
		28	19.6	23.995	0.011	0.199	0.402	0.595	0.781
		32	22.4	27.323	0.010	0.178	0.356	0.531	0.695
		36	25.2	30.621	0.007	0.153	0.309	0.463	0.610
80	85.458	15	12.0	14.832	0.015	0.295	0.596	0.883	1.158
		18	14.4	17.743	0.018	0.283	0.570	0.841	1.104
		21	16.8	20.639	0.017	0.264	0.533	0.786	1.034
		24	19.2	23.502	0.016	0.245	0.494	0.725	0.956
		27	21.6	26.374	0.017	0.225	0.456	0.667	0.880
		30	24.0	29.212	0.010	0.198	0.404	0.599	0.792

Literature Cited

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Determining the Maturity of Frozen Vegetables

A Rapid Objective Method for Whole-Kernel Corn

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A rapid objective method for determining maturity of whole-kernel corn, involving the determination of specific gravity by difference in weight in air and in a weak salt solution of specific gravity 1.000, was found successful. Coefficients of correlation of these results with corresponding organoleptic tests and tentative standards are listed.

THE three essential factors in the quality grading of frozen vegetables are maturity, absence of defects, and color. Maturity is very important. An objective test tends to obviate the personal element always present in organoleptic tests, and, therefore, a rapid objective test for maturity is desirable. In a recent paper (*3*), one of the authors showed that specific gravity is a reliable index of the maturity of frozen peas.

At the present time there are no recognized methods for frozen whole-kernel corn, such as exist for frozen peas (*4*). The work reported in this paper is the result of an effort to promulgate a standard method for the measurement of maturity of frozen whole-kernel corn.

The determinations were made on whole-kernel Golden Cross frozen corn packed under commercial conditions during the 1939 and 1940 seasons. The corn was blanched on the cob for 7.5 minutes at 210° to 212° F., the standard commercial practice. Duplicate lots of the same samples were saved for organoleptic testing by qualified disinterested groups. The maturity was determined by means of specific gravity, which was determined by the difference in weight in air and in a mixture of xylene and carbon tetrachloride adjusted to a specific gravity of 1.000, an adaptation of a method described before (*3*). During the season of 1940, the same liquid was used for this purpose, and, in addition, another set of determinations was run making use of a weak salt brine of specific gravity 1.000. This brine was made to have a constant specific gravity, regardless of the temperature at which the determination was being run, and, therefore, the amount of salt in the brine varied with the temperature of the liquid used. Water has been used (*1*) in the determination of the specific gravity of potatoes as an index of the starch and solids content.

$$\text{Specific gravity} = \frac{\text{weight in air} \times \text{specific gravity of liquid}}{\text{difference of weights in liquid and in air}}$$

The following equipment is used: A suitable balance weighing to 0.1 gram can be supported on a stand or shelf and the basket can be attached to the hook under the pan. The sample basket is made of 16-mesh brass screen 8.125 cm. (3.25 inches) high and 5.625 cm. (2.25 inches) in diameter, and conveniently holds a 100-gram sample.

The samples of corn are thawed and then drained for 2 minutes on an 8-mesh sieve before starting the work. The specific gravity can be found as follows. The corn is weighed in air. The weight in the liquid mixture is determined by subtracting the weight of the basket in the liquid used from that of the corn and basket in this same liquid. The weight in air minus the weight in the liquid mixture gives the difference of weight in this liquid.

Table I gives the data accumulated on this test during the 1939 season. Table II gives the same for the year 1940, and in addition, the specific gravity data in which brine was used in place of the xylene–carbon tetrachloride mixture. Table III shows the several coefficients of correlation. In calculating these coefficients of correlation for the 1939 series, the immature samples were not included in the calculation, in order to get a straight-line relationship. In making the calculations for the 1940 series, the overmature were omitted for the same reason.

TABLE I. DATA FOR 1939 SEASON

Specific Gravity of Thawed Corn in Xylene–CCl_4	Organoleptic, Based on 50	Specific Gravity of Thawed Corn in Xylene–CCl_4	Organoleptic, Based on 50
1.059[a]	33	1.102	47
1.066[a]	33	1.102	47
1.068[a]	33	1.102	47
1.083	49	1.102	43
1.088	49	1.103	49
1.088	49	1.103	47
1.088	49	1.104	49
1.090	49	1.105	49
1.091	45	1.105	49
1.092	49	1.105	43
1.093	49	1.106	49
1.093	49	1.107	43
1.094	49	1.109	49
1.095	49	1.109	47
1.095	49	1.110	47
1.095	49	1.111	49
1.095	43	1.111	49
1.096	49	1.112	49
1.096	47	1.115	49
1.096	47	1.116	49
1.096	47	1.120[b]	39
1 097	47	1.121[b]	39
1.097	47	1.128[b]	41
1.097	45	1.128[b]	35
1.097	43	1.137[b]	33
1.098	49	1.137[b]	33
1.100	47	1.138[b]	33
1.101	49	1.139[b]	33
1.101	49	1.141[b]	33
1.101	49	1.142[b]	33
1.101	47	1.143[b]	33
1.102	49	1.149[b]	33
1.102	49	1.149[b]	33
		1.150[b]	33

[a] Immature.
[b] Overmature.

Discussion

The organoleptic ratings for maturity after cooking, together with the values assigned to them for use in the calculation of coefficients of correlation for the 1939 series are: A, satisfactory; B, satisfactory but not first grade; C, low quality; A = 49; A− = 47; A−B+ = 45; B+ = 43; B = 41; B− = 39; B−C+ = 37; C+ = 35; C = 33. Samples were added to one-half cup of boiling water and removed 2 minutes after the second boil.

The organoleptic ratings after cooking (*2*) for the 1940 series are based upon a numerical basis of 100 points. Scores of 0 to 40 are designated as C grade or unfit for food, 40 to 70 as B grade or good food but not the highest quality, and 70 to 100 as A grade or high quality. This difference in scoring is accounted for by the fact that two different, disinterested groups judged the samples.

The point will always arise in the determination of maturity of whether the seasonal variation affects the quality of the product. This work on corn was done in two successive seasons of opposite climatic conditions. In 1939 the season was hot and dry, while in 1940 it was cool and wet. Little variation, if any, was found in the indexes of quality for the two seasons. Although the length of ripening periods may vary from season to season, the specific gravity determinations

[1] Died October 9, 1940.

show no variation for the corn harvested in prime condition. It is evident, therefore, that restandardization is not necessary from year to year. Check tests indicate that the figures given here are applicable to all similar sweet corn varieties.

Table II indicates that the specific gravities as determined by means of the xylene–carbon tetrachloride mixture are somewhat higher than those determined by means of the water containing enough sodium chloride to give a specific gravity of 1.000. This is possibly caused by the fact that water adhering to the vegetable is weighed when it is immersed in the xylene–carbon tetrachloride mixture because of its failure to dissolve in this mixture. When the vegetable is weighed in the aqueous mixture, only the vegetable itself is weighed. The final result would be a slight error in both methods, and a different set of standards would have to be prepared for each method.

The brine method is somewhat the better of the two because of its low cost, because it is easier to prepare, and because it is less likely to change in use, although it should be tested with the hydrometer from time to time to be sure the specific gravity is 1.000.

Table II. 1940 Series

Specific Gravity of Thawed Corn In brine	In xylene–CCl_4	Organoleptic, Based on 100
1.042[a]	1.063	58
1.048[a]	1.053	45
1.050[a]	1.051	46
1.053[a]	1.062	55
1.058[a]	1.065	57
1.059[a]	1.065	48
1.062[a]	1.059	53
1.066[a]	1.074	56
1.066[a]	1.081	57
1.070[a]	1.076	66
1.071[a]	1.078	55
1.073[a]	1.058	69
1.074[a]	1.084	59
1.076[a]	1.082	60
1.080	1.089	73
1.084	1.098	69
1.085	1.096	62
1.086	1.103	80
1.087	1.087	74
1.088	1.094	77
1.089	1.093	85
1.091	1.098	77
1.092	1.101	71
1.096	1.101	70
1.096	1.102	75
1.097	1.101	77
1.097	1.102	77
1.097	1.111	77
1.098	1.101	77
1.098	1.101	68
1.098	1.106	80
1.098	1.106	80
1.098	1.109	74
1.098	1.111	91
1.099	1.103	70
1.099	1.105	68
1.099	1.106	82
1.100	1.097	79
1.100	1.102	75
1.101	1.105	76
1.101	1.110	86
1.102	1.100	89
1.102	1.105	72
1.102	1.110	71
1.102	1.111	74
1.104	1.103	70
1.106	1.112	87
1.106	1.114	82
1.107	1.110	78
1.107	1.114	71
1.108	1.110	88
1.108	1.116	79
1.109	1.111	82
1.109	1.112	92
1.109	1.116	84
1.110	1.120	89
1.112	1.116	89
1.113	1.110	80
1.113	1.115	80
1.117	1.113	80
1.118	1.117	77
1.121[b]	1.128	64
1.122[b]	1.122	64
1.130[b]	1.134	63
1.149[b]	1.147	34
1.150[b]	1.154	39
1.151[b]	1.155	35

[a] Immature.
[b] Overmature.

Table III. Coefficients of Correlation for Specific Gravity of Thawed Corn

Correlation between:	Coefficient
Xylene–carbon tetrachloride method and organoleptic tests (1939)	-0.8592 ± 0.0221
Brine method and organoleptic tests (1940)	$+0.8456 \pm 0.0246$
Xylene–carbon tetrachloride method and organoleptic tests (1940)	$+0.8408 \pm 0.0253$

The following tentative standards are suggested, based upon the results using the brine solution for the determination of the specific gravity:

Fancy	1.080 to 1.118
Reject, immature	1.079 and lower
Reject, overmature	1.119 and higher

These standards can be revised if and when other grades are generally packed.

Acknowledgment

The authors wish to thank the Frosted Foods Sales Corporation, New York, N. Y., for financial assistance, and the Snider Packing Corporation, Rochester, N. Y., for making available facilities at one of its plants for this work. They also wish to thank the group of tasters connected with this corporation for running the organoleptic tests for the 1939 set of samples, and to thank L. S. Fenn of the Agricultural Marketing Service, United States Department of Agriculture, and his associates for running the organoleptic tests for the 1940 set of samples.

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Approved by the director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 472.

Determination of the Maturity of Frozen Peas

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A REVISION of the method for determining the maturity of frozen peas (*1*) has been worked out, in which the mixture of xylene and carbon tetrachloride is replaced by water containing enough sodium chloride to give a liquid of specific gravity 1.000. This liquid is considerably less expensive than the former, is easier to prepare, and has the added advantage of undergoing less change in specific gravity in use.

The following set of standards is suggested to replace those published before:

Fancy	1.072 and lower
Standard	1.073 to 1.084
Substandard	1.085 and higher

These standards can be revised if and when an extra standard grade is generally packed.

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(1) Lee, F. A., Ind. Eng. Chem., Anal. Ed., **13**, 38–9 (1941).

Approved by the director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 480.

Separation of Lithium from Potassium and Sodium

By Treatment of the Chlorides with Higher Aliphatic Alcohols

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OF THE various methods for the quantitative separation of lithium from potassium and sodium by extraction of the mixed chlorides with organic solvents, the isoamyl alcohol method of Gooch (*2*) appears to have had the widest use. Because the boiling point of isoamyl alcohol is considerably above that of water, the aqueous solution containing the alkali chlorides may be evaporated in the presence of this solvent; the water is not only evaporated away without much loss of solvent but the salts are completely dehydrated in the process. Moreover, the lithium chloride gradually dissolves in the isoamyl alcohol while the other alkali chlorides are gradually precipitated out. Hence, for quantitative separation by this general method such a solvent is better than one, such as dioxane, whose boiling point is only slightly above that of water, and much better than one, such as acetone, whose boiling point is below that of water. The only other solvent of relatively high boiling point that has been advocated for this separation is pyridine (*3*), but its disagreeable odor and the difficulty of obtaining it readily in a highly pure state have apparently restricted its use.

The method of Gooch has the disadvantage that neither potassium chloride nor sodium chloride is quantitatively insoluble in isoamyl alcohol, so that relatively large corrections must be applied for the amounts of these salts that dissolve along with the lithium chloride. Furthermore, these corrections differ in accordance with the particular combination of salts present. Though the corrections may be applied with considerable confidence, there is, nevertheless, some uncertainty involved, especially in regard to the amounts of salts dissolved in the washings, and for this no corrections are attempted. Furthermore, the measurement of the volume of solvent present after extraction in order to apply these corrections is somewhat inconvenient in practice. A solvent that required no such corrections and yet had the high boiling point and other desirable characteristics of isoamyl alcohol would manifestly be better from the standpoint of both accuracy and convenience.

Since the solubilities of lithium, potassium, and sodium chloride in the aliphatic monohydric alcohols are known to decrease progressively in ascending the series from methyl to amyl alcohol, it seemed apparent that the solubilities of potassium chloride and sodium chloride in similar alcohols of more than five carbon atoms might become very small, whereas the solubility of lithium chloride might still remain large enough for analytical separation. Experiment showed this to be true. The higher alcohols chiefly considered were *n*-hexanol and 2-ethylhexanol, since these are now readily available in a technical grade at low cost. For the purpose of this separation no advantage would probably result from the use of a grade of these alcohols entirely free from isomers. By reason of these considerations, the experiments here described were all made with the so-called practical or technical grades of these two alcohols.

Solubility Experiments

The solubilities of lithium, sodium, and potassium chloride in the two alcohols at 25° C. are shown in Table I. These results were obtained by conventional procedures.

Before being used for these determinations the solvents were boiled to ensure their freedom from water, and the salts were carefully dehydrated. With one exception each result shown is the result of several concordant determinations. The result for the solubility of potassium chloride in 2-ethylhexanol is merely an estimate based upon the probable limit of measurement of the analytical method used for the determination, since the solubility of the salt in this solvent was so slight that the attempted measurements gave the same results as blank determinations. For convenient comparison the solubilities of the three salts in isoamyl alcohol are also given, calculated from data published by Turner and Bissett (*4*).

With the increase in number of carbon atoms the solubility of sodium chloride in these three alcohols decreases more than the solubility of lithium chloride, and the solubility of potassium chloride decreases much more. From the standpoint of probable sharpness of separation, the *n*-hexanol is obviously better than isoamyl alcohol, and the 2-ethylhexanol obviously much better. However, since the solubility of lithium chloride in octyl alcohol is considerably less than the solubility of this salt in either amyl or hexyl alcohol, it seemed possible that in actual practice hexyl alcohol might be a more satisfactory solvent. Therefore, analytical experiments were made with both solvents.

Analytical Experiments. The general procedure recommended by Gooch was followed except for certain minor variations. The potassium and sodium were weighed out in the form of their chlorides, and the lithium in the form of carbonate, the lithium carbonate in the weighed test mixture then being converted to chloride by the addition of slightly more than the calculated amount of hydrochloric acid before evaporating the aqueous solutions of the salts with the alcohol. After evaporation and dehydration, the residual potassium chloride, sodium chloride, or both, was collected on a weighed sintered-glass crucible, and washed thoroughly with successive small portions of the cold alcohol. No attempt was made to restrict the total volume of alcohol used in washing, as is the usual practice in using amyl alcohol for this separation. After washing, the crucible and salts were dried in an air oven at a temperature sufficient to volatilize rapidly the residual solvent. In separations made with *n*-hexanol a temperature of 180° C. was used, and in those with 2-ethylhexanol, a temperature of 210° C.

The accuracy of separation was judged from the agreement or discrepancy between the weights of the residual salts and their original weight. The lithium chloride that dissolved in the alcohol was not converted to sulfate and weighed, as is the usual procedure in actual analysis, since this seemed unnecessary for measuring the accuracy of separation. However, in order to make sure that the observed results were not due to a compensation of errors, the weighed residual salts were all tested for lithium with a spectroscope. Except when the lithium was present in unusually large amounts, these experimental separations were made by

Table I. Solubilities of Chlorides of Lithium, Sodium, and Potassium

(In certain amyl, hexyl, and octyl alcohols at 25° C., expressed in grams dissolved by 100 ml. of solvent)

Salt	Isoamyl Alcohol	*n*-Hexanol	2-Ethylhexanol
LiCl	7.3	5.8	3.0
NaCl	0.0016	0.0008	0.0001
KCl	0.0006	0.00004	<0.00001

TABLE II. SEPARATIONS OF SIMILAR AMOUNTS OF LITHIUM AS CHLORIDE BY *n*-HEXANOL AND 2-ETHYLHEXANOL

Solvent	NaCl Taken	KCl Taken	LiCl Present	Weight of Extraction Residue	Error of Separation in Terms of: NaCl	Na	KCl	K	LiCl	Li
	Gram	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*
n-Hexanol	0.2507	None	0.0608	0.2500	−0.0007	−0.0003	.....	.....	+0.0007	+0.0001
	0.2475	None	0.0629	0.2470	−0.0005	−0.0002	.....	.....	+0.0005	+0.0001
	None	0.2213	0.0592	0.2208	.....	.....	−0.0005	−0.0003	+0.0005	+0.0001
	None	0.2641	0.0663	0.2637	.....	.....	−0.0004	−0.0002	+0.0004	+0.0001
	0.1613	0.1524	0.0743	0.3122	.....	.....	.....	.....	+0.0015	+0.0002
	0.1702	0.1754	0.0755	0.3447	.....	.....	.....	.....	+0.0009	+0.0001
2-Ethylhexanol	0.2072	None	0.0640	0.2069	−0.0003	−0.0001	.....	.....	+0.0003	±0.0000
	0.2378	None	0.0636	0.2375	−0.0003	−0.0001	.....	.....	+0.0003	±0.0000
	None	0.3077	0.0548	0.3073	.....	.....	−0.0004	−0.0002	+0.0004	+0.0001
	None	0.2134	0.0679	0.2132	.....	.....	−0.0002	−0.0001	+0.0002	±0.0000
	0.1583	0.1049	0.0570	0.2628	.....	.....	.....	.....	+0.0004	+0.0001
	0.1147	0.1056	0.0435	0.2198	.....	.....	.....	.....	+0.0005	+0.0001
	0.1099	0.0791	0.0587	0.1884	.....	.....	.....	.....	+0.0006	+0.0001
	0.0714	0.1202	0.0621	0.1918	.....	.....	.....	.....	−0.0002	±0.0000

TABLE III. SEPARATIONS BY 2-ETHYLHEXANOL WITH LITHIUM PRESENT IN UNFAVORABLE RATIO OR AMOUNT

Analysis No.	No. of Extractions	NaCl Taken	KCl Taken	LiCl Present	Wt. of Extraction Residue	Error of Separation in Terms of: LiCl	Li
		Gram	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*
1	1	0.0493	0.0561	0.1584	0.1210	−0.0156	−0.0026
2	1	0.0547	0.0524	0.1366	0.1270	−0.0199	−0.0033
3	1	0.0605	0.0744	0.1010	0.1372	−0.0023	−0.0004
4	2	0.0568	0.0556	0.1278	0.1118	+0.0006	+0.0001
5	2	0.0757	0.0612	0.1240	0.1364	+0.0005	+0.0001
6	2	0.0699	0.0569	0.1113	0.1261	+0.0007	+0.0001
7	1	0.2296	0.2187	0.0546	0.4475	+0.0008	+0.0001
8	1	0.1673	0.2449	0.0541	0.4117	+0.0005	+0.0001
9	1	0.2269	0.2413	0.0510	0.4677	+0.0005	+0.0001
10	1	0.2831	0.1224	0.0502	0.4058	−0.0003	±0.0000
11	1	0.0829	0.1117	0.0122	0.1943	+0.0003	±0.0000
12	1	0.0968	0.0807	0.0118	0.1769	+0.0006	+0.0001
13	1	0.0886	0.0812	0.0118	0.1705	−0.0007	−0.0001
14	1	0.0812	0.0951	0.0117	0.1766	−0.0003	±0.0000

means of a single extraction only, and no solubility corrections were applied to any of the results obtained.

In Table II are shown typical results obtained in separations with *n*-hexanol and with 2-ethylhexanol under similar conditions. The results of the separations with *n*-hexanol are fairly satisfactory, though as might be expected from the solubility data, the results for sodium are slightly low, and those for potassium slightly less so. With both sodium and potassium present, the slightly high results for lithium reflect the combined noticeable solubility of the sodium and potassium chlorides. However, these uncorrected results are obviously much better than could be obtained by the use of isoamyl alcohol without application of solubility corrections, and not very much worse than those ordinarily obtained by the isoamyl alcohol method when such corrections are applied. The results obtained with 2-ethylhexanol are obviously better than those obtained with *n*-hexanol, and for an analytical method of this type every one of these results is very satisfactory. In accordance with the solubility data, 2-ethylhexanol is therefore a better solvent than *n*-hexanol for the separation of lithium chloride from admixtures with potassium chloride and sodium chloride, and good results are obtained in spite of the low actual solubility of lithium chloride in this octyl alcohol.

In order to test further the value of 2-ethylhexanol as a solvent in this separation, a number of analyses were made of mixtures in which lithium was present either in relatively large amount or in small proportion. Typical results are shown in Table III.

As might be expected from the known inaccuracy of the isoamyl alcohol method when an attempt is made to separate 100 mg. or more of lithium chloride by a single extraction, poor results are also obtained with 2-ethylhexanol when an attempt is made to separate such quantities by a single extraction. This is shown by the first three results in Table III. However, the results with 2-ethylhexanol, especially analyses 1 and 2, are much poorer than analogous results obtained by Gooch (*2*) with isoamyl alcohol. This difference is apparently not due to the difference in the solubility of lithium chloride in the two solvents, for in the experiments there was always more 2-ethylhexanol than was needed to dissolve the lithium chloride present. It is probably due to difference in temperature of final dehydration when this dehydration is performed at the boiling points of the two solvents, a greater proportion of insoluble lithium hydroxide being formed by hydrolysis at the high boiling point of 2-ethylhexanol.

The remedy, of course, is to avoid dehydrating the salts with 2-ethylhexanol at too high a temperature. Dehydration at a temperature 50° C. below its boiling point will remove the water as well as dehydration with boiling isoamyl alcohol. That better results are obtained when the temperature of dehydration is not too high is shown by analysis 3 of Table III. However, by a double extraction, which is the usual procedure, satisfactory results are obtained when 2-ethylhexanol is used to separate these larger amounts of lithium, even when not much attention is given to the temperature used for final dehydration. This is shown by analyses 4, 5, and 6. That satisfactory results are obtained by even a single extraction when lithium is present in small amount and proportion is shown by the other analyses of Table III.

All the salt residues from which the lithium chloride had been extracted with 2-ethylhexanol were tested for the presence of lithium by means of a hand spectroscope and a special light filter. With the exception of those from analyses 1, 2, and 3 of Table III, no lithium was detected in these residues. From this evidence of complete separation and from the solubility data it seems likely that the so-called errors of separation found by the test analyses are in large part only errors of manipulation.

Applicability of Ammonium Stearate Reaction

In a previous paper by the senior author (*1*) it was shown that minute amounts of lithium present in an isoamyl alcohol extract of mixed alkali chlorides could be detected or estimated by means of a reagent consisting of solution of ammonium stearate in this solvent. The same reaction may be applied in 2-ethylhexanol solution, even though ammonium stearate is considerably less soluble in 2-ethylhexanol than in isoamyl alcohol. Only about 0.55 gram of ammonium stearate dissolves in 100 ml. of 2-ethylhexanol at room temperature. In spite of this low solubility a sufficient concentration of salt is present in the saturated reagent to give precipitates or turbidities with very dilute solutions of lithium chloride in 2-ethylhexanol. Lithium stearate is apparently somewhat less soluble in 2-ethylhexanol than is isoamyl alcohol, since

the sensitivity of the reaction appears, in spite of the more dilute reagent, to be slightly greater in the octyl alcohol solution. From a series of experiments it was found that when 1 or 2 ml. of a saturated solution of ammonium stearate in 2-ethylhexanol was added to like volumes of a solution of lithium chloride in this same solvent, a slight but definite reaction was still obtained when the amount of lithium present was as low as 0.01 to 0.02 mg.

Summary

These experiments show that 2-ethylhexanol yields results equal to or better than those obtained by the use of isoamyl alcohol with the advantage that no solubility corrections are necessary and therefore no attention need be paid to the exact volume of solvent used in the extraction or for washing. Another advantage of 2-ethylhexanol over isoamyl alcohol for this separation is that the former need not be boiled in order to dehydrate the salts properly. As a consequence the danger of loss from bumping is less, and quantities of disagreeable fumes are not evolved, which some chemists regard as a serious objection to the isoamyl alcohol method of Gooch. On the whole, 2-ethylhexanol appears to be the best solvent yet proposed for the separation of lithium from potassium and sodium by extraction of the mixed alkali chlorides. The ammonium stearate reaction for the detection or estimation of lithium may be applied with at least equal success in 2-ethylhexanol solution.

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PRESENTED before the Division of Analytical and Micro Chemistry at the 102nd Meeting of the AMERICAN CHEMICAL SOCIETY, Atlantic City, N. J. Constructed from a senior thesis submitted by Herbert D. Axilrod to the Department of Chemistry, Princeton University, 1941.

Improved Manometer Design

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IN CHEMICAL and metallurgical plants there are numerous applications for vertical manometers, to indicate pressure, vacuum, liquid level, or fluid flow rate. The diagram illustrates a manometer that can be made in any plant having ordinary machine shop and welding facilities. It is inexpensive, can be made in any length desired, is easily maintained, and will not lose any appreciable amount of manometer liquid even when pressures greatly in excess of the maximum scale reading are accidentally applied.

The body of the manometer consists of two pieces of standard $1^1/_2$-inch wrought iron pipe separated by a 1.56-cm. (0.625-inch) mild steel partition to which they are welded. A length of standard $^1/_4$-inch wrought iron pipe is inserted through a central hole in the partition plate and welded to it. This tube extends 6.25 cm. (2.5 inches) above the plate and down to a point 1.25 cm. (0.5 inch) above the bottom outlet hole to the gage glass. The top outlet hole to the gage glass is drilled so that the bottom of this hole is flush with the top of the partition plate. Bosses are welded to the sides of the manometer body and are drilled and tapped for the high- and low-pressure connections and the filling plug. The high- and low-pressure connections can be spaced for convenient installation of an equalizing valve. The open ends of the meter body are closed by welding on end plates, which are drilled and tapped for $^1/_4$-inch pipe plugs for ease of cleaning and draining. A 0.16-cm. (0.0625-inch) thick mild steel baffle plate is welded to the top end plate before it is put in place.

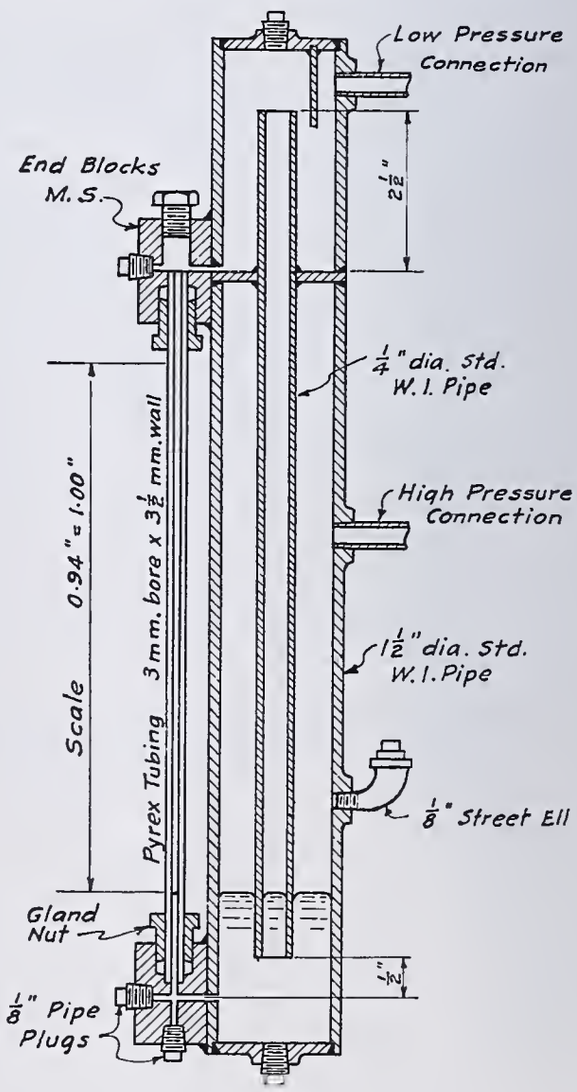
The end blocks for the gage glass are drilled and tapped as shown for the gland nuts and clean-out plugs, and welded to the manometer body. Care must be taken to hold these blocks rigidly in correct alignment where they are being welded in place, as gage glasses are easily broken by tightening up incorrectly aligned gland nuts.

A satisfactory gage glass is Pyrex tubing of 3-mm. bore and 3.5-mm. wall.

An aluminum or plastic scale with zero adjustment is mounted behind the gage glass. In this type of manometer a correction must be made on the scale for the depression of the liquid level in the high-pressure chamber. In the manometer described above the correction factor is 0.94.

OPERATION. When a pressure in excess of the maximum scale reading is applied, the liquid seal in the lower chamber is broken at the bottom of the central tube. The manometer liquid in this tube is blown into the upper chamber, where it collects in the annular space surrounding the tube. The baffle plate prevents this liquid from being blown out of the manometer. The central tube is then free of liquid, and permits continued passage of gas with no further disturbance of the liquid. When the excess pressure is released, the liquid in the upper chamber drains back to the lower chamber through the gage glass and restores the seal.

This type of manometer is particularly suitable for use in chemical plants on materials with high crystallizing temperatures, as the body of the instrument can readily be maintained at a temperature above the point of crystallization by means of a steam jacket or electric heater.



Chromatographic Adsorption Analysis

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The separation of mixtures by passage of their solutions through towers or columns of powdered adsorbents (Tswett's chromatographic adsorption analysis) is applicable to the detection, preparation, and estimation of both inorganic and organic substances. This method, in macro and micro modifications, is useful for concentration of solutes from dilute solution, for comparison of substances suspected of being identical, and for estimation of the structure of organic molecules. The new technique is beginning to find application in industrial operations.

IN 1906 at the then Russian city of Warsaw, a botanist named Tswett invented a unique adsorption method of chemical analysis that was destined to find wide application in many branches of the natural sciences (*52*). Results obtained through improvement and application of the method have broken the hardpan in barren fields and have made virgin regions tillable by the chemist.

Separation of Colored Compounds

In the course of his experiments on the pigments of green leaves, Tswett performed a simple experiment that has formed the basis for chromatographic adsorption technique.

In the narrow end of a constricted glass tube he placed a wad of cotton, and above this he tamped successive portions of finely powdered, adsorptive material such as precipitated chalk. This packed tube or adsorption column (Figure 1) was attached to a suction flask, and then a green, petroleum ether extract of dried leaves was passed through it. Under these conditions, some of the pigments, particularly the yellow carotene hydrocarbons, were weakly adsorbed and passed rapidly through the column. Other more strongly adsorbed pigments, the green chlorophylls and yellow xanthophylls, were held near the top of the adsorbent. In this way, the migrating pigments gradually separated from one another, forming a series of colored bands or zones, called a "chromatogram". The bands always occurred in the same sequence, analogous to the colors in the spectrum.

Tswett observed that the separation of the several bands was increased if only a small quantity of the solution was passed into the column and if this was then followed with fresh solvent. The completed separation of the leaf pigments is shown by Figure 2.

Utilization of fresh solvent to complete the separation of the bands is now recognized as an essential step in the resolution of mixtures. It is known as the "development" of the chromatogram. A diagrammatic representation of the development of a chromatogram is provided by Figure 3.

In order to recover the leaf pigments separated on the adsorption column, the development with fresh solvent was continued until the pigments in each band were carried successively into the percolate. This provided solutions of the pure pigments. Another procedure was to push the moist adsorbent from the tube. The bands in the cylinder of cohesive adsorbent were then separated from one another with a knife. Each colored band or zone of adsorbent was agitated with a little alcohol or other polar solvent (now called the "eluant"). This liberated the adsorbed pigment. Solutions of the eluted pigment were separated from the adsorbent by filtration. Still another procedure was to dig out the bands separately from the column with a spatula and to elute the pigments from the respective portions with a polar solvent.

As indicated by Figure 2, Tswett separated one yellow carotene, three or four yellow xanthophylls, and two green chlorophylls from leaf extracts. However, only minute quantities of these pigments were obtained. Isolation of crystalline leaf xanthophyll at this time by use of other methods provided a single pigment and gave credence to the assumption that Tswett's adsorption method had produced alteration of a single leaf xanthophyll (*54*, *55*). As a consequence, the adsorption method fell into disrepute, and not until 1931 was its great usefulness rediscovered.

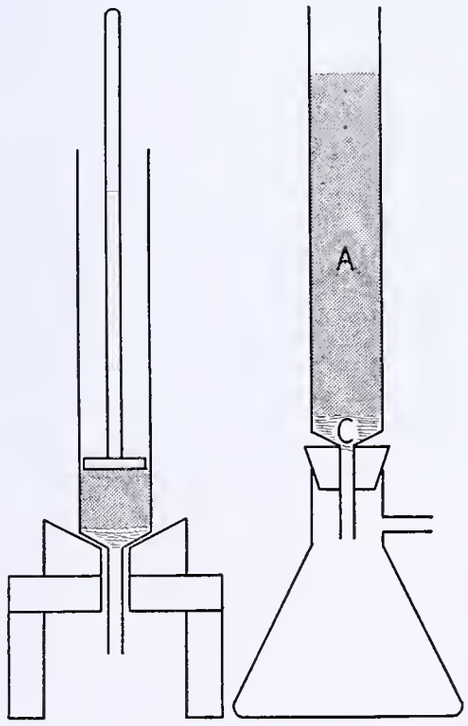

FIGURE 1. (*Left*) CORK RING SUPPORT AND METAL PLUNGER USED FOR PACKING ADSORPTION COLUMN. (*Right*) COMMONEST ARRANGEMENT FOR CHROMATOGRAPHIC ADSORPTION ANALYSIS (*40*)

A, adsorbent; *C*, cotton

Separation of crystalline, presumably homogeneous, carrot root carotene into several isomeric hydrocarbons by adsorption on columns of specially prepared adsorbents marked the beginning of a new era in chromatographic investigations (*19*, *22*, *26*, *43*). It demonstrated the great selectivity of the method because the carotenes were found to differ only in the position of one double bond in molecules containing 40 atoms of carbon. It proved that sufficient quantities of material for subsequent chemical analysis could be prepared quickly and in good yield. It indicated that the method of preparation and activation of the adsorbent exerted a profound effect upon the applicability of the method.

Because of the importance of the carotenes as provitamins A (*14*), their resolution by adsorption attracted the attention of workers in many different fields. One result was the substantiation of Tswett's observations pertaining to the resolution of the leaf pigments. With the most selective adsorb-

ents, about a dozen xanthophylls were finally separated from the leaf extracts (*45*). These advances stimulated a great deal of work on the preparation of various adsorbents for the separation of mixtures of many different types of compounds ranging from the elements themselves to the most complex materials found in organic nature. This in turn led to many new applications of the method, to the perfection of macro- and microcolumns, to improvement in the design of the columns, and to the separation of colorless as well as of colored compounds. Many of the applications of the adsorption method have been reviewed (*4, 9, 28, 57, 62, 63*), and thorough discussions of the method and extensive bibliographies are to be found in two books (*40, 61*).

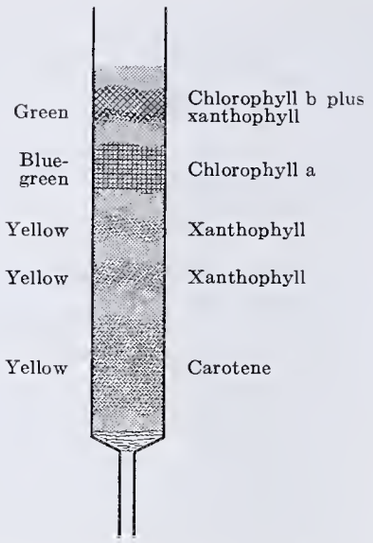

FIGURE 2. CHROMATOGRAM OBTAINED BY ADSORPTION OF LEAF PIGMENTS UNDER CONDITIONS SIMILAR TO THOSE DESCRIBED BY TSWETT

For student practice, the separation of dyestuffs by adsorption upon columns of alumina has been recommended (*36*). Separation of other materials, including the leaf pigments and the carotenes of carrot roots (*40, 43*) may be made the basis of lecture demonstrations. As a rule, the bands in a chromatogram gradually become diffuse and indistinct when the flow of solvent through the column is discontinued; hence, for permanent exhibits, columns may be packed with mixtures of stable, dry pigments and siliceous earth, thus yielding permanent replicas of the original chromatograms.

TABLE I. MIXTURES AND THE ADSORBENTS AND SOLVENTS USED FOR THEIR SEPARATION

Mixture	Adsorbent	Solvent
Cations	8-Hydroxyquinoline	Water (*13*)
	Alumina	Water (*38*)
Lithium isotopes	Zeolites	Water (*47*)
Halogens	Magnesium silicate	Carbon tetrachloride (*40*)
Carotenes	Magnesia	Petroleum ether (*43*)
	Lime	Petroleum ether (*22*)
	Alumina	Petroleum ether (*26*)
Vitamins A_1 and A_2	Lime	Petroleum ether (*19*)
Chlorophylls	Sucrose	Petroleum ether (*59*)
	Inulin	Petroleum ether (*30*)
	Magnesium citrate	Ether and petroleum ether (*30*)
Xanthophylls	Magnesia	1,2-Dichloroethane (*45*)
	Calcium carbonate	Carbon disulfide (*60*)
Fatty acids	Charcoal	Petroleum ether (*8*)
Fats	Alumina	Petroleum ether (*51*)
Polycyclic hydrocarbons	Alumina	Petroleum ether (*58*)
Thiamin and riboflavin	Decalso and Supersorb	Water (*11*)

Separation of Colorless Substances

Colorless compounds are separable on the adsorption columns, but special methods must be employed to locate these invisible materials. According to one procedure, successive portions of the percolate are collected and analyzed by chemical, optical, or biological methods. In order to use the Toepler *Schlieren* optical method for determination of colorless substances in the percolate, the solution may be passed upward through the adsorbent (*49*). By another procedure, the column containing the colorless compounds is sectioned empirically and the compounds eluted from the respective sections are examined as just described. Colorless, fluorescent compounds may be observed on the adsorption columns in ultraviolet light (*20, 58*).

Previous to adsorption some colorless compounds may be converted into colored derivatives that can then be separated on the columns (*27, 41*). Other colorless compounds may be located on the columns by means of reagents with which they produce color changes. These reagents may be passed through the column (*38*) or they may be applied to the cylinder of moist adsorbent after it has been pressed from the tube (*63*). A clever innovation is the adsorption of cations on columns of 8-hydroxyquinoline, with which they form colored compounds and thus become visible (*13*). Adsorption of a colorless compound with a colored indicator serves to locate the former with respect to the latter (*5, 6*). The closer together the two are adsorbed, the more precisely one can locate the colorless compound.

TABLE II. SUBSTANCES PREPARED BY ADSORPTION OF EXTRACTS OF PLANT AND ANIMAL PRODUCTS

Substance	Adsorbent	Solvent	Source
Vitamin A	Lime	Petroleum ether	Fish liver oil (*18*)
Vitamin B_1	Decalso	Water	Rice hulls (*10*)
Vitamin B_2 (acetylated)	Alumina	Ethyl acetate	Alfalfa (*25*)
Vitamin D	Alumina	Petroleum ether	Fish liver oil (*6*)
Vitamin E	Alumina	Petroleum ether	Wheat germ oil (*15, 50*)
Vitamin K_1	Decalso	Petroleum ether	Fish meal (*3*)
Vitamin K_2	Decalso	Petroleum ether	Putrified fish meal (*29*)
Sex hormones	Alumina	Carbon tetrachloride	Human urine (*7*)
Glucosidase and chitinase	Bauxite	Water	Snails (*64*)
Anthocyanins	Alumina	Water (*21*)	.....
Pterins	Frankonit	Water	Insects (*2*)
Carotenes	Magnesia	Petroleum ether	Leaves (*30, 44*)

Applications of the Method

From the analyst's point of view it is important to know what can be accomplished by use of the adsorption method. To this end it has seemed desirable to classify the various procedures and to illustrate them with selected experiments.

One of the principal uses of adsorption columns is the resolution of mixtures into their constituents. Here all the components are recovered after qualitative or quantitative separation of the mixture. Some examples are summarized in Table I.

Very often it is necessary to use the adsorption method for the preparation of only one or two substances from complex mixtures. Many examples of this application of the method are to be found among the procedures utilized for preparation of specific substances from extracts of biological materials. Minute quantities of enzymes, hormones, vitamins, pigments, and similar materials have been prepared from extracts containing large quantities of other materials. Unwanted materials in these extracts frequently complicate the adsorption procedure, necessitating the use of relatively large quantities of adsorbent. Examples of important biological products prepared or investigated by adsorption methods are reported in Table II.

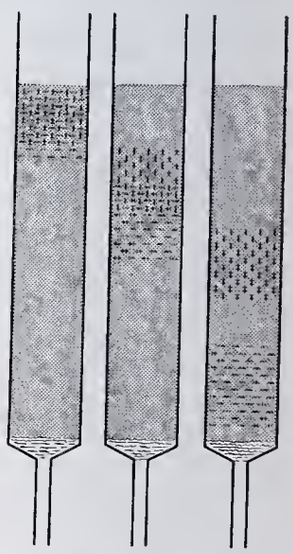

FIGURE 3. SUCCESSIVE STAGES IN DEVELOPMENT OF A CHROMATOGRAM

A solution of substances + and − is passed into the column. When the column is washed with fresh solvent, + and − move through the tube at different rates, finally separating completely from each other (*40*).

Table III. Adsorbents and Solvents for Separation of Carotenes from Other Leaf Pigments

Adsorbent	Solvent
Inulin	Petroleum ether (*35, 53*)
Sucrose	Petroleum ether (*53, 59*)
Calcium carbonate	Petroleum ether (*53*)
Starch	Petroleum ether (*46*)
Calcium phosphate	Petroleum ether (*33*)
Soda ash	Petroleum ether (*23*)
Magnesia (partly activated)	Petroleum ether (*17*)
Magnesia (activated)	1,2-Dichloroethane (*45*)

If quantitative separations of one or two components from complex mixtures are to be made, the adsorption method is often modified so that the materials to be estimated pass through the column without being adsorbed while the contaminants remain on the column. This reduces losses that often result from incomplete elution of adsorbed compounds. One of the best examples of this application of the adsorption method is the separation of the carotenes from other pigments in leaf extracts. A number of adsorbents and solvents have been utilized for this purpose, as is illustrated in Table III. The method is not restricted to use in this way, because it has been shown that riboflavin and thiamin (vitamins B_2 and B_1) in food products can be adsorbed and eluted quantitatively (*11*).

Some materials containing traces of impurities may be purified by passage of the solutions through adsorption columns that retain the impurities. This application of the method is similar to the well-known decolorization of solutions by percolation through towers of charcoal or fuller's earth, as in the industrial clarification of sugar solutions and lubrication oils.

Chromatographic adsorption is often used to determine the purity of chemical compounds. Substances are said to be chromatographically homogeneous if they cannot be purified further with adsorption columns.

Still another use of the adsorption technique is the concentration of solutes from dilute solution, thus eliminating or supplementing the tedious and sometimes destructive process of evaporation. Materials adsorbed by passage of the dilute solution through the column are recovered in greatly concentrated form upon elution from the adsorbent. This procedure has been utilized for the concentration of certain alkaloids and urinary pigments (*16, 24*).

Two substances suspected of being identical may be compared quickly by use of the chromatographic method. A solution of each compound is adsorbed on separate columns to make certain that the materials yield only one band and are therefore homogeneous. Solutions of the two compounds are then mixed and adsorbed on a fresh column of adsorbent. Formation of a single band indicates that the two substances are very similar or identical. Formation of two bands proves that the compounds are different (*28, 37, 40, 61*).

The great selectivity of the chromatographic adsorption method makes it extremely useful for the detection of adulterants in technical products. It has been used to prove the presence of artificial coloring in wine and in butter (*32, 48*).

Determination of the molecular structure of certain types of organic molecules has been facilitated by the use of adsorption methods. Experience has shown that there is a relation between the adsorbability of organic molecules and the architecture of their molecules. The more polar the molecule, the greater the number of polar groups, and the greater the number of double bonds the more strongly is the compound adsorbed. Chemical reactions that alter the molecule also change its relative position upon the adsorption column. The greatest use of these relationships has been made in studies of the carotenoid pigments (*40*).

Chromatographic adsorption has found some use for the separation of hydrocarbons and sterols from their addition compounds with trinitrobenzene or picric acid. The nitro compounds are held by the adsorbent and the liberated materials pass into the percolate (*34, 35*).

For the separation of water-soluble, ionized, or charged compounds the adsorption method may be combined with the electrophoretic method. When electrical potential is applied to the ends of a column upon which ionized substances have been adsorbed, the materials migrate over the surface of the adsorbent although no liquid is permitted to flow through the tube. Thus far, the method has been applied only to the separation of a few dyes (*42*).

Recent developments indicate that the chromatographic adsorption method may find considerable use in industrial processes (*1*). Alumina has been utilized for the dehydration of organic solvents (*12*), and magnesia has been recommended for continuous purification of dry cleaning solvents (*39*).

Apparatus and Procedure

In spite of the hundreds of applications of the adsorption method in the past ten years, utilization of this technique remains an art rather than a science. This is due to the fact that a great many different conditions affect the procedure, often in several unrelated ways. Moreover, there is no adequate theoretical basis for the method to serve as a guide for its use (*8, 56*).

Some two dozen different types of adsorption columns have been described, a few of which are illustrated in Figure 4. The diameters of the adsorption columns range from 1 or 2 mm. to many centimeters. The lengths are usually 6 to 12 times the diameter, but this proportion is by no means fixed.

The amount of material adsorbed on the columns varies with the activity of the adsorbent, with the solvent, and with the adsorbability of the compound itself. It ranges from a few micrograms with the smallest columns to a few tenths of a gram with columns several centimeters in diameter. With still larger columns, several grams of material may be prepared.

Adsorbents, Solvents, and Eluants

No universal adsorbent or solvent has yet been discovered. Each application of the adsorption method depends upon the careful selection of adsorbents, solvents, and eluants, upon the basis of experience and trial and error. Adsorbents and solvents useful for adsorption of a given substance are

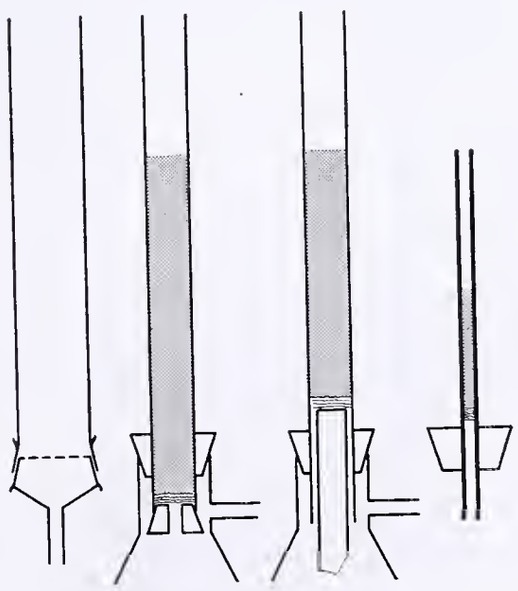

Figure 4. Various Types of Adsorption Tubes (*40, 61*)

usually suitable for adsorption of other materials of the same class.

Particular attention must be paid to the properties of the adsorbent. The adsorptive capacity of most solids varies enormously with the method of preparation and with the presence of impurities and polar solvents such as water. To a considerable extent it is characteristic of the solid itself, as is indicated by the following list of adsorbents arranged in approximate order of their adsorption capacity, the most active recorded first (*40*):

Activated alumina, charcoal, and magnesia (Micron brand)	Calcium carbonate
Lime	Talc
Magnesia (Merck)	Magnesium citrate
Magnesium carbonate	Inulin
Calcium phosphate	Sucrose, starch

The adsorption capacity of all solids varies with the solvents that are employed. Adsorption is strongest from aliphatic hydrocarbons such as petroleum ether, and is progressively weaker from more and more polar solvents, as illustrated by the following list of solvents arranged in approximate order of their effect on adsorption (adsorption is greatest from those listed first):

Petroleum ether, b. p. 30–50°	1,2-Dichloroethane
Petroleum ether, b. p. 70–100°	Alcohols
Carbon tetrachloride	Water
Cyclohexane	Pyridine
Benzene	Acids

Substances adsorbed from a nonpolar solvent may be caused to move through the column at a faster rate by the addition of a more polar liquid to the solvent. This accelerates the development of the chromatogram (*51*).

For each application of the adsorption technique, the adsorbent must be of just the right activity. If it binds the adsorbed compounds too firmly, a chromatogram cannot be developed. If it is too weakly adsorptive, sufficient quantities of material will not be adsorbed.

The particle size of the adsorbent is of importance because this determines the rate of percolation of the solvent through the column, the definition and evenness of the bands, and the method to be used in packing the adsorption column. Granular adsorbents (about 200-mesh) can be poured into the glass tube and tamped gently. More finely divided materials such as precipitated chalk must be tamped firmly into the column in small portions. Very fine powders should be mixed with a filter aid such as the heat-treated siliceous earth known as Hyflo Super-Cel. These mixtures are pressed rather than tamped into the tubes. Occasionally the adsorbent is made into a slurry with the solvent and this is poured into the glass tube.

Selection of adsorbents and solvents is determined, to a large extent, by the nature of the compounds to be resolved. Some idea of the combinations of adsorbent and solvent used for preparation of various compounds may be gained by perusal of Tables I and II.

Once substances have been separated by adsorption upon Tswett columns, it is essential to be able to release or elute them from the adsorbent. The polar solvents usually used for this are alcohols, or pyridine with alcohols or acetic acid. Materials adsorbed from water can frequently be eluted by changing the pH of the solutions.

Possible Future Developments

In view of the spectacular advances made in the preparation of active and selective adsorbents in the past ten years, it seems highly probable that similar developments may continue. The new organic polymers that function as exchange adsorbents have yet to be tested in adsorption columns. Future investigations may be expected to reveal methods whereby adsorbed compounds can be eluted in good yield, thus extending the usefulness of the chromatographic adsorption technique in quantitative analysis. Much remains to be done in order to perfect the methods for location of colorless compounds on the adsorption columns. With the clarification of concepts regarding the process of adsorption itself, further useful applications of the chromatographic method will undoubtedly be made.

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Sodium Salt of Quinizarin-6-Sulfonic Acid as an Acid-Base Indicator

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THE red sodium salts of quinizarin sulfonic acid have been used as dye intermediates by the I. G. Farbenindustrie (*3*), and for the formation of metallic lakes by Bayer & Co. (*1*). The author observed that when the sodium salt of quinizarin-6-sulfonic acid was treated with acid, the yellow quinizarin-6-sulfonic acid was formed, while treatment with alkali produced a blue-violet coloration. As these reactions indicated that the sodium salt of quinizarin-6-sulfonic acid might be used as an indicator in acidimetry-alkalimetry, this investigation was undertaken.

Preparation of Reagents

The quinizarin is prepared according to the method of Bigelow and Reynolds (*2*). To make the sodium salt of quinizarin-6-sulfonic acid (*4*) quinizarin, boric acid, and 20 per cent fuming sulfuric acid are heated to 90–100° C. for 1.5 hours, after which 60 per cent fuming sulfuric acid containing mercuric sulfate is added; the temperature is raised to 170–180° C., and kept constant until the melt becomes soluble in water. The mass is then poured into water, and the quinizarin-6-sulfonic acid is neutralized with sodium carbonate and salted out with sodium chloride. Red crystals are obtained, which are purified by recrystallization from alcohol. The sodium salt of quinizarin-6-sulfonic acid is soluble in water and concentrated sulfuric acid.

Approximately 0.1 *N* solutions of hydrochloric acid, sulfuric acid, nitric acid, acetic acid, oxalic acid, sodium hydroxide, and sodium carbonate, and a 0.2 *N* solution of sodium hydroxide were prepared, as was the universal buffer mixture of Prideaux and Ward (*5*), which gives pH values between 2 and 12. A 0.1 per cent aqueous solution of the sodium salt of quinizarin-6-sulfonic acid is used.

Color Ranges and Constants of Indicators

This indicator changes from a yellow color under distinctly acid conditions to pink and finally to blue-violet as alkali is added. The change from yellow to pink is sharp, and that from pink to blue-violet is gradual. The color ranges were obtained through the use of a series of buffer solutions, made by titrating the Prideaux and Ward universal buffer solution with 0.2 *N* sodium hydroxide. All buffer solutions employed in this investigation were checked by means of a Beckman pH meter. The pH range for the change from yellow to pink was found to be 7.1 to 9.1, and from pink to blue-violet 9.1 to 11.5 at 20° C.

The ionization constants were determined by using a Bausch & Lomb hydrogen-ion colorimeter. The pKa for the first hydrogen was found to be 8.2 and for the second hydrogen 10.7 at 20° C.

Titration

Acid and base titrations were made by using the appropriate indicator in each case. These were compared with similar titrations made by using a 0.1 per cent aqueous solution of the sodium salt of quinizarin-6-sulfonic acid as the indicator, the end point being the color change from yellow to pink.

Discussion

The preparation of the indicator required no special apparatus. The cost of preparation of the compound was less than that of phenolphthalein. The author found that in titrating acids with bases, the color change was sharper when using the sodium salt of quinizarin-6-sulfonic acid than when using phenolphthalein. In titrating bases with acids, the sodium salt of quinizarin-6-sulfonic acid gave a sharp color change, whereas phenolphthalein cannot be used so efficiently. This indicator can be used for colorimetric determinations of pH over the range from 7 to 11.5.

Table I. Titrations of Acids with Bases[a]

Acid *Ml.*	Base, NaOH *Ml.*	Acid-Base Ratio	Mean Deviation	Indicator
HCl 29.00	27.81	0.960	0.000	Na salt of quinizarin-6-sulfonic acid
28.66	27.47	0.959	0.000	Phenolphthalein
29.40	27.91	0.951	−0.001	Methyl orange
H_2SO_4 27.32	30.86	0.896	−0.001	Na salt of quinizarin-6-sulfonic acid
27.98	31.19	0.898	−0.001	Phenolphthalein
27.98	31.01	0.902	0.000	Methyl orange
HNO_3 28.32	27.03	1.047	+0.001	Na salt of quinizarin-6-sulfonic acid
28.99	27.61	1.050	+0.001	Phenolphthalein
29.00	27.47	1.055	0.000	Methyl orange
$HC_2H_3O_2$ 28.66	29.77	0.962	+0.001	Na salt of quinizarin-6-sulfonic acid
28.99	30.24	0.959	−0.001	Phenolphthalein
$H_2C_2O_4$ 28.66	32.43	0.884	−0.001	Na salt of quinizarin-6-sulfonic acid
28.32	32.02	0.883	0.000	Phenolphthalein

[a] Values are means of several titrations, using volumes between 20 and 40 ml.

The sodium salt of quinizarin-6-sulfonic acid can be used as an indicator in the titration of strong acids with strong bases, and weak acids with strong bases.

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The High-Vacuum Still in Medicine

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THE place of the high-vacuum still in medical research is largely a matter of definition. Various organic substances of high molecular weight used in the preparation of drugs have been purified in the high-vacuum still and most of the materials which are produced commercially by molecular distillation are applied in popular medicine—the vitamins (*23*), for instance. When, however, we examine the acceptance which the latest kind of molecular distillation apparatus and method has found in laboratories devoted to medical research we find that the technique has not yet come into widespread use, which is a pity because the methods would seem to offer much to the biochemist and pharmacologist.

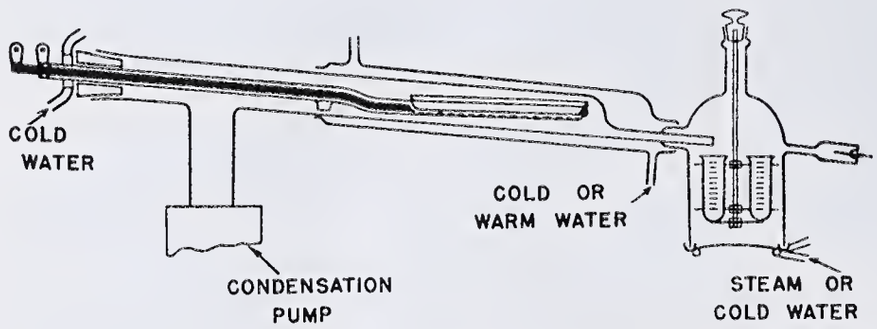

FIGURE 1. BURCH TRAY STILL

Molecular distillation is a term applied as loosely as its real meaning is restricted. In the narrow sense it refers to distillation between a parallel evaporator and condenser, the gap between the two being held under high vacuum. In the wider terminology of the literature almost any kind of a distillation device, whether pot or tube or a falling-film apparatus, is referred to as a molecular still if the operating pressure is believed to be less than 0.001 mm. Operating pressures are generally quoted, not from measurements made during and at the site of distillation but from the theoretical pressure which the pump attached to the apparatus could produce under ideal conditions when the apparatus contained nothing to be distilled. This state of affairs must be the subject of a separate paper, since it is in urgent need of airing. For the purpose of this review we may cover the distillations which have been done in pot stills, tubular stills, falling-film stills, and revolving-plate stills.

Figure 1 shows the first tray still of Burch (*4*) and Figures 2 and 3 show various pot stills after Waterman (*25*), Washburn (*24*), Hickman (*19*), Nelson and Haller (*21*), Carothers (*5*), and others. The material is placed in the pot, which may have a detachable lid; vacuum is applied and the material is warmed while the lid is cooled or frozen. The distillate is removed at intervals by dismantling the still or allowing drippings from the lid to pass out through a side arm into a receiver. This kind of still has been in operation since about 1929 in laboratories throughout the world.

The tubular stills, shown in Figure 4, are of two general varieties, the simpler kind having a single stationary source of heat, the others having a graduated or moving evaporative zone. The material is placed at the far closed end of the tube which is maintained nearly horizontal. In the simple type, heat is applied gently below the material and later the sublimate is scraped off the walls near the mouth after dismantling. In the compound type (*2*, *10*) the material is volatilized at the far end and the vapor allowed to condense fractionally by controlling the heat throughout the length of the tube. The proper description for this kind of apparatus would seem to be a high-vacuum diffusion still, since it is not truly molecular no matter how low the pressure of operation.

In the falling-film molecular still, Figure 5, the substance, generally dissolved in oil, is allowed to fall in a thin film over a heated surface which is maintained parallel to a cooled condenser. The revolving plate still, Figure 6, employs centrifugal force to whirl the liquid in a thin layer across the surface of a heated plate held in front of a cooled condenser. The thermal exposure in a falling-film still is perhaps a hundred times less than in a pot still. In the centrifugal still the exposure may be a thousand or ten thousand times less and the distillation method is extended in corresponding degree to the separation of unstable substances.

Reviewing the substances of interest in medicine, calciferol (*3*), or vitamin D_2, was first distilled free from ergosterol in a tubular diffusion still. Vitamin K was separated by Almquist (*2*, *10*) in a compound tube still and by Dam (*6*) in a pot still. Progesterone (*1*) was separated from an extract of pigs' ovaries in a tiny pot still in the early 1930's. Farmer and van den Heuvel (*13*) investigated the marine fatty acids in glass pot stills. Vitamins A (*20*), D (*18*), and E (*17*) have been produced industrially during the last few years in relatively large quantities in the falling-film stills. A valuable but by no means complete bibliography of high-vacuum distillation has been compiled by Detwiler and Markley (*7*, *8*); and a revised compilation is reported to be near completion.

At the present time the molecular still can serve medicine in three capacities: as a manufacturing process for vitamins A and E and sterols; as an occasional adjunct to organic research; and as a quantitative tool in the central focus of medicinal research. The usefulness as an adjunct to organic research has existed for about twelve years, and as a manufacturing process for the last three or four years, but this application is expanding rapidly. During the year 1941 more than five tons of vitamin A in the form of ester concentrate (largely palmitate) will have been distilled from over 100,000 gallons of crude fish liver oils. About half a ton of mixed tocopherols will have been distilled from vegetable sources in the same period. However, the importance of molecular distillation is not its past accomplishments, which are insignificant compared with those

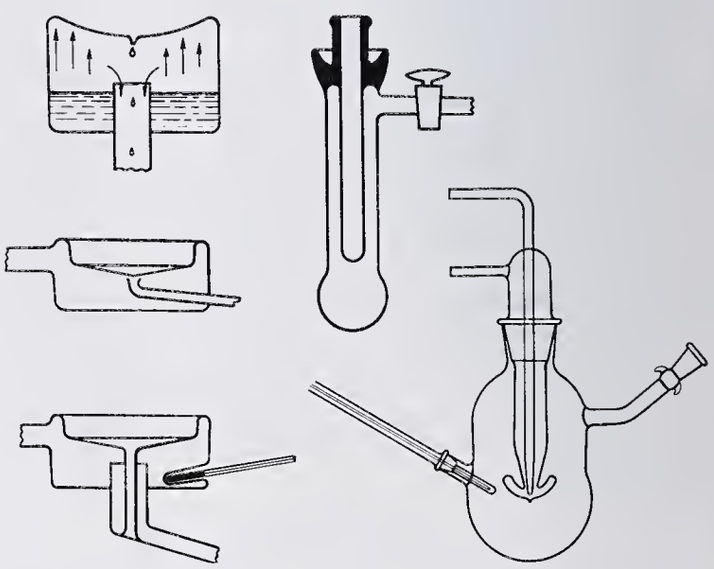

FIGURE 2. POT STILLS

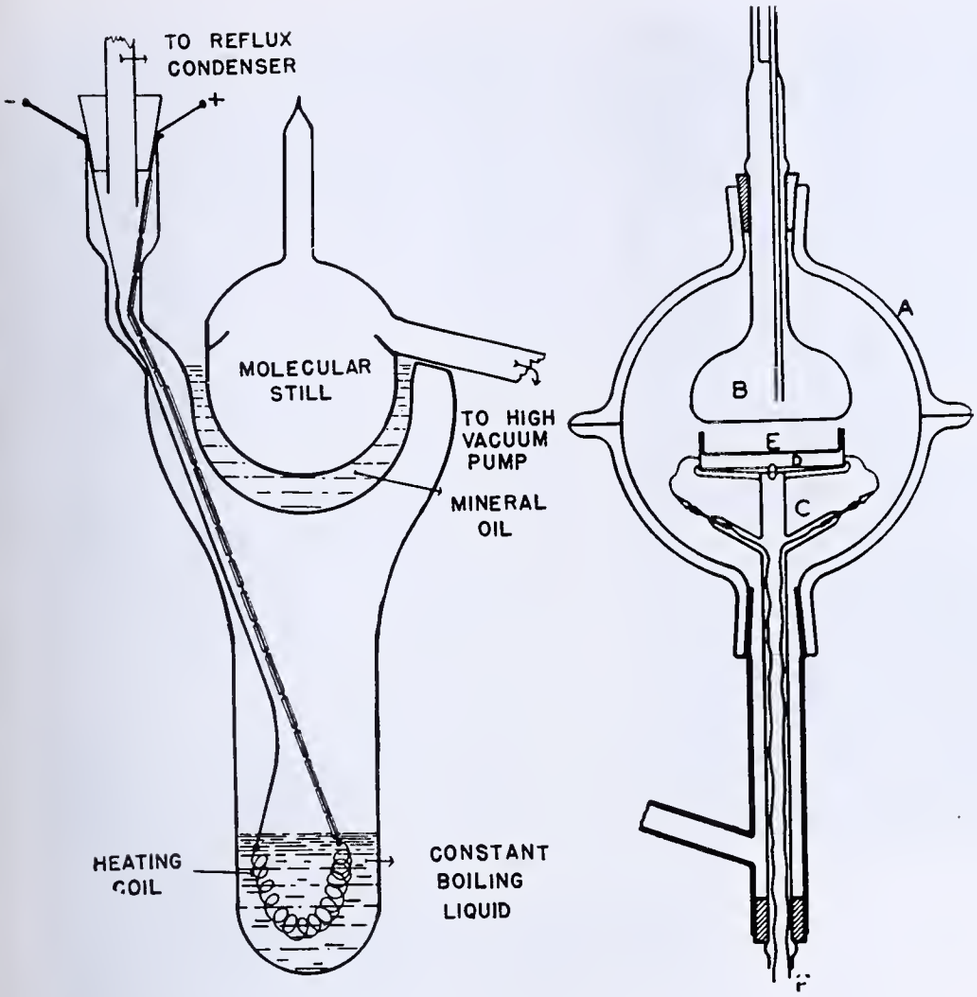

FIGURE 3. POT STILLS

of the Twsett column or the polarograph, but its future promise. How can it be useful in the reader's laboratory? The brightest prospects are as a quantitative tool in medicinal research.

In the distillation of one or more compounds from a mixture the first requirement is a sharp and complete degree of separation and the second is the power to identify the fractions and follow the course of separation. Ordinary distillation methods employ fractionating columns for the first and boiling point measurements for the second. In the molecular still there is no sharp separation and, ordinarily speaking, there is no boiling point. Our first concern, therefore, is to find an analog of boiling point. As a rough generalization we may equate separating power, S, and identifying power, T, with utility, U, as follows: $S \times T = U$. Without going into the reasons why the separation is poor and why there is no boiling point we may say that the conventionalized distillation described below grants us so much power under T that we are able to accept severe compromises with S for separation.

The conventionalized method uses a cyclic molecular still (*16*). That this apparatus has reached some finality of design is evidenced by the fact that Figure 7 is a photograph of apparatus built five years ago and in the meantime few modifications in the essential layout have been required. The cyclic still of the near future will have a centrifugal plate instead of a falling-film column and may operate automatically; otherwise major changes are not foreseen. The material under investigation is for convenience dissolved in a carrier fluid. This at present limits the method to working with oil-soluble substances, but fortunately these comprise more than half the field of interest in current biochemical research. The oily solution is given a series of distillations at succeeding temperatures, the process being known as stepwise distillation. The time of passage through the still is controlled accurately and the temperatures are raised by known and constant amounts, generally 5° or 10° C. over a range of 80° to 260° C., though these limits are purely arbitrary. From 15 to 30 fractions result and each fraction is weighed and analyzed for the wanted constituent. The product of weight and concentration is plotted graphically against temperature and the line connecting the points forms an elimination curve (*11*). This elimination curve is merely a grand name for a special kind of distillation curve and distillation curves, of course, are as commonplace and old as manipulative chemistry itself. The elimination curve, however, because of its specialized mode of production tells us very much more than a common distillation curve. From it we may learn some or all of the following: (1) the exact fractionating power of the apparatus; (2) the efficiency with which the apparatus is being employed; (3) the purity of the substance under investigation; (4) the nature of the substance, whether polar or nonpolar, whether stable or labile, high or low latent heat, etc.

Embree (*11*) of this laboratory has worked out from theoretical considerations the expected shape of the elimination curve for a substance of average latent heat of evaporation. The curve looks like Figure 8. From the falling-film still have been obtained the curves for various volatile dyes (Figure 9), the concentration of which can be measured in the fraction with the minimum of labor. The curves are similar to the

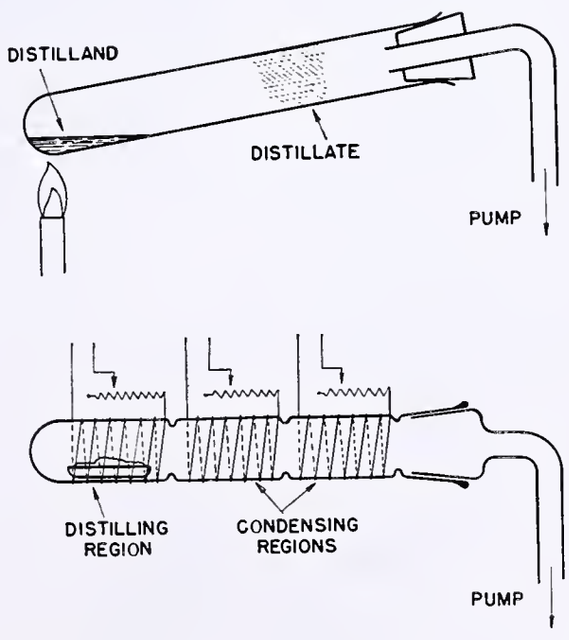

FIGURE 4. TUBULAR STILLS

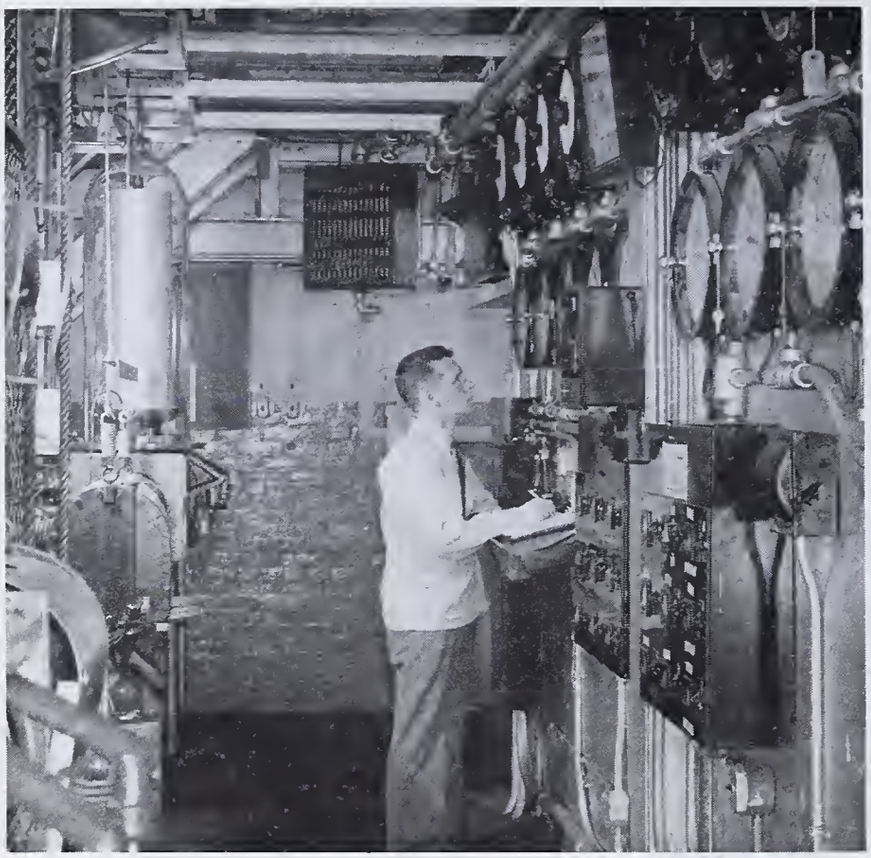

FIGURE 5. FALLING-FILM MOLECULAR STILL

well-known probability distribution curve and possess a sharply defined maximum situated along the distillation temperature axis. This maximum would seem to be a good substitute for boiling point. Unfortunately the position of the maximum depends on the kind and details of the apparatus, the rate at which distillation is done, and the relative affinity of the test substance and the solvent oil. It is, therefore, not a measure of absolute boiling point. However, comparative scales have been constructed using colored indicators as arbitrary standards and in this way it is possible to determine with some exactness the relative boiling points of substances, identify them during distillation, and predict the degree of separation or the composition of any chosen fraction.

The dye elimination curves soon showed that the theoretical shape was realized in practice to a very considerable extent; but it was not easy to tell whether the best possible separation was being given by the apparatus. Recently a new method has been found for investigating the matter. Pilot dyes were distilled from different kinds of solvents from centrifugal plates rotating at different speeds. The curve was found to become sharper and sharper until with slow rates of flow and high speeds of rotation the shape of the curve became constant (Figure 10). This shape revealed a better separation than had been obtained in the falling-film cyclic stills and indicated the improvement which can be introduced to this technique in the future.

The curves so far described are those of pure substances. The term "pure" is used in a specialized sense, since in a mixture the components are scarcely in pure condition. As here used the term means that no other substances are present which respond to the test used for the chosen substance. Thus if a blue pilot dye is under examination it is assumed that there is only one blue dye present; if a test for a sterol is being applied, that there is only one sterol present, the other substances in solution not affecting the sterol test. When there is more than one substance affecting the test reagent, a complex curve is produced and by the degree of departures from the standard shape it is possible to judge the number and the probable difference in boiling points between the interfering substances. Here then is a place where the analytical molecular still can be useful in pharmacology—indeed, in the whole range of high molecular weight chemistry—as a criterion of purity. The author has distilled commercial samples of dyes and obtained complex elimination curves such as shown in Figure 11. The dye has then been recrystallized and again distilled, only to reveal that there are still two or three homologs present. It is then necessary to purify the dye by a multiple redistillation, chromatographic absorption, etc., but all that can be said at the finish is that the dye is pure so far as boiling point characteristics are concerned. This sometimes turns out to be absolute purity by other standards.

The technique has been used for examining the complexity of many allegedly single substances. The most fruitful of these researches in this laboratory has been on the fish liver

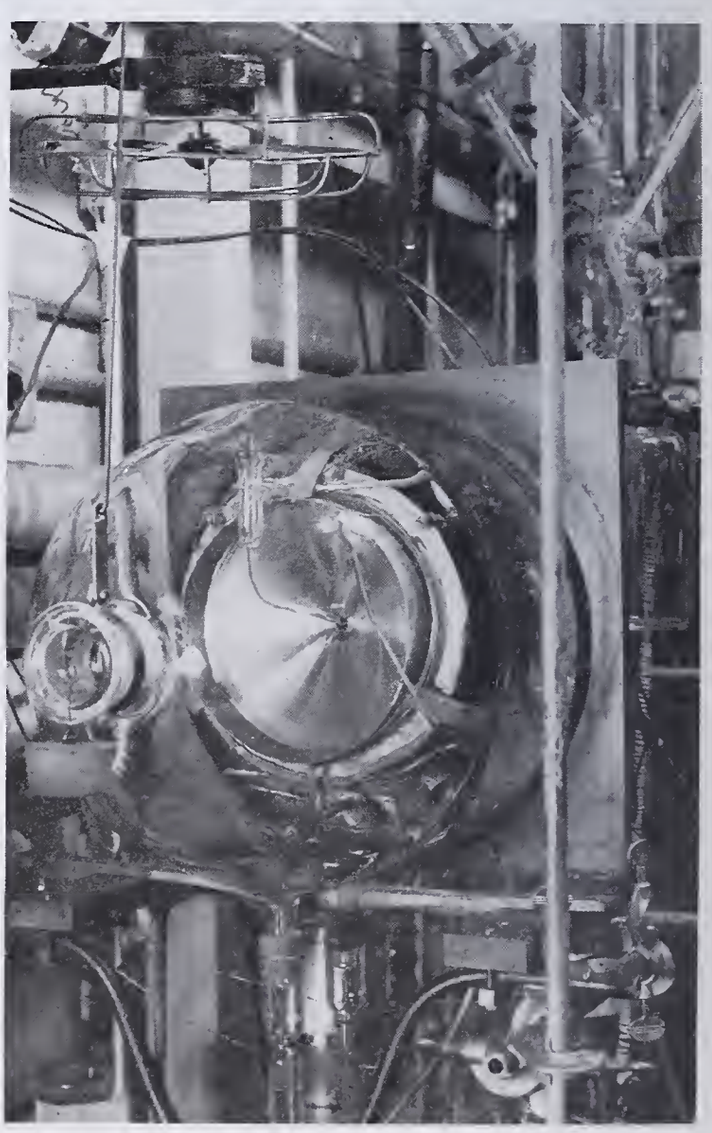

FIGURE 6. REVOLVING-PLATE STILL WITH CONDENSER REMOVED

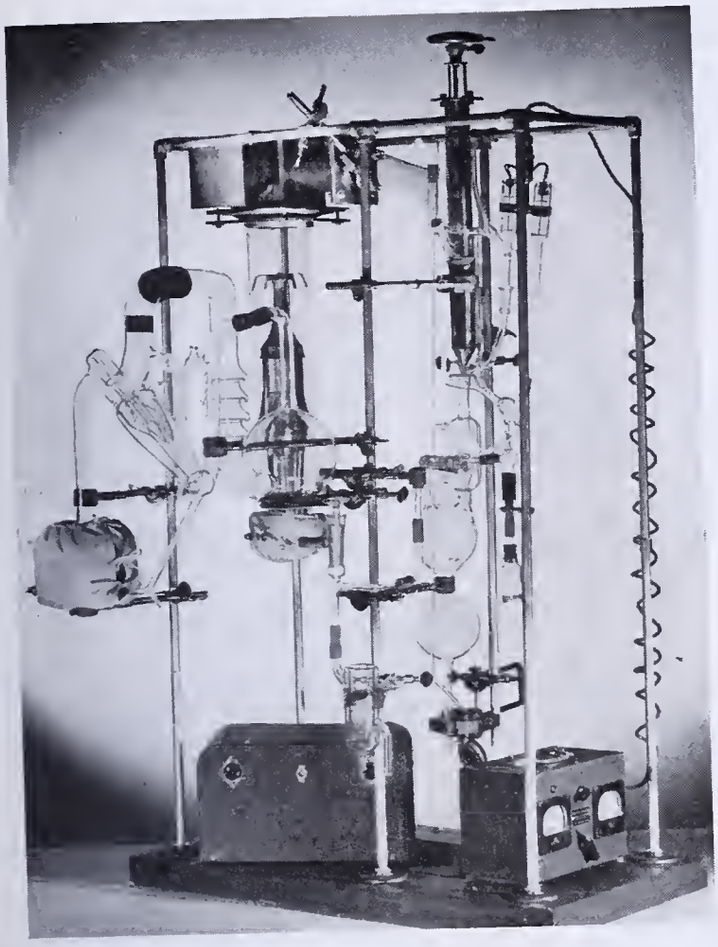

FIGURE 7. CYCLIC MOLECULAR STILL

oils to determine the complexity of vitamins A and D. Vitamin A is found to have a double elimination curve (*11*), from which it has been deduced that the vitamin is present in the free form, maximum 123° C., and as a mixture of esters, maximum 190° to 230° C. Natural vitamin D has been shown to be a mixture of at least four, probably six, antirachitic substances existing partly in free and partly in esterified condition (*18*). Table I lists various substances for which the elimination curves have been traced and for many of which the technique of molecular distillation has been largely responsible for their separation and the knowledge which we have about them today. (For more exact data concerning

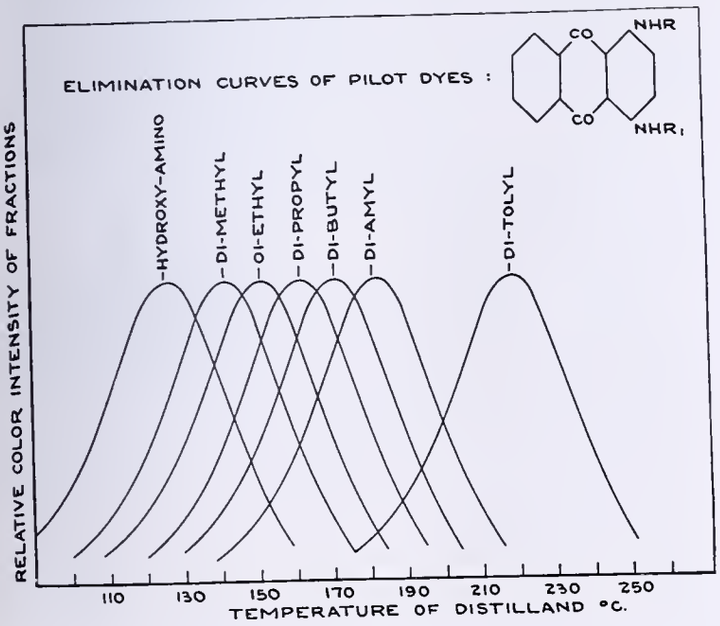

FIGURE 9

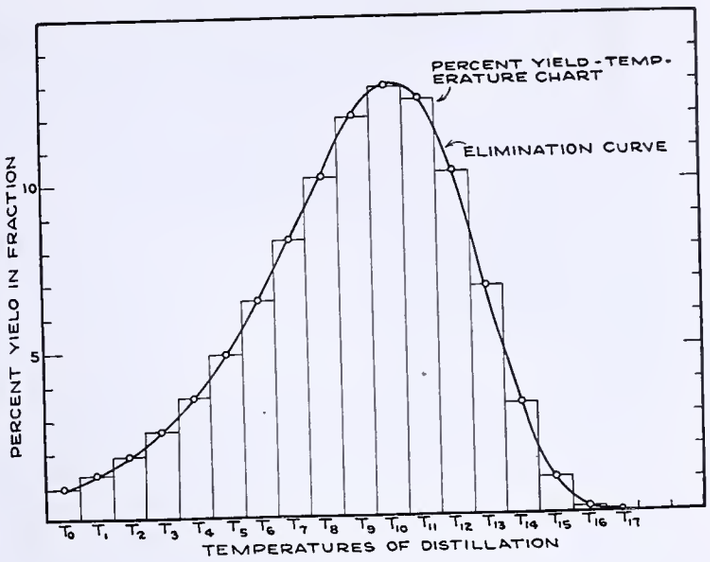
PERCENT YIELD - TEMPERATURE CHART
ELIMINATION CURVE
PERCENT YIELD IN FRACTION
10
5
T0 T1 T2 T3 T4 T5 T6 T7 T8 T9 T10 T11 T12 T13 T14 T15 T16 T17
TEMPERATURES OF DISTILLATION

FIGURE 8

these and other substances reference must be made to a paper to appear in *Chemical Reviews*.) Through the aid of the molecular still it has been possible to determine the constitution of vitamin A compounds metabolized in the animal body under normal circumstances or after administration of large doses of the vitamin. The still has been used to trace the progress and successful completion or otherwise of the esterification of vitamin A with various substances. The preparation of vitamin A succinate and half-succinate is a case in point (*9*). The half-succinate gave an elimination curve with a maximum at 173° C., the double succinate of vitamin A failed to distill completely at 250° C., and the submaximum at 120° C. showed that not all of the reaction mixture had become esteri-

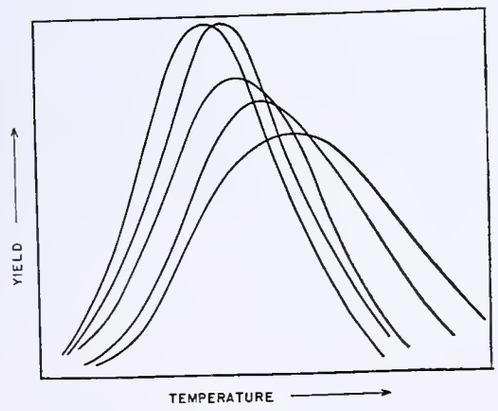

FIGURE 10

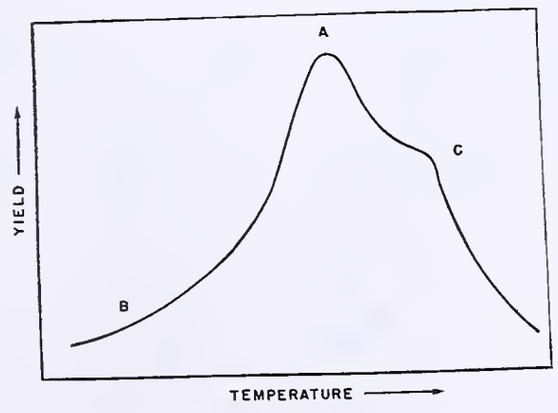

FIGURE 11

TABLE I. SUBSTANCES INVESTIGATED[a] QUANTITATIVELY[b] BY THE MOLECULAR STILL

Vitamin A Class	Vitamin D Class	Sterol Class	Hormone Class	Dye Class
Vitamin A[b]–alcohol (*16*)	D_2 (*18*)–Calciferol[b]	Cholesterol (*17*)	Progesterone (*1*)	Substituted diamino anthraquinones[b] (*16*)
Vitamin A[b]–acetate (*15*)	D_3 (*18*) (Windaus)[b]	Sitosterols[b] (*17*)	Bone marrow anemia factor (*22*)	Indigo and derivatives[b] (*16*)
Vitamin A[b]–palmitate (*15*)	Cod liver oil *D*[b] (*18*)	Stigmasterol[b] (*17*)	....	....
Vitamin A[b]–naphthoate	Tuna liver oil D[b] (*18*)	α-Tocopherol[b] (*17*)	....	Butter coloring matters (*9*)
Vitamin A[b]–half-succinate (*9*)	Swordfish D[b] (*18*)	γ-Tocopherol	....	....
Vitamin A[b]–succinate (*9*)	....	Tocoquinones	....	....
Vitamin A[b]–fish liver esters (*16*)	....	Vitamin K	....	....
Vitamin A[b]–rat liver esters (*15*)	....	....	....	....
Vitamin A_2[b] (*14*)	....	....	....	....
Cyclized vitamin A[b] (*12*)	....	....	....	....
Subvitamin A[b] (*9*)	....	....	....	....
β-Carotene[b] (*9*)	....	....	....	....
α-Carotene (*9*)	....	....	....	....
Xanthophyll (*9*)	....	....	....	....

[a] This list does not include many investigations of natural glycerides and heavy petroleum residues.
[b] Only these substances have been examined quantitatively. Elimination maxima are not given because figures do not yet give a concordant series.

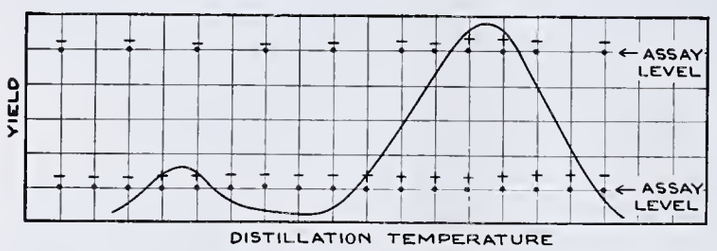

FIGURE 12. METHOD OF SETTING ASSAY LEVELS

fied. When both esters were hydrolyzed and redistilled a maximum at 120° C. only was obtained, showing that vitamin A had been liberated from what must have been a true A-ester.

The elimination technique used with the tocopherols has been applied to the separation of the tocopherols, their quinones, and various other oxidized forms, but with no great success.

A useful method of applying the molecular still in medical or biological investigation is as follows.

An unknown substance, *X*, is being traced. *X* is known to be oil-soluble, moderately stable to heat, and has a well-characterized effect on a certain test animal, plant, or mold, which effect can be used as a semiquantitative test. A rich concentrate of *X* is dispersed in constant yield oil and the mixture is colored faintly blue with a trace of diethyldiaminoanthraquinone. The mixture is distilled and fractions are taken off at 10° intervals between 80° and 260° C. The fractions are now weighed and aliquot portions are fed or applied to the biological test at one level or preferably two widely separated levels. The responses are plotted against the temperature and, providing the levels have been chosen properly, the responses will pass from negative to positive and back to negative as the fractions cross the rising and falling limits of the elimination curve of the unknown substance, *X*. This is shown in Figure 12. By the position and width of the region of positive response it is possible to tell approximately how much of *X* is present and indicate its boiling point relative to the pilot dye.

Larger quantities of the concentrate *X* are now distilled, in carrier oil if necessary, and fractions are collected for 20° C. in 5° intervals on either side of the maximum suggested by the pilot distillation. It is convenient to include a trace of the pilot dye, or better, a dye chosen to coincide with the expected maximum of *X*. A high concentration of *X* will then be found in or near the deepest colored fraction. It may then be found that after saponification of the richer fraction the substance is obtained crystalline for the first time, after which its purification and characterization are relatively easy. This technique has been used for many of the substances listed in Table I.

The analytical distillations just described provide information from the simplest of all forms of distillation and one having the poor separating power of approximately a single theoretical plate. The next steps in improving technique are plain. A molecular fractionating still is needed which gives better separations and the long and tiresome task of cyclic distillation should be avoided by the construction of an automatically operated still.

Suggested Applications

In conclusion, a number of applications may be suggested for the molecular still in pharmacology and in nutritional and biochemical research. Those marked with an asterisk would be benefited by the analytical technique.

Investigating essential fatty acids
Investigating fats, alcohols, sterols, and vitamins recovered after feeding to animals
* Testing for homogeneity or purity of new drugs, plant extracts, dyes, etc., with special reference to sulfonamides and barbiturates
* Separation and characterization of steroids and other lipids from bone marrow, pituitary, suprarenal, and other glands
* Extending the chemistry of the carotenoids
Analysis of fecal, visceral, and tissue extracts, etc.

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PRESENTED before the Division of Agricultural and Food Chemistry, Symposium on New Analytical Tools for Biological and Food Research at the 102nd Meeting of the AMERICAN CHEMICAL SOCIETY, Atlantic City, N. J. Communication No. 28 from the laboratories of Distillation Products, Inc.

Column for Stripping Solvents from Extracted Oils

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OLCOTT (*1*) has recently pointed out that the removal of traces of solvent from laboratory-extracted oils is difficult even when vacuum is employed at temperatures up to 50° C. He found that traces of solvent still persisted. No adequate device to facilitate the removal of the small amounts of solvent is generally available; hence, the stripping column described below meets a definite need. It has been successfully used without causing any detectable change in the unsaturated oils given in Table I.

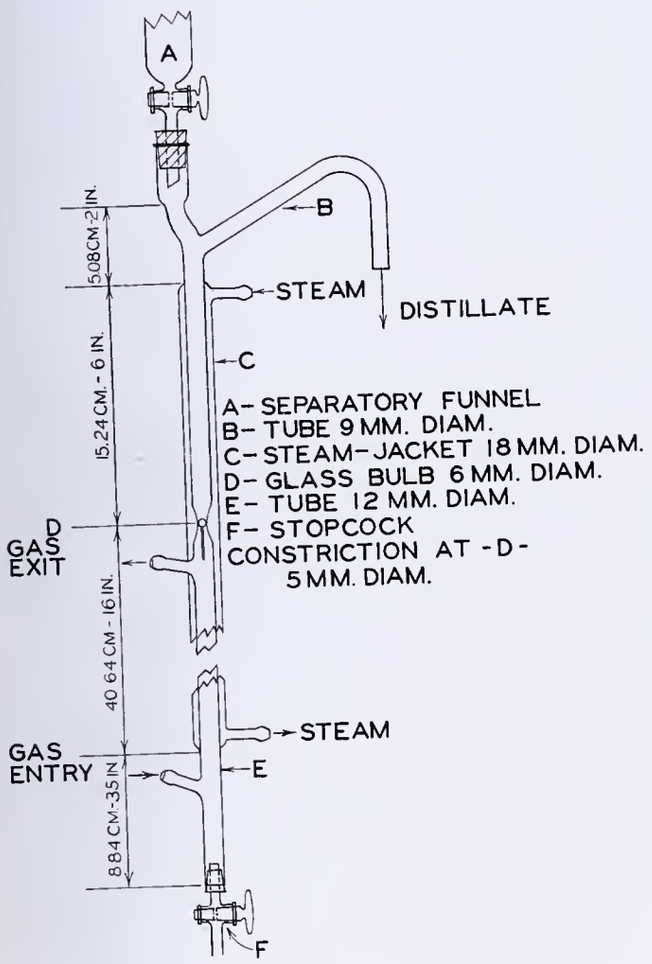

FIGURE 1. DIAGRAM OF COLUMN

The apparatus illustrated in Figure 1 consists of a steam-jacketed column made of Pyrex glass. The inner tube is constricted at *D* and a small loosely fitting glass bulb at this point permits the oil to pass through at a slow rate. At the same time the oil forms a seal against the passage of solvent vapors. The section of the inner tube below *D* is provided with side arms. A current of air is drawn in through the lower arm, or nitrogen under pressure may be admitted, at the rate of 800 ml. per minute. The stopcock, *F*, at the bottom of the column is opened enough to permit the oil as it collects here to drain into a receiving vessel.

The filtered extract oil-solvent mixture is fed into the column from the separatory funnel, *A*, at the flow rate of 1.6 to 2 ml. per minute for solvents such as ethyl or petroleum ether, and more slowly for the higher boiling solvents such as ethylene dichloride. As the oil-solvent mixture enters the area surrounded by the steam jacket, the solvent volatilizes and passes out at *B* through a water-cooled condenser to a receiving vessel. The oil drains past bulb *D* and forms a thin film on the lower portion of the column. Here any residual traces of solvent are removed by the combined action of heat and the countercurrent of air or nitrogen.

Experimental

The following data show that the stripping column completely removes the solvent from the extracted oil-solvent mixture without any detectable influence on the recovered oil.

Seventy-five milliliters (69 grams) of refined corn oil (Mazola) were mixed with 400 ml. of petroleum ether, Skellysolve F, boiling range 30° to 60° C. This mixture was passed through the stripping column with a recovery of 68 grams of oil and 340 ml. of solvent.

To confirm the completeness of separation of solvent from the stripped corn oil, charges of about 21 grams of the original and of the stripped oil were subjected to drying in a vacuum oven 6 hours at 30° C. at about 1-mm. pressure, followed by 4 hours' drying at 50° C. at 20-mm. pressure. The changes in weight on the charges of each oil were 0.0115 and 0.0122 gram, respectively. These losses are insignificant and indicate that the solvent had been completely removed by the stripping column. Further, the organoleptic tests of odor and taste for the presence of solvent in the recovered oil were negative, and the values of the fat constants of specific gravity, refractive index, and iodine number of the corn oil both before and after treatment remained the same and showed no change in unsaturation (Table I).

A sample of crude soybean oil, No. 2537, was extracted from Tokyo soybeans with petroleum ether and stripped free of solvent by passing through this apparatus. Sample 2537a was extracted from the same lot of soybeans with ethylene dichloride. The fat constant gave no indication that the unsaturation of these oils had been changed. The odor and taste of the stripped oils gave no detectable evidence of the presence of any remaining solvent.

While efficient solvent recovery in laboratory stripping operations is generally considered unimportant, the amounts of solvent recovered were recorded because the column might be adapted to industrial usage by suitable modification. The amount of solvent recovery from 5 samples varied from 85 to 93 per cent. A cold trap placed in the gas exit line below *D* did not recover any more solvent.

TABLE I. DATA ON EXTRACTED OILS STRIPPED OF SOLVENTS

Sample No.	Oil	Solvent Used	Solvent Recovery	Specific Gravity, 25°/25° C.	Refractive Index, $n_D^{20°}$	Iodine No. (Hanus)	Extract Mixture Flow Rate
			%				*Ml./Min.*
2707	Refined corn oil[a]	....	...	0.9190	1.4743	123.6	...
2707a	Refined corn oil[b]	Petroleum ether	85.0	0.9188	1.4741	123.5	1.6
2537	Crude Tokyo soybean oil	Petroleum ether	93.0	0.9203	1.4751	131.5	1.6
2537a	Crude Tokyo soybean oil	Ethylene dichloride	88.0	0.9229	1.4753	130.8	0.7

[a] Mazola; 21.1804 grams of refined corn oil lost 0.0115 gram when dried in a vacuum oven 6 hours at 30° C. at 1-mm. pressure, followed by 4 hours' further drying at 50° C. at 20-mm. pressure.

[b] A charge of 21.2922 grams of stripped corn oil lost 0.0122 gram in weight when dried under the same conditions.

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Dissolved Oxygen Recordings with the Dropping Mercury Electrode

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THE importance of dissolved oxygen in several phases of water and sewage chemistry has long been recognized. The satisfactory use of the dropping mercury electrode for determining dissolved oxygen in sewage and activated sludge–sewage mixtures has been demonstrated by the author (*2*). Petering and Daniels (*5*) have used the dropping mercury electrode for the determination of dissolved oxygen in studies upon photosynthesis, while Manning (*4*) has adapted this method to the study of the dissolved oxygen cycle in lake water throughout a 24-hour period. Manning was the first to demonstrate that dissolved oxygen can be determined continuously in water by using a constant voltage and noting the shift in current flow with a sensitive recording galvanometer. This paper will demonstrate the use of the constant voltage method to obtain continuous dissolved oxygen values in laboratory experiments and during the regular operation of the aeration tank of activated sludge–sewage plants.

Instrument

The instrument supplied by the Cambridge Instrument Company, New York, N. Y., for this study uses a simple potentiometer circuit with a sensitive, suspension, mirror galvanometer to measure the current flow in the dropping mercury electrode circuit. A light beam impinging upon the mirror is reflected to a photoelectric cell. The current from the photoelectric cell is amplified and then recorded at minute intervals with a recording thread galvanometer. The electrodes consist of a calomel half-cell as the inert reference electrode and the dropping mercury electrode consisting of a separatory funnel with a long stem and a short length of capillary tubing of 0.04-mm. bore to obtain a drop rate of approximately 1 drop per second. For outside work both were encased in a steel shell to protect the electrical connections and prevent contamination of either reservoir. A cup was placed 5 cm. (2 inches) below the capillary to catch the used mercury. A long, heavy, high-conductance cable was used to connect the electrodes to the instrument for outdoor work. The unit is illustrated in Figure 1.

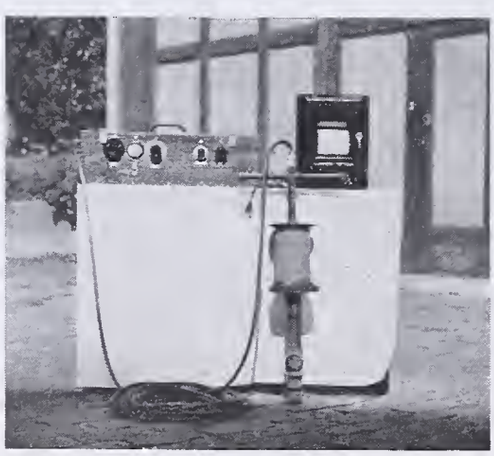

FIGURE 1. DISSOLVED OXYGEN RECORDING INSTRUMENT

Results

The dissolved oxygen recording apparatus has been used in the laboratory principally to obtain type curves. The difference in the rate of increase of dissolved oxygen in activated sludge–sewage mixtures studied in different laboratory units is illustrated in Figure 2; curve *A* shows how rapidly the dissolved oxygen rises when 1 liter of the activated sludge–sewage mixture is in a tall glass tube agitated with air at the rate of 80 ml. per liter per minute (4 cubic feet per gallon per 6 hours) through a sintered glass diffuser at the bottom. Each accentuated horizontal line is equivalent to 1.0 p. p. m. of dissolved oxygen, while each vertical division is 1 hour. Curve *B* shows how the dissolved oxygen in the same apparatus is affected by lowering the oxygen in the diffusing gas from 25 per cent (air) to 5 per cent (air and nitrogen), so that it is equivalent to 20 ml. per liter per minute (1 cubic foot of air per gallon per 6 hours). Curve *C* shows how the dissolved oxygen increases in a shallow aeration tank, 30 × 30 × 60 cm. (1 × 1 × 2 feet), with spiral flow using a small amount of air for agitation. It was not possible to measure the air used in the tank, but it was adjusted visually to the minimum needed to keep the sludge in motion. The results in the aeration tank (curve *C*) with a slow rise in dissolved oxygen are more comparable to plant conditions than the results in curve *A* with the tall glass tube with the rapid rise in dissolved oxygen. Since much laboratory work upon activated sludge has been done with efficient aeration vessels, it is small wonder that the results have not been transferable to the less efficient aeration tanks of sewage plants. The important effect of the dissolved oxygen level upon nitrification has been shown (*2*) and any study which includes rates of carbon oxidation or nitrification should include the dissolved oxygen level at which the study was conducted. The use of diluted air (5 per cent oxygen gas of curve *B*) gives an approximation of plant condition in the laboratory in small, efficient, more readily controlled apparatus which does not use too much sample or space.

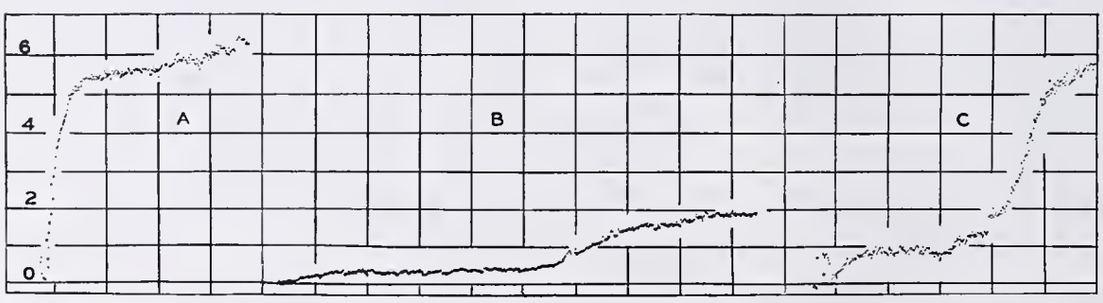

FIGURE 2. DISSOLVED OXYGEN CURVES OBTAINED IN LABORATORY UNITS WITH ACTIVATED SLUDGE PLUS SEWAGE

A. 1 liter of sewage-sludge mixture in cylinder agitated with air
B. In same cylinder agitated with 5 per cent O_2 gas
C. In tank 1 × 1 × 2 feet, agitated with minimum amount of air

The glass tubes and 5 per cent oxygen gas as shown in curve *B* were used to study the effect upon the dissolved oxygen of the addition of different quantities of food to activated sludge. Return activated sludge was brought into the laboratory and diluted with balanced mineral dilution water, described previously (*3*), to 1500 p. p. m. of suspended solids; 100 p. p. m. of sodium propionate were added to the sludge mixture.

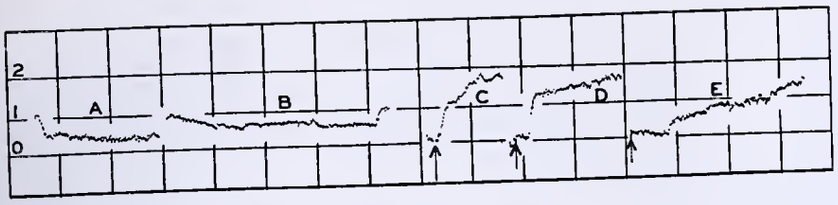

FIGURE 3. DISSOLVED OXYGEN CURVES

Different amounts of food fed to activated sludge.
A. 100 p. p m. sodium propionate on 1st dose
B. 200 p. p. m. sodium propionate for 2nd dose
C. 10 p. p. m. sodium propionate after 3 doses
D. 30 p. p. m. sodium propionate
E. 100 p. p. m. sodium propionate

The solution was agitated with nitrogen till the instrument showed no further drop, indicating a zero dissolved oxygen and the 5 per cent oxygen gas was then used, producing curve *A* (Figure 3). Between curves *A* and *B*, 200 p. p. m. more of sodium propionate were added to the activated sludge, but the dissolved oxygen levels in *A* and *B* indicate that the food in sodium propionate is not sufficiently available to the sludge organisms to use all the available oxygen at first and that increasing the quantity of food does not increase the quantity of oxygen required. The nitrogen necessary for the utilization of sodium propionate was supplied by the addition of urea to yield a carbon-nitrogen ratio of 1 to 5. The sludge was aerated overnight with more sodium propionate and then settled. The supernatant was withdrawn and more mineral dilution water added. Curve *E* was obtained by the addition of 100 p. p. m. of sodium propionate to the conditioned sludge and indicates by the absence of dissolved oxygen how much higher the oxygen demand is over a shorter period of time. Curves *C* and *D* were obtained with 10 and 30 p. p. m. of sodium propionate additions, respectively, and indicate that increasing the amount of food increases the period of high oxygen demand.

Studies of the effect of feeding different amounts of food to activated sludge with methods to indicate either oxygen consumption or carbon dioxide production produce curves the reverse of those shown. The effects of different types of laboratory equipment upon dissolved oxygen level are best studied with the dropping mercury electrode which measures the dissolved oxygen directly.

An opportunity presented itself at an activated sludge plant where the air supply was temporarily impeded, resulting in deterioration of sludge and a turbid effluent, to test changes in the liquor under these conditions. The electrodes were placed in the aeration tank within 90 cm. (3 feet) of the outfall weir and several curves were obtained, of which the lowest curve in Figure 4 is typical. There was no dissolved oxygen present in the liquor during the 24-hour period. Spot tests in other parts of the aeration tank indicated that dissolved oxygen was absent.

In order to show positive variations in the dissolved oxygen of aeration tanks, tests were made at the activated sludge plant at Bernardsville, N. J. (*1*). The electrodes were placed in the first of eight compartments of the aeration tank and the center curve of Figure 4 was obtained. The onset of the rise in dissolved oxygen agrees with the shift from day to night flow. The sudden drop and subsequent rapid rise in the dissolved oxygen at 8:30 A.M. are due to the use of one-third of the air supply to pump sludge from the primary settling tank to the digester for a 15-minute period. The curve indicates that operation might be altered somewhat, without greatly increasing the cost, by increasing the flow of air during the day and decreasing it at night, if the operator has a positive indication of the actual conditions in the aeration tanks and could follow immediately and continuously the effect of any changes in operation. During the dissolved oxygen rise in the curve, a wider band of dots is shown, indicating that the liquid is more variable. When the electrodes are placed nearer the diffusers, the variability in the current flow increases, owing to the surging of some better or poorer aerated liquid past the electrodes.

When the electrodes are placed in the final compartment of the aeration tank, the variations in dissolved oxygen shown in the top curve of Figure 4 are obtained. The curve shows approximately 2 parts per million dissolved oxygen throughout the day, and a rise in the dissolved oxygen with the lower sewage flow during the night. During the strong day flow, there is a rise of 2 parts per million in the dissolved oxygen through the aeration tank as purification progresses and oxygen demand decreases. Because there is dissolved oxygen present during the day in the final compartment of the aeration tank, there is less variation in the dissolved oxygen between the day and night flow than between the day and night flow in the first compartment.

Summary

The importance of maintaining dissolved oxygen in the activated sludge process of sewage treatment is well known.

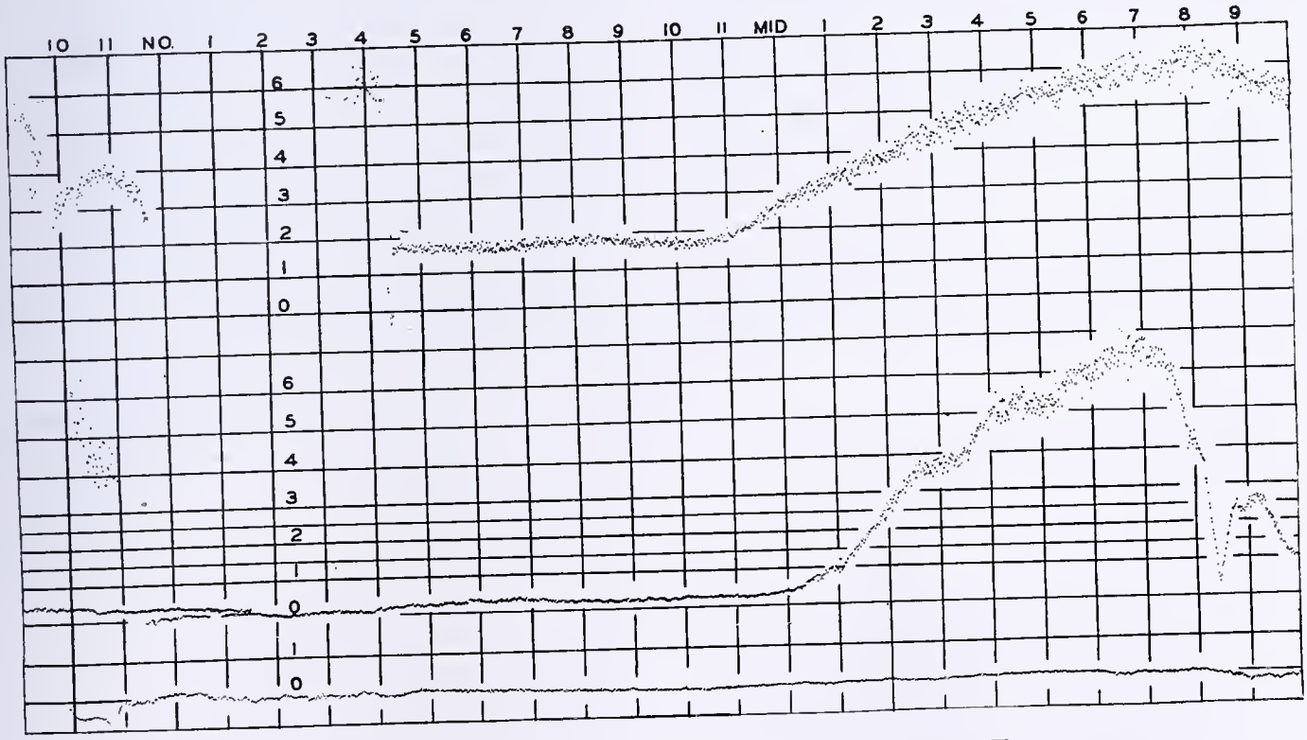

FIGURE 4. DISSOLVED OXYGEN CURVES OBTAINED IN AERATION TANK

Top. In final compartment of aeration tank at Bernardsville
Middle. In first compartment of tank at Bernardsville
Bottom. In aeration tank at plant with temporary inadequate blower capacity

An instrument to record the dissolved oxygen level continuously is briefly described and some results of laboratory experiments and activated sludge–sewage plant operation studies are given. The variations in the dissolved oxygen produce changes in the current flow which are amplified and recorded upon a thread galvanometer for a 24-hour period. The curves showing the dissolved oxygen in activated sludge–sewage mixtures in the laboratory under different conditions demonstrate that the shape of the vessel can largely determine the efficiency of the gas used for aeration. The effect upon the dissolved oxygen of the addition of different quantities of the same food to activated sludge indicates that when more food is added there is no effect upon the dissolved oxygen unless or until the enzyme capacity is large enough to handle the increased load. The electrodes were placed in the aeration tank of an activated sludge–sewage treatment plant, and the dissolved oxygen levels at several points in the tank recorded continuously for 24-hour intervals. The first section of an aeration tank showed no dissolved oxygen during the day period while the dissolved oxygen increased during the night. There was a relatively large amount of dissolved oxygen in the final section of the aeration tank with only slight variations over the 24-hour period.

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Presented before the Division of Water, Sewage, and Sanitation Chemistry at the 102nd Meeting of the American Chemical Society, Atlantic City, N. J. Journal series paper of the New Jersey Agricultural Experiment Station, Rutgers University, Department of Water and Sewage Research.

A Simplified Photometer for Determining Nitrogen Dioxide Concentrations

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The concentration of nitrogen dioxide in gaseous mixtures is conveniently determined with a simple photometer. The installation and calibration of the system are described here. The photometric system permits measurement of the partial pressures of nitrogen dioxide to within 0.05 mm.

THE authors have developed a simple photometric method for the determination of the partial pressure of nitrogen dioxide in gaseous mixtures. A means of determining the concentration of nitrogen dioxide was required for the study of its reactions with other gases (*4*). Since the other gases used do not absorb radiation between 400 and 700 mμ and nitrogen dioxide absorbs strongly in this region of the spectrum, a photometric method was ideally suited for the solution of the problem.

The photometric technique of determining concentrations of gases is well developed (*5*), and its application to the determination of the concentration of nitrogen dioxide has been described. Dixon (*3*) has made a study of the absorption of nitrogen dioxide in the visible spectrum with a General Electric recording spectrophotometer. Narrow spectral regions (15 and 40 Å. wide) were isolated with the "built-in" monochromator. Willey and Foord (*6*) used a filtered incandescent source and measured the intensity of light transmitted with a potassium photocell and an amplifying system.

The system described here has the advantage of being more easily adaptable to problems involving the determination of nitrogen dioxide concentrations than those heretofore described. The necessary parts are readily available, inexpensive, and easy to install. The reproducibility of the measurements exceeds that previously reported and recalibration of the photometer is unnecessary if an accuracy of 0.2 mm. in the partial pressure of nitrogen dioxide is adequate. A calibration of the system should be made, however, if determinations of ±0.05 mm. in the partial pressure of nitrogen dioxide are required.

Since nitrogen dioxide absorbs radiation throughout the visible spectrum, it is possible to use wide continuous bands of radiation for the measurements without the usual loss in sensitivity attendant on such a procedure. The increased intensity thus available permits the use of less refined measuring instruments than are ordinarily required. Thus, an incandescent source is used, bands about 1000 Å. wide in the blue and green are isolated by filters, and the intensity of the transmitted light is measured directly by the e. m. f. developed by a photoelectric cell (Weston Photronic cell, Type 1).

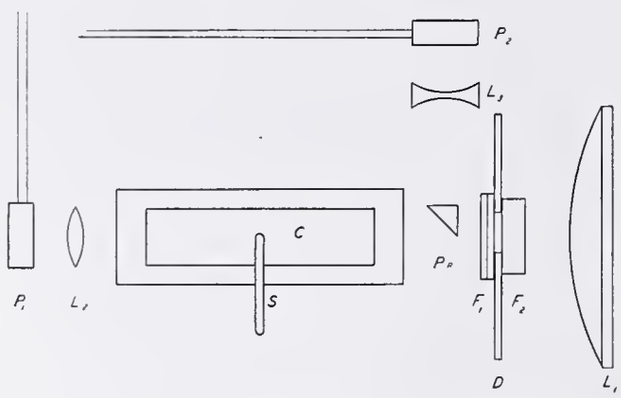
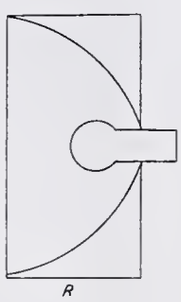


Figure 1. Experimental Arrangement



Experimental Work

The experimental arrangement is shown in Figure 1.

Illuminating System. The source of illumination is a 50-candlepower single-filament automobile headlight bulb operated at 6.0 amperes (5.6 volts) with a steady current supplied by storage batteries of large capacity. The optical system consists of a parabolic, chromium-plated reflector, *R* (diameter 11 cm.), housing the

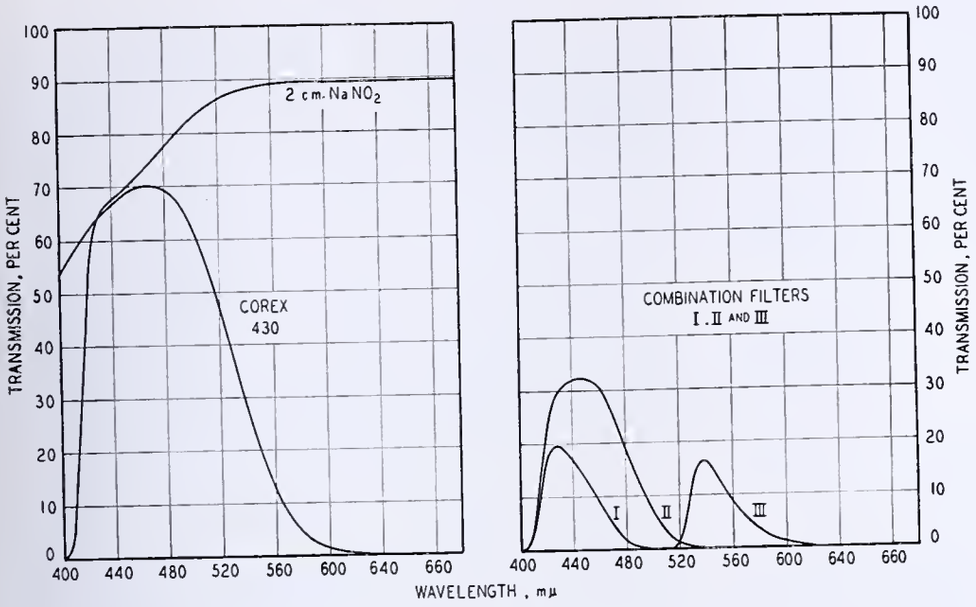


FIGURE 2. TRANSMISSION OF FILTERS

lamp, an inexpensive plano-convex lens, L_1 (focal length 25 cm., diameter 11 cm.), placed 1.4 meters in front of the reflector, and a diaphragm with a 2-cm. aperture, D, placed 3 cm. in front of the lens. This optical arrangement produces a beam which does not diverge more than 3 cm. for a distance of 40 cm. beyond the diaphragm.

FILTERS. There are three filters, each composed of three components, all held at F_1 and F_2 by the diaphragm which has been designed to hold the filters and make their interchange simple. Two components are common to all three filters: a cell 2-cm. long containing a saturated aqueous solution of sodium nitrite, and a Corning Corex filter No. 430.

The three compound filters used are:

I. Corex filters, Nos. 585 and 430, and 2 cm. of $NaNO_2$ (saturated solution)
II. Corex filters, Nos. 554 and 430, and 2 cm. of $NaNO_2$ (saturated solution)
III. Corex filters, Nos. 351 and 430, and 2 cm. of $NaNO_2$ (saturated solution)

A set of filters transmitting sharp bands covering the visible spectrum has been listed by Clifford and Brice (*2*). A set of filters could be chosen from this list which would give transmission bands similar and sharper, but which have a smaller per cent transmission and narrower bands than the ones used by the authors. These factors would reduce the response, and since the calibrations had been completed on the above set of filters before the publication of the set by Clifford and Brice, no measurements have been made with their filters.

It is only necessary to interchange the single filters Nos. 585, 554, and 351 to obtain any one of the three compound filters. The transmission of the filters is shown in Figure 2. Since the incandescent source gives much more radiation in the green than in the violet, bright brass screens are used with filters II and III to produce approximately equal e. m. f. for each of the three filter combinations.

MEASUREMENT OF LIGHT INTENSITY. The beam, having passed through the diaphragm aperture and filters, is split by a right-angle prism, P_R, into two beams which are adjusted to give approximately the same e. m. f. (1.5 millivolts) from the photronic cells, P_1 and P_2, when the absorption cell, C, is empty. The e. m. f. response of the photronic cells is measured on a Type K Leeds & Northrup potentiometer to the nearest microvolt. The absorption cell has parallel windows 22.3 cm. apart.

Certain precautions are necessary when photronic cells are used for quantitative measurements. Here the response of the cells was calibrated with screens and found to be linear for the low intensity of radiation used (about 0.1 to 0.2 lumen). It was found advisable to illuminate the cells for 5 to 10 minutes before recording the e. m. f. to allow for the large decay in the response of the cells. In addition, the beam of light was diffused over the whole face of the photronic cells by lenses L_1 and L_2, but the more sensitive outer edges of the cells, at which the copper contacts are exposed, were covered with opaque rings. The photronic cells were housed in aluminum boxes to prevent any sudden temperature changes.

The photronic cell, P_2, is used as a check both of any variation in the intensity of the incident beam and the decay and fluctuation of response, for the decays in the two cells are similar. Measurements of the ratio $\frac{\text{e. m. f. } P_1}{\text{e. m. f. } P_2}$ with the absorption cell empty are constant within 1 per cent.

CALIBRATION OF PHOTOMETER. Nitrogen dioxide which had been prepared by the decomposition of lead nitrate and stored with an excess of oxygen was dried by passing it slowly over phosphorus pentoxide. The residual oxygen was pumped off the nitrogen dioxide, maintained at −78° C. The nitrogen dioxide was charged into the quartz absorption cell, C, through an all-Pyrex system with glass valves; so that at no time did the nitrogen dioxide come in contact with any stopcock grease or mercury. The cell itself was surrounded by a lagged copper sheath.

An excess of nitrogen dioxide was sublimed into the cell (a small amount of liquid remained in the side arm at room temperature), and the cell was sealed off just above the side arm of the cell. The total pressure (of the nitrogen dioxide plus nitrogen tetroxide) in the cell was varied by controlling the temperature of the liquid or solid phase in the side arm. The pressures were measured with a mercury manometer and Société Génevoise cathetometer, pressure balance being indicated by a quartz spiral between the absorption cell and the mercury manometer. The partial pressure of the nitrogen dioxide was calculated from the measurements of total pressure and from the equation of Bodenstein (*1*) for the equilibrium $N_2O_4 \rightleftharpoons 2NO_2$. The cell temperature was recorded to 0.1° C.

The transmission of light at each nitrogen dioxide pressure was calculated from four measurements:

E_{1f}, e. m. f. of photocell P_1 when the absorption cell contains nitrogen dioxide.

E_{1e}, e. m. f. of photocell P_1 when the absorption cell contains no nitrogen dioxide, all the nitrogen dioxide having been frozen in the side arm to −195° C.

E_{2f}, e. m. f. of photocell P_2, measured just before and just after E_{1f} (and averaged).

E_{2e}, e. m. f. of photocell P_2, measured just before and just after E_{1e}.

Transmission $I/I_0 = E_{1f}/E_{1e} \times E_{2e}/E_{2f}$

Results

The transmissions at 23° C. for different partial pressures of nitrogen dioxide, for a path length of 22.3 cm., are given for each of the compound filters. The calibration is not af-

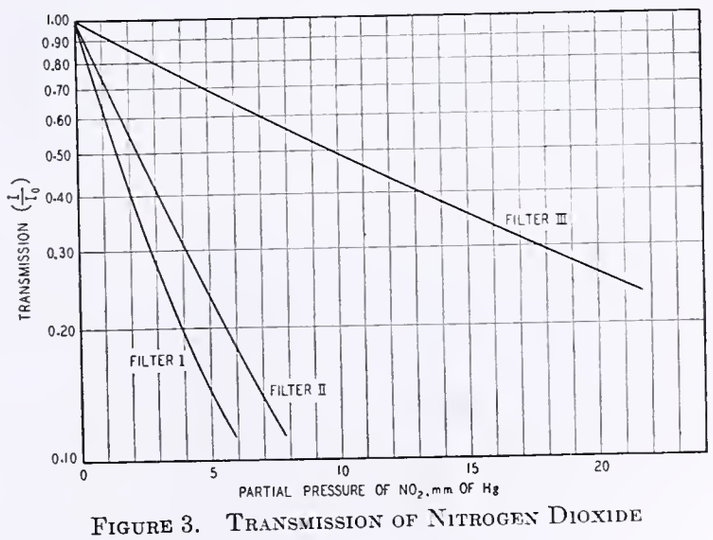


FIGURE 3. TRANSMISSION OF NITROGEN DIOXIDE

fected by temperature changes of ±3° C. for pressures of nitrogen dioxide below 10 mm.

It will be observed that the logarithm of the transmission is not a linear function of the partial pressure of nitrogen dioxide. The linear relation is to be expected only for strictly monochromatic radiation or for the case where the absorbing species has an absorption coefficient that is constant over the whole spectral band used for analysis. Dixon (*3*) has shown that the absorption coefficient of nitrogen dioxide changes rapidly with wave length, so that the nonlinearity of this function is to be anticipated.

The limiting slopes (at high transmission values) of the curves of Figure 3 lead to the following limiting absorption coefficients, k:

Filter	k
I	0.0089
II	0.0057
III	0.0014

k is calculated from the relation $k = -\frac{\log_{10} I/I_0}{p} \times \frac{1}{l}$, where p is the partial pressure of nitrogen dioxide (reduced to 0° C.) in millimeters of mercury, and l = 22.3 cm., the cell length.

The authors have used the procedure described to follow the nitrogen dioxide partial pressure in various gas mixtures capable of reaction. It is applicable to other systems for determining the partial pressure of nitrogen dioxide. An accuracy of ±0.05 mm. in the measurement of the partial pressure of nitrogen dioxide can be obtained where the filters are used under the following conditions:

Filter	Product of p_{NO_2} (Mm. Hg) × l (Cell Length, Cm.)
I	1 to 90
II	45 to 130
III	110 to 220[a]

[a] Accuracy with filter III is ±0.1 mm. in the measurement of the partial pressure of NO_2 for $p \times l$ products between 220 and 450.

To attain this accuracy, however, it is advisable to calibrate the cell and filters because of the possible variation in the transmission of the commercial filters.

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Contribution No. 475 from the Research Laboratory of Physical Chemistry, Massachusetts Institute of Technology.

A Cutter for Spectroscopic Electrodes

E. S. HODGE, Kentucky Agricultural Experiment Station, Lexington, Ky.

MANY workers using carbon electrodes for spectrographic analysis employ a small crater to hold either a dry solid sample or a small liquid volume to be evaporated. Two devices for cutting such craters, described previously (*1*, *2*), require either a lathe of special design or a drill press capable of turning the tool and holding the carbon in line in a chuck. The tool described here is simpler than either of these, both in manufacture and in use. Its use requires neither a lathe nor a drill press with a stationary chuck whose axes must be kept aligned.

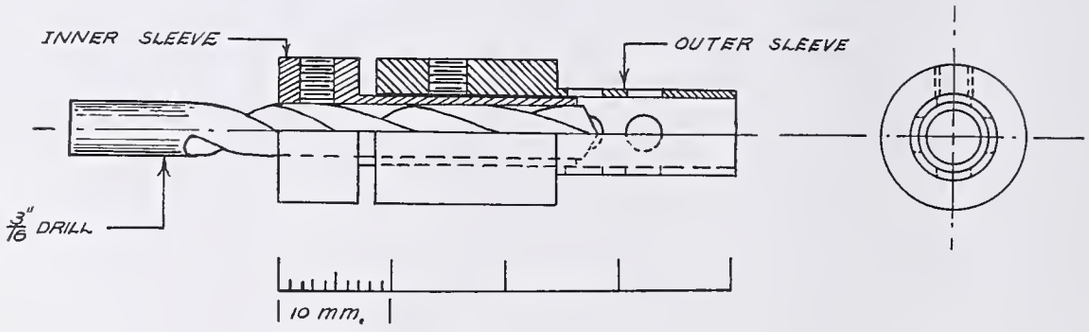
The accompanying figure shows an inner sleeve which is fastened by a setscrew to an ordinary twist drill of the size of the crater to be made. The depth of the crater is regulated by the position of the sleeve along the drill. An outer sleeve for guiding and centering the electrode is fastened to the inner one. The inside diameter of this outside sleeve is the same as the diameter of the rods to be drilled.

In use the inner sleeve is fastened to the drill to give the desired crater depth, then the guide sleeve is attached by means of a setscrew. The drill is placed in the headstock of a lathe or the chuck of a drill press or even a chuck of a polishing wheel shaft. A desirable speed is about 1200 r. p. m. The electrodes to be cratered should be cut with a fine-toothed saw (a coping saw is excellent) in a right-angle miter guide, so that the ends are smooth and perpendicular to the axis of the rod. Any roughness should be taken off with emery paper. The uniformity of depth in the crater will be determined by the flatness and the right-angle surface of the electrode. A carbon rod is drilled by slowly pushing it into the rotating tool until the maximum depth is cut.

In preparing craters on the spectroscopic carbons and special graphite electrodes a sharp drill holds its edge very well. However, because of the hardness of the special carbon spectroscopic electrodes the drills become dull and it is desirable to have several well-sharpened drills of the desired size at hand. Drills should be replaced after 20 to 30 cuttings, or when the crater walls begin to break. It is important to use a sharp drill and not to attempt to turn down the crater wall.

This device has been found especially satisfactory since it does not require that two axes be kept aligned. By using drills only for this purpose a regular shop drill press can be used without fear of contamination of the electrodes, even those of the highest purity. While similar devices are known to have been used for some time by Cholak, Mankovich, and others, this is presented as a very satisfactory means of cutting the harder special spectroscopic carbon electrodes.

The writer wishes to thank Fred Mangelson of the College of Engineering, University of Kentucky, for technical assistance.



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The investigation reported here is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the director.

Visual Fluid Flowmeters with Straight-Walled Tubes

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Several variations of a visual fluid flowmeter are described and calibration data for fluids of varying density and viscosity are given. A comparison of the data with those of the rotameter is made, based on the Schoenborn-Colburn equations. The meters are easy to construct and inexpensive, as a tapered glass tube is not required. Accuracies of 1 to 3 per cent of the volume of fluid flowing were observed.

SEVERAL papers have been published on the visual fluid flowmeter, usually referred to as the "rotameter". Stout and Rowe (*3*) described a meter which operated on similar principles and which involved a straight-walled glass tube and a central cone in its construction. This paper describes several variations of a meter of the visual flow type which has been developed in this laboratory. Calibration data for fluids of varying viscosity and density are included and a comparison with the rotameter, based on the equations of Schoenborn and Colburn (*2*), is given. The meters are easy to construct and inexpensive, as a tapered tube is not required.

Apparatus

TYPE P. In constructing the meter, shown diagrammatically in Figure 1, two parallel-sided slots were cut along the 180° axes of a piece of standard 0.75-inch brass pipe, after which the pipe was reamed to a uniform internal diameter. Above, but connected with the slots, a number of large holes were cut through the pipe walls to allow the fluid to return to the pipe after passing around a float. It was found that unless the area of these holes was made many times the entire area of the slots, the upper readings would be useless. A rubber stopper was fitted snugly around the pipe above these holes and a second stopper placed below the bottom of the slots. The two stoppers held a glass tube 2 inches in internal diameter in place to confine the flow, through which the position of the float could be noted with reference to a scale inscribed on the slotted brass pipe. Four tie rods, not shown on the diagram, were used to hold the stoppers securely in place. Two types of floats were used: A simple sphere of glass or molybdenum of slightly smaller diameter than the interior of the slotted pipe, and an aluminum cylinder modified as shown in Figure 2, held in place by a central guide rod.

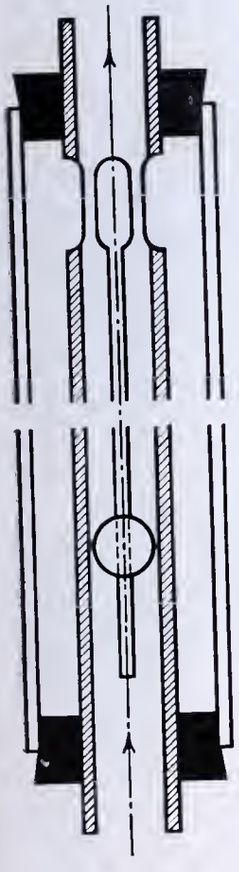

FIGURE 1. DIAGRAM OF TYPE P METER

The fluid rises in this instrument in the metal pipe until it meets the float, passes out through the slots into the annular space between the pipe and the glass jacket, and reenters the metal pipe above the float, both through the slots and through the large holes provided for the purpose. The whole may be said to act like a variable-sized orifice as set forth by Schoenborn and Colburn (*2*), the available orifice being the annular ring between the float and the interior surface of the metal pipe together with the area of the slots below the float. In the case of floats using a central guide rod, an additional small annular ring exists between the guide rod and the float. Since the kinetic energy of the flowing fluid must just balance the potential energy of the float, there must be an equilibrium position of the float for each rate of flow because of the increasing area provided by the slots at higher levels.

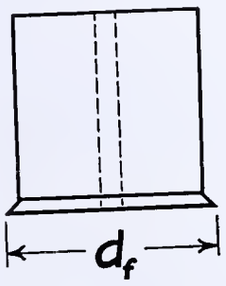

FIGURE 2. TYPE OF CYLINDRICAL FLOAT

TYPE V meters were made similar in all respects to the Type P meters, except that the slots in the metal tube were V-shaped, varying in width from zero at the bottom to 0.4375 inch at a height of 9.375 inches. This was done in order to provide a smaller available area for flow per unit of height at the lower readings and a relatively larger area per unit of height at the higher readings.

Experimental

Water was allowed to flow from a constant-head tank through the instrument to a weighing tank. A number of runs were made and the results averaged for each position of the float. For the runs on liquids other than water, the fluid was pumped at constant rate from a broad shallow tank through the instrument to the weighing tank.

The results of these tests are presented in tabular form in Tables I to IV. A typical calibration curve of rate of flow for each meter reading is shown in Figure 3.

Correlation of Data

Let us consider the parallel-slot meter designated as Type P. We might think of the slot as a weir, the height of the float being a measure of the head of fluid above the bottom of the weir. The construction of the meter violates all the re-

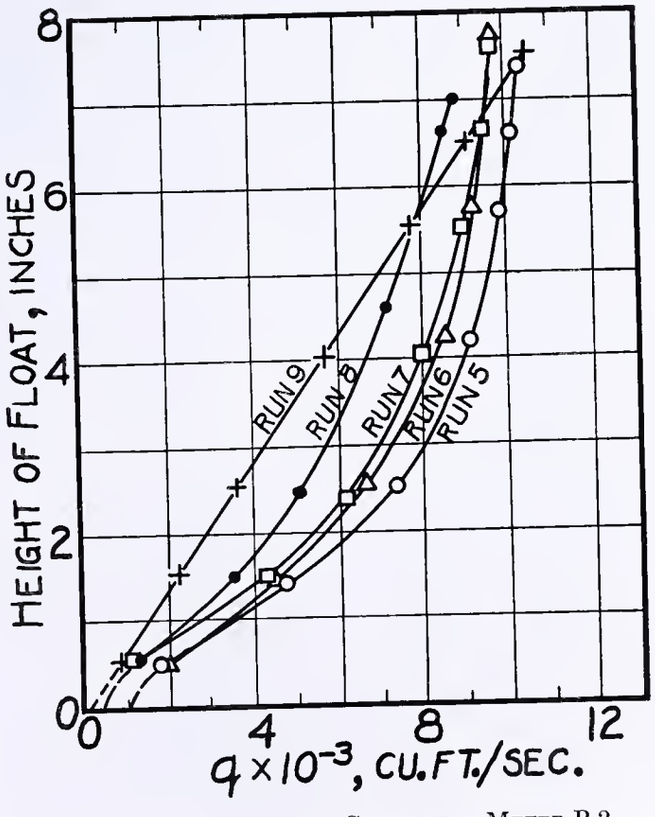

FIGURE 3. CALIBRATION CURVES FOR METER P-2

Table I. Calibration Data

Meter P-1, Spherical Float
d_t = 0.8075 inch
w = 0.063 inch
d_f = 0.7615 inch
ρ_f = 151.7

x (Inches)	$\frac{S_0}{1000}$	$\frac{D}{100}$	$\frac{q}{1000}$	V	Re	C
Run 1, Water (ρ = 62.36, μ = 0.00074, H = 0.0604)						
0.67	0.65	0.1284	1.010	1.554	168	0.788
3.07	2.75	0.211	3.745	1.36	242	0.690
5.67	5.02	0.231	6.02	1.199	234	0.608
7.72	6.88	0.239	6.74	0.988	199	0.501
9.53	8.40	0.2425	7.52	0.896	183	0.454
12.05	10.60	0.247	7.54	0.711	148	0.360
15.28	13.43	0.250	8.03	0.598	126	0.303
Run 2, Water (ρ = 62.26, μ = 0.000614, H = 0.0606)						
0.71	0.683	0.1317	1.013	1.480	198	0.750
3.11	2.78	0.211	3.94	1.416	303	0.717
5.47	4.85	0.231	5.91	1.218	284	0.616
7.79	6.97	0.239	6.95	1.010	245	0.512
10.31	9.09	0.244	7.64	0.841	208	0.426
13.70	12.05	0.248	7.97	0.661	166	0.335
15.58	13.70	0.250	8.26	0.604	153	0.306
Run 3, Water (ρ = 62.10, μ = 0.000506, H = 0.0609)						
0.315	0.398	0.0967	0.664	1.668	198	0.843
2.60	2.34	0.203	3.33	1.422	355	0.719
5.32	4.72	0.230	5.91	1.250	353	0.632
8.27	7.30	0.240	7.17	0.983	290	0.497
10.82	9.54	0.246	7.60	0.797	240	0.403
11.92	10.50	0.247	7.76	0.738	223	0.373
15.23	13.40	0.250	8.11	0.605	186	0.306
Run 4 Water (ρ = 61.86, μ = 0.000414, H = 0.0613)						
0.827	0.788	0.141	1.176	1.492	314	0.753
2.875	2.58	0.208	3.58	1.390	431	0.701
5.67	5.02	0.231	6.12	1.218	420	0.614
8.35	7.37	0.240	7.46	1.012	363	0.510
9.92	8.75	0.246	7.49	0.856	312	0.432
12.68	11.16	0.248	7.84	0.703	260	0.354
15.40	13.54	0.250	8.20	0.606	226	0.305

Table II. Calibration Data

Meter P-2, Cylindrical Float
d_t = 0.8297 inch
w = 0.125 inch
d_h = 0.073 inch
d_r = 0.0682 inch
d_f = 0.8140 inch
ρ_f = 167.9
v_f = 0.3068 cu. in.
S_f = 0.516 sq. in.

x (Inches)	$\frac{S_0}{1000}$	$\frac{D}{100}$	$\frac{q}{1000}$	V	Re	C
Run 5, Water (ρ = 62.23, μ = 0.000631, H = 0.0843)						
0.50	1.011	0.638	1.770	1.750	1105	0.751
1.41	2.58	1.102	4.72	1.830	1990	0.785
2.53	4.52	1.385	7.35	1.627	2225	0.695
4.21	7.28	1.585	9.125	1.254	1970	0.538
5.70	10.05	1.697	9.84	0.979	1643	0.420
6.60	11.62	1.742	10.13	0.877	1500	0.374
7.37	12.96	1.770	10.37	0.800	1400	0.343
Run 6, Salt Solution (ρ = 70.6, μ = 0.00087, H = 0.0683)						
0.51	1.000	0.65	1.900	1.900	1003	0.905
1.47	2.68	1.122	4.50	1.680	1530	0.800
2.54	4.55	1.288	6.66	1.464	1530	0.697
4.24	7.50	1.595	8.51	1.135	1470	0.540
5.76	10.15	1.700	9.20	0.906	1250	0.431
7.76	13.65	1.785	9.70	0.711	1032	0.338
Run 7, Corn Sirup (ρ = 72.25, μ = 0.000925, H = 0.0657)						
0.56	1.100	0.690	1.704	1.550	835	0.753
1.50	2.75	1.135	4.275	1.555	1380	0.755
2.40	4.30	1.360	6.16	1.433	1520	0.696
4.05	7.18	1.580	7.99	1.113	1385	0.540
5.52	9.75	1.688	8.99	0.922	1215	0.448
6.64	11.70	1.745	9.45	0.807	1100	0.392
7.60	13.38	1.780	9.65	0.722	1004	0.351
Run 8, Corn Sirup (ρ = 77.85, μ = 0.00268, H = 0.0575)						
0.52	1.025	0.660	1.273	1.242	238	0.645
1.49	2.62	1.032	3.49	1.332	400	0.692
2.47	5.42	1.375	5.09	0.939	375	0.487
4.59	9.12	1.623	7.16	0.785	370	0.408
5.69	11.03	1.697	7.92	0.718	354	0.373
6.62	12.65	1.742	8.54	0.675	342	0.351
7.01	13.33	1.760	8.82	0.661	338	0.343
Run 9, Corn Sirup (ρ = 81.3, μ = 0.01235, H = 0.053)						
0.52	1.025	0.660	0.858	0.837	56.4	0.452
1.54	2.80	1.150	2.255	0.805	61.0	0.435
2.54	4.45	1.387	3.61	0.810	73.8	0.437
4.05	7.17	1.578	5.69	0.810	84.0	0.437
5.54	9.77	1.690	7.76	0.795	88.5	0.429
6.50	11.45	1.737	9.10	0.795	90.8	0.429
7.54	13.26	1.777	10.54	0.795	92.8	0.429

Table III. Calibration Data

Meter V-1, Spherical Float
d_t = 0.8125 inch
Max. x = 9.375 inches
Max. w = 0.4375 inch
d_f = 0.7418 inch
ρ_f = 151.5

x (Inches)	$\frac{S_0}{1000}$	$\frac{D}{100}$	$\frac{q}{1000}$	V	Re	C
Run 10, Water (ρ = 62.4, μ = 0.000815, H = 0.0603)						
0.6	0.717	0.474	1.34	1.869	677	0.946
1.2	1.067	0.535	1.81	1.695	694	0.859
2.4	2.47	0.832	3.38	1.370	872	0.695
3.6	4.80	1.216	6.32	1.317	1225	0.667
4.8	8.07	1.640	7.92	0.981	1155	0.497
6.0	12.29	2.08	8.67	0.706	1125	0.358
7.2	17.42	2.53	9.18	0.527	1021	0.267
8.4	23.5	2.99	9.67	0.411	941	0.208

Meter V-2, Spherical Float
d_t = 0.8125 inch
Max. x = 9.375 inches
Max. w = 0.4375 inch
d_f = 0.7690 inch
ρ_f = 151.5

Run 11, Water (ρ = 62.4, μ = 0.000815, H = 0.0611)						
0.6	0.492	0.310	1.01	2.05	486	1.030
1.2	0.842	0.415	1.41	1.675	532	0.843
2.4	2.25	0.745	3.12	1.388	790	0.698
3.6	4.58	1.141	5.65	1.233	1078	0.620
4.8	7.85	1.570	8.22	1.047	1259	0.527
6.0	12.07	2.02	8.97	0.743	1150	0.374
7.2	17.20	2.47	9.52	0.553	1045	0.278
8.4	23.3	2.94	10.04	0.431	969	0.217

Meter V-3, Spherical Float
d_t = 0.8125 inch
Max. x = 9.375 inches
Max. w = 0.4375 inch
d_f = 0.7500 inch
ρ_f = 636

Run 12, Water (ρ = 62.4, μ = 0.000815, H = 0.383)						
0.6	0.650	0.427	3.30	5.08	1660	1.020
1.2	1.000	0.495	4.21	4.21	1590	0.846
1.8	1.583	0.629	5.98	3.78	1820	0.760
2.4	2.40	0.795	8.28	3.45	2095	0.694
3.0	3.45	0.980	10.95	3.17	2380	0.637

Table IV. Calibration Data

Meter V-4, Cylindrical Float
d_t = 0.816 inch
Max. x = 9.375 inches
Max. w = 0.4375 inch
d_h = 0.073 inch
d_r = 0.0682 inch
d_f = 0.780 inch
ρ_f = 168.7
v_f = 0.314 cu. in.
S_f = 0.478 sq. in.

x (Inches)	$\frac{S_0}{1000}$	$\frac{D}{100}$	$\frac{q}{1000}$	V	Re	C
Run 13, Water (ρ = 62.3, μ = 0.000678, H = 0.0935)						
1	0.652	0.335	1.35	2.07	637	0.845
2	1.619	0.585	3.12	1.93	1004	0.787
3	3.24	0.905	5.25	1.62	1350	0.661
4	5.50	1.253	7.81	1.42	1635	0.579
5	8.43	1.620	9.83	1.166	1735	0.475
6	11.97	1.990	10.80	0.902	1650	0.368
7	16.19	2.37	11.52	0.712	1555	0.291
8	21.1	2.76	11.81	0.560	1420	0.229
9	26.6	3.14	12.05	0.453	1310	0.185
Run 14, Salt Solution (ρ = 68.05, μ = 0.000968, H = 0.0810)						
1	0.652	0.335	1.07	1.64	386	0.719
2	1.619	0.585	2.55	1.576	647	0.690
3	3.24	0.905	4.65	1.435	913	0.630
4	5.50	1.253	6.58	1.198	1054	0.525
5	8.43	1.620	8.33	0.988	1125	0.433
6	11.97	1.990	9.56	0.799	1118	0.351
7	16.19	2.37	10.38	0.641	1068	0.281
8	21.1	2.76	10.94	0.518	1005	0.227
9	26.6	3.14	11.28	0.424	934	0.186
Run 15, Salt Solution, (ρ = 70.9, μ = 0.001345, H = 0.0756)						
1	0.652	0.335	1.20	1.84	325	0.836
2	1.619	0.585	2.45	1.515	467	0.689
3	3.24	0.905	4.65	1.435	685	0.652
4	5.50	1.253	7.52	1.367	903	0.621
5	8.43	1.620	9.13	1.084	925	0.493
6	11.97	1.990	9.82	0.820	859	0.372
7	16.19	2.37	10.32	0.638	794	0.290
8	21.1	2.76	10.75	0.509	740	0.231
9	26.6	3.14	11.03	0.415	638	0.189
Run 16, Corn Sirup (ρ = 78.0, μ = 0.00370, H = 0.0636)						
1	0.652	0.335	0.60	0.920	69	0.455
2	1.619	0.585	1.45	0.895	111	0.443
3	3.24	0.905	2.90	0.895	171	0.443
4	5.50	1.253	4.80	0.873	231	0.432
5	8.43	1.620	7.15	0.848	290	0.419
6	11.97	1.990	10.15	0.847	355	0.419
7	16.19	2.37	13.70	0.846	424	0.418
8	21.1	2.76	18.0	0.852	496	0.421
9	26.6	3.14	22.7	0.853	565	0.422

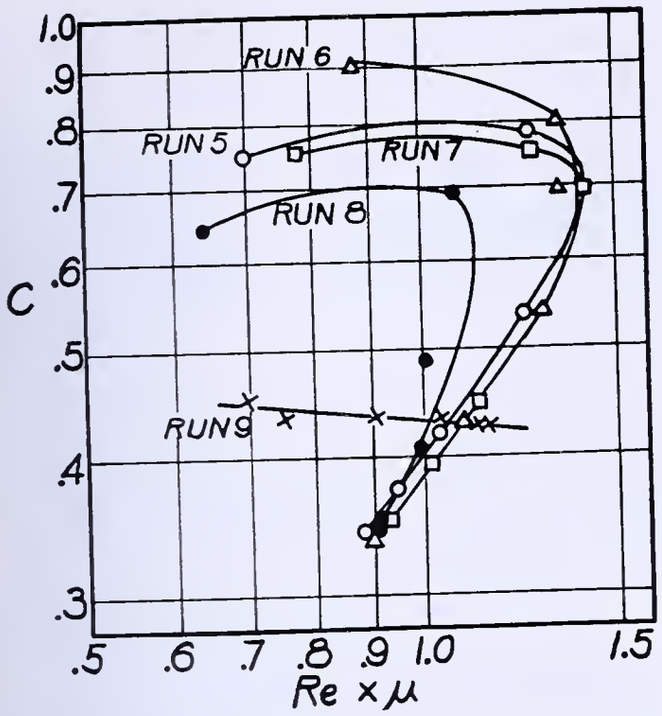

FIGURE 4. PLOT OF C *vs.* $Re \times \mu$ FOR METER P-2

quirements for even a poor weir and the meter should therefore give very unreliable results. Furthermore, a plot of log q *vs.* log x does not even approximate a straight line. This conception was therefore rejected.

The work of Schoenborn and Colburn (*2*) in which the rotameter was considered as a variable size orifice, offers a convenient method of correlation as well as a means of comparison of these meters with the rotameter. The equation developed by them

$$C = \frac{V}{(2gH)^{1/2}} = \frac{q}{S_0\left[\frac{2gv_f(\rho_f - \rho)}{S_f\rho}\right]^{1/2}} \qquad (1)$$

was used. The constant, C, involves the properties of the instrument and when plotted against some characteristic of the flowing fluid, such as Reynolds number, gives a smooth curve. Schoenborn and Colburn found that a nearly universal curve could be drawn from data on various rotameters. Since the flow rate appears in both ordinate and abscissa, they plotted C *vs.* Re/C to eliminate this undesirable feature.

Such a plot for the present meters showed a series of curves, very similar in shape but spaced at different values of Re. Several of the runs are plotted in Figure 4 as C *vs.* $Re \times \mu$, the abscissa having been modified in order to bring the curves together, so that several curves could be shown in one illustration. At very high viscosity C was nearly constant.

Several definitions are necessary in order to calculate the quantities involved. The available area for flow is considered as the total area of both slots and the annular areas within the pipe. For Type P meter with spherical floats this becomes

$$S_0 = 2wx + \frac{\pi}{4}(d_t - d_f)^2 \qquad (2)$$

In the case of the cylindrical floats, the additional area $\pi/4 \times (d_h - d_r)^2$ must be added to the right-hand side of Equation 2. The wetted perimeter, p_w, is considered as the entire series of lines outlining S_0. For the spherical float this becomes

$$p_w = 4x + \pi(d_t + d_f) \qquad (3)$$

and for the cylindrical float

$$p_w = 4x + \pi(d_t + d_f + d_h + d_r) \qquad (4)$$

The equivalent diameter, D, used in the Reynolds number then becomes

$$D = \frac{4S_0}{p_w} \qquad (5)$$

In the case of the Type V meter, the angle is so small that the sides of the V-shaped slot were considered equal to the reading. The 2 wx term in Equation 2 must be replaced by wx for this type of slot and twice the width of the slot at reading x must be subtracted from the right-hand side of Equations 3 and 4.

Discussion

In all cases the meters were accurate to 3 per cent of the quantity flowing and those in which the clearance between float and tube was smallest were accurate within 1 per cent. The ranges of the meters were from a minimum flow of 150 to about 2400 pounds per hour of water. The range can be varied by varying the dimensions of the slots, the tube, and the float as well as the material of the float. The clearance between the metal tube and glass jacket must be at least 0.5 inch for a 0.75-inch pipe with slots up to 0.125 inch. For wider slots, the clearance should be greater. The minimum flow rate is determined by the clearance between metal float and pipe. The cylindrical float was tried with the object of eliminating the effect of viscosity as set forth by Fisher *et al.* (*1*). No such result was shown, the constant, C, varying as much as with the spherical float. Although only two slots were tried, a greater number could be distributed on corresponding axes around the tube.

These meters, operating on the principle of variable available area for flow, as does the rotameter, are easy to construct and are inexpensive. One Type P installation in which two floats of molybdenum were used simultaneously in a 0.75-inch tube to measure the cooling water in a commercial heat transfer investigation gave satisfactory service for several months and was doing well when the investigation was ended. The meters may be made of various metals to meet existing corrosion conditions.

The curves of Figure 4 serve as a measure of comparison of this meter with the rotameter. While no universal curve was obtained, a single type of hooked curve resulted in all but the two cases of very high viscosity. Two values of C were obtained for each value of Re, the higher value of C being applicable at low meter readings and the lower values of C corresponding to the higher meter readings. The higher values of C at the low meter readings are not deemed reliable.

A logarithmic plot of C *vs.* $DV\rho$ for the upper three fourths of the readings for any single meter operating on liquids of ordinary viscosity (0.0006 to 0.0025 pound per foot second) can be drawn as a straight line from which the experimental points for that particular meter will vary but little.

Nomenclature

C = meter coefficient
d_f = diameter of float
d_t = diameter of tube
d_h = diameter of hole through float
d_r = diameter of guide rod
D = equivalent diameter of flow area
g = acceleration of gravity
H = difference in fluid head
p_w = wetted perimeter
q = quantity of flow, volume
Re = Reynolds number
S_0 = area available for flow
S_f = area of float at largest diameter
V = velocity of flow at constriction
v_f = volume of float
w = width of slot
x = meter reading; height of float above bottom of slots

ρ = density of fluid
ρ_f = density of float
μ = viscosity

All values are in terms of feet, pounds, and seconds unless otherwise designated in the text.

Acknowledgment

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A Support for Reflux Condensers

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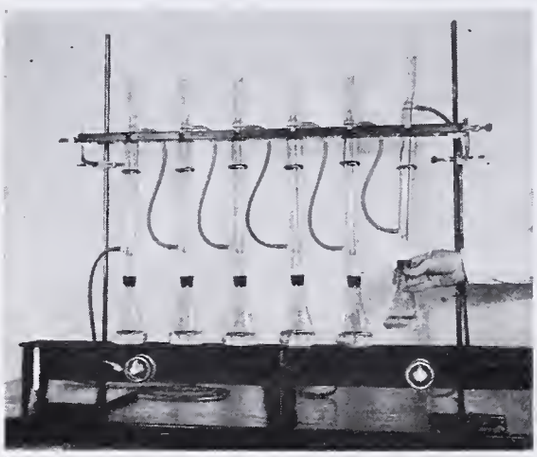
FOR extraction procedures and other operations involving refluxing, it is frequently necessary to arrange a battery of condensers in such a manner that the attached flasks can be rotated individually in order to mix their contents. Since the condensers must usually be clamped rigidly to ensure their safety, it is often difficult to obtain sufficient elasticity in the mounting to permit vigorous rotation of the attached flasks without having to loosen clamps.

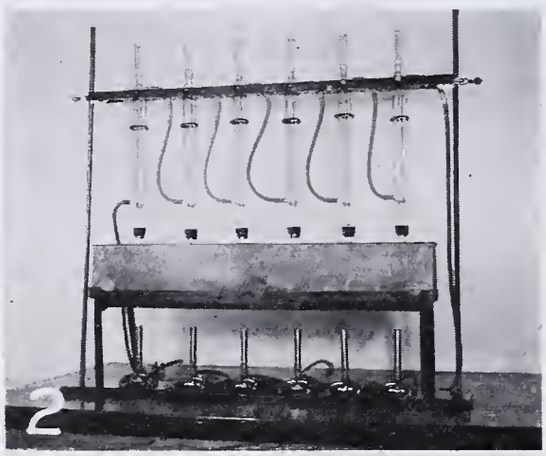
The author was confronted with the task of digesting a large number of samples of plant material, and in order to minimize this difficulty, executed the support shown in the accompanying figures. Such a support is easy to construct and has proved convenient and effective.

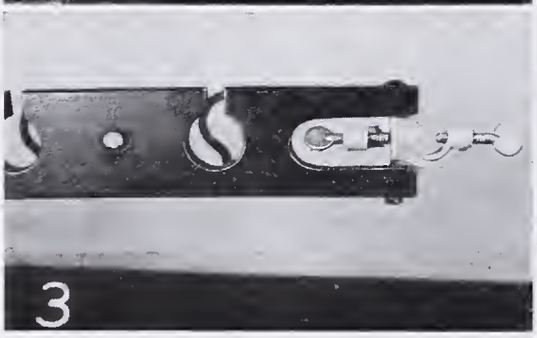
Figures 1 and 2 show a support for a battery of six condensers in use with an electric hot plate and water bath, respectively. Each flask can be raised and rotated freely without interfering with the others or having to loosen clamps. With pliable connecting stoppers, such a support can be used with a water bath without having to clamp the flasks, since the buoyant force of the water supports them from below. The author and associates have used this arrangement for hundreds of digestions without mishap. The support can be used also for condensers with ground-glass connections without clamping the flasks, if a hot plate is the source of heat.

Figures 3 and 4 show the construction of the support. Holes large enough to accommodate the condensers to be used are bored in a piece of board of appropriate dimensions, depending upon the number of reflux units desired, and notches large enough to permit the upper nipple of the condenser to pass through are cut from one side of the board to the holes. Other holes of correct size to permit the condenser tubing to pass through freely without being constricted are bored between the large ones at a slight angle with respect to the upper side of the board. A notch should be made in each end of the board, and a 0.375-inch bolt passed through in such a way that an ordinary clamp holder can be used to attach the support to ringstands. A small ring for holding a screw is bent on one end of a piece of moderately stiff wire of appropriate length and a ring that will almost encircle the jackets of the condensers to be used is bent on the other. The large ring should not be closed completely but should have a gap at the back big enough for the upper nipple of the condenser to pass through. Small-bore rubber tubing should be passed around the large ring to act as a cushion against the condenser jacket. One of these holders should be screwed onto the back of the support in line with the center of each large hole.

To place the condenser in position, the upper end is passed through the large hole from below and the condenser is turned in such a way that the upper nipple will pass through the gaps in the wire ring and in the board as it is raised upward. When it has passed through the upper notch, the nipple is placed on the board so that it points in the direction the water is to flow. After all the condensers are in place and equipped with rubber tubing, they should be aligned by adjusting the wire holders.







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Polarographic Determination of Arsenic in Biological Material

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THE uncertainties and inconveniences attending the use of the Gutzeit method (*1*) have resulted in the introduction of a number of improved procedures (*3, 4, 7, 8, 11, 12*) for the microdetermination of arsenic in biological material. Although these methods are much better suited for general work than the Gutzeit procedure, they possess certain disadvantages which prompted the investigation of polarographic methods.

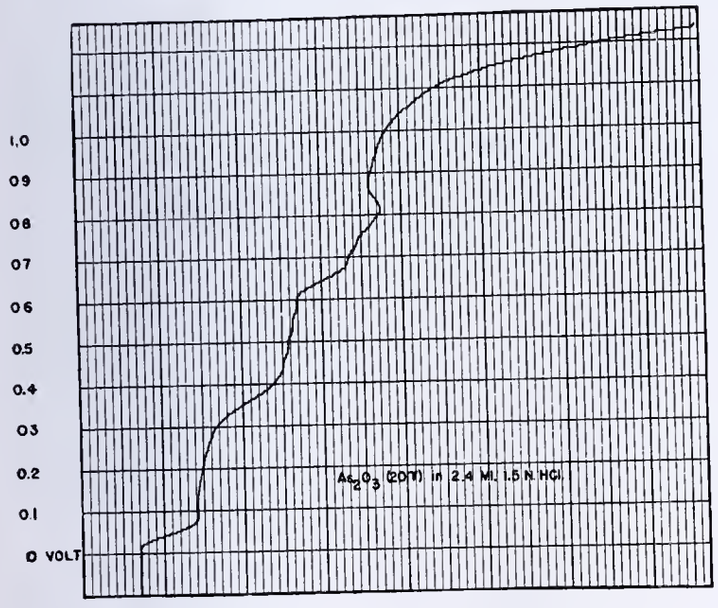

FIGURE 1. POLAROGRAPHIC CURVE GIVEN BY REDUCTION OF ARSENIC

20 micrograms as As_2O_3 in 2.4 ml. of 1.5 *N* hydrochloric acid

The literature contains a few references to polarographic studies on arsenic. Bayerle (*2*) worked with sodium arsenite in alkaline solutions but gave no definite information regarding the possibility of obtaining quantitative results with the procedure, while Kacirkova (*9*) reported that arsenic in relatively high concentrations gives a number of steps that are difficult to separate quantitatively. Preliminary work confirmed this report, but the author found that when the quantities of arsenic (trivalent) were kept below 100 micrograms per milliliter, well-defined steps were obtained, one of which was suitable for use in quantitative analysis (Figures 1 and 2).

Arsenate ion apparently is not reduced by the polarograph (*10*), although it is possible that reduction could be accomplished if a suitable electrolytic base were found. No definite steps due to quantitative reduction were observed with sodium arsenate in neutral aqueous solution, 5 per cent acid, 10 per cent tartaric acid, 1 *N* hydrochloric acid, 0.125 *N* hydrochloric acid, 25 per cent sulfuric acid, or alkaline solutions varying from slight alkalinity to about 0.2 *N* sodium hydroxide.

The polarographic method depends upon the isolation and concentration of the arsenic by the evolution of arsine and its absorption in mercuric chloride solution in a manner similar to that described by Cassil (*3, 4*). The mercury arsenides are changed to arsenious oxide by heating with the excess mercuric chloride, the mercury is precipitated by reduction with hydroxylamine, and the solution is then ready for the polarographic determination.

Procedure

PREPARATION OF SAMPLE. The sample of biological material is prepared by the wet-ashing procedure described by Hubbard (*8*). Nitrogen oxides should be removed from the sample solution by treatment with saturated ammonium oxalate solution, as in the Gutzeit method. An aliquot or the entire prepared sample may be used in the determination; the method cited ends with the sample dissolved in 50 ml. of approximately 40 per cent sulfuric acid, a solution satisfactory for use in the arsenic isolation step.

ISOLATION OF ARSENIC. The prepared sample is mixed with 10 to 15 ml. of hydrochloric acid in a 125-ml. Erlenmeyer flask and diluted with water to about 70 ml., after which 5 ml. of potassium iodide solution (*4*), 1 ml. of stannous chloride solution (*4*), and 10 to 12 grams of stick zinc are added. A Kjeldahl trap fitted with a glass delivery tube, which dips to the bottom of an ordinary 15-ml. graduated centrifuge tube containing 2 ml. of mercuric chloride solution (1.6 grams per 100 ml.), is immediately attached to the flask, which is then placed on a hot plate or in a water bath. It is advisable to provide some means of removing hydrogen sulfide from the evolved gas, although small quantities do not interfere. The simplest method is to put a gas-washing bottle with a fritted-glass disk, containing about 100 ml. of lead acetate solution (10 grams per 100 ml.), between the trap and the delivery tube. No arsine is retained in this bottle, so that it need not be cleaned frequently.

The evolution of hydrogen is allowed to proceed for about 30 minutes; then the delivery tube is disconnected and the centrifuge tube, containing both the mercuric chloride and the delivery

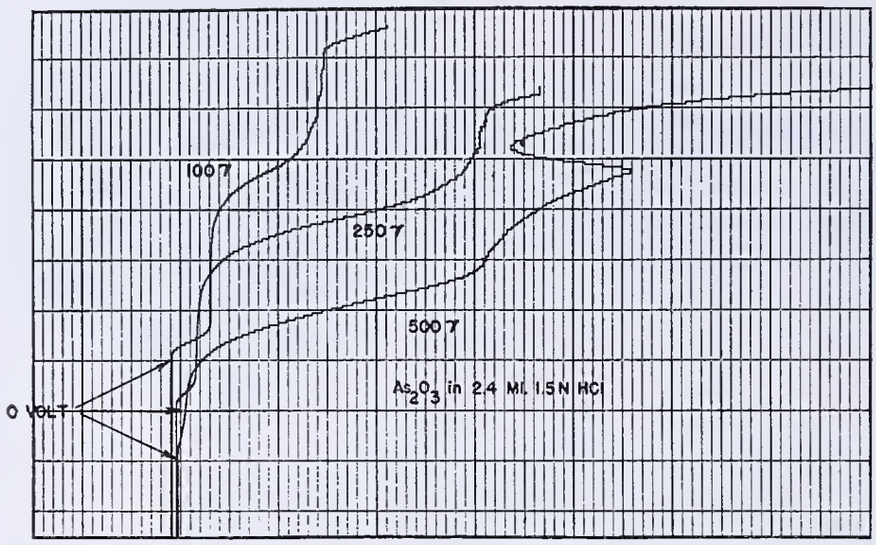

FIGURE 2. STEPS GIVEN BY ARSENIC

Quantities indicated as As_2O_3 in 2.4 ml. of 1.5 *N* hydrochloric acid

tube, is placed in a water or steam bath. The centrifuge tube is heated for about 5 minutes in order to change the mercury arsenides to arsenious oxide and mercurous chloride, and the delivery tube is rinsed off with hot water and removed. The solution is concentrated to about 2 ml., 1 drop of bromothymol blue solution (0.2 gram per 100 ml.) and 0.2 ml. of hydroxylamine sulfate solution (40 grams per 100 ml.) are added, and ammonium hydroxide is added to the hot solution carefully, in small drops, until the mercury precipitates. Upon addition of the first portion of ammonium hydroxide a flocculent precipitate appears; when more alkali is added it causes the mercury to be precipitated in a finely divided black form. At this step arsenic may be lost if the solution becomes too alkaline; it should not turn blue but should show the yellow-green color indicative of about pH 6. It is then heated until evolution of nitrogen ceases, it is cooled, and the volume is noted.

DETERMINATION OF ARSENIC. Two milliliters of the clear supernatant liquid are placed in the electrolysis cell, 0.4 ml. of 9 *N* hydrochloric acid is added, nitrogen is bubbled through the solution for no longer than 5 minutes, and the mixture is polarized under nitrogen from 0 to −0.7 volt. [If the quantity of arsenic is expected to be low (less than 20 micrograms), as judged from the appearance of the mercury arsenides in the centrifuge tube in the previous step, 3 *N* hydrochloric acid is used instead of the 9 *N* acid.] The half-wave of the arsenic step occurs at −0.35 volt (standard calomel electrode) in 1.5 *N* hydrochloric acid and at −0.5 volt in 0.5 *N* acid. The step height is measured and compared with a calibration curve made by taking known quantities of standard arsenious oxide solution, adding them to 2-ml. portions of the mercuric chloride solution contained in centrifuge tubes, and following the procedure described above, beginning with "The solution is concentrated.....". These determinations should all be made at the same temperature, with the same capillary and drop rate. Such precautions would not be necessary if it were possible to use an internal standard (*6*), but the complexity of the arsenic curve (Figure 1) prevents this.

Discussion

The arsenic isolation procedure has been shown by Cassil and others (*3*, *4*) to be quantitative, but the author was not able to confirm Cassil's recovery of quantities of arsenic above 30 micrograms with his method. Losses of arsenic of about 10 per cent usually occurred when granulated zinc and the 5-minute evolution were employed. Similar low results have been reported elsewhere (*13*) since the completion of this work. Without going into the possible causes of the losses which were experienced with the equipment described, suffice it to say that they were eliminated by the substitution of stick zinc for granular and by increasing the period of evolution to 30 minutes.

The precipitation of the mercury after the arsenic evolution does not cause any loss of arsenic, provided that the mercuric chloride solution is not made too alkaline (green or blue to bromothymol blue). It is necessary to remove mercuric ions from the solution before polarizing. Various methods were tried, including precipitation as the iodide, phosphate, oxide, and hydroxide, but all either caused loss of arsenic or failed to remove the mercuric ions completely. Reduction by hydroxylamine was the only method found which removed the mercury without resulting in loss of arsenic.

The arsenic solutions are polarized in acid of two different strengths: 0.5 *N* and 1.5 *N*. The weaker acid is used when it is necessary to employ high recorder sensitivities (with small quantities of arsenic); this gives a polarographic curve which is much smoother than that obtained with strong acid. With larger quantities of arsenic it is necessary to use 1 *N* or stronger acid, in order to bring the half-wave potential of the quantitative arsenic step as low as possible, so that it does not fuse with the next step. Even under these conditions, the steps fuse when the quantity of arsenic in the cell is greater than 100 micrograms per ml. (Figure 2).

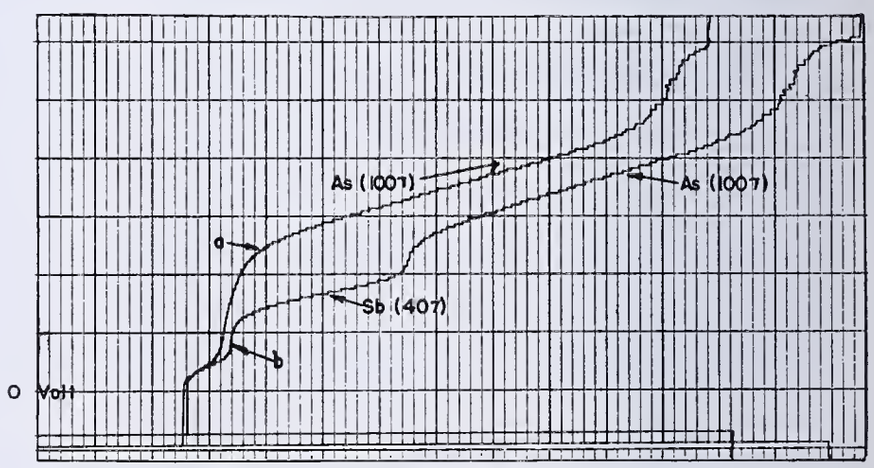

FIGURE 3. EFFECT OF ANTIMONY

a. 5 mg. of antimony plus 100 micrograms of arsenic carried through complete analysis
b. Same solution in electrolysis cell as *a* plus 40 micrograms of antimony

Antimony is an interfering element in most methods that depend upon the isolation of arsenic as arsine. It does not interfere in the polarographic method, however. Quantities of antimony as high as 5 mg. (antimony sulfate in sulfuric acid) were added to the flask before evolution of arsine; the arsenic step was not affected and no step due to antimony appeared (curve *a*, Figure 3). When, however, antimony sulfate was added directly to the electrolysis cell, an antimony step occurred at −0.16 volt in 1.5 *N* hydrochloric acid and at −0.22 volt in 0.5 *N* acid (curve *b*, Figure 3). Therefore, even if antimony were introduced into the solution being polarized, it would always be detected by its characteristic step and would

not interfere with the arsenic step unless present in large quantities.

The evolution and determination of known quantities of arsenic and comparison of results with some results obtained by Hubbard (*8*) with his procedure, indicate that the error of the polarographic method lies between 3 and 5 per cent in the case of quantities of arsenic above 10 micrograms; quantities below 10 micrograms can be determined within 1 microgram (see Tables I to III).

The polarographic method has certain advantages over other methods for the microdetermination of arsenic in biological material. The Gutzeit and Marsh methods, while sensitive, are neither flexible nor accurate; the former gives results accurate to 10 per cent only under the best conditions, while greater errors are often encountered. Also, it is frequently necessary to repeat analyses because the range of ascertainable quantities is quite small. Colorimetric methods employing the molybdenum blue color are as accurate as the polarographic procedure but usually are considerably more time-consuming. Total elapsed time between the completion of sample preparation and the final arsenic determination is usually about 24 hours; this time is consumed mainly by the evaporation of the arsenic trichloride distillate and preparation for the color development. During this manipulation all dust contamination must be avoided, since phosphate and silica, as well as arsenic, give the blue color. The total elapsed time with the polarographic procedure is less than an hour, and with the proper equipment a trained operator could run over twenty analyses per day. The titrimetric method used by Cassil (*3*, *4*) is probably the most rapid when a large number of analyses must be run daily and it compares favorably in accuracy with the polarographic and colorimetric procedures, provided antimony is absent. However, the polarographic technique has a distinct advantage when samples need be run only occasionally, as in a clinical laboratory. No standard solutions are necessary, so that time is not used in standardizing volumetric solutions for one or two analyses.

The calibration curves, once made, are valid indefinitely, so long as the same capillary is used in the polarograph. In addition, as little as 1 microgram of arsenic can be determined by the polarographic method, while the titrimetric procedure is not useful for quantities of arsenic below 5 micrograms. With the polarograph, determinations on samples with high arsenic content need be repeated only if the quantity of arsenic evolved is over 1 mg., so that the method covers a wide range. Dangers of contamination are slight, since the unique specificity of polarographic procedures eliminates interference from all common substances, including antimony. Considering these many factors, the polarographic procedure seems to be the method of choice for clinical work.

The Leeds & Northrup Electro-Chemograph was used in this work.

Table I. Recoveries of Known Quantities of Arsenic

Arsenic Taken *Micrograms*	Arsenic Found *Micrograms*
5	5.6
5	4.5
10	10.0
10	9.6
20	19.5
20	19.5
30	30.0
30	30.5
100	99
100	100
300	296
300	285

Table II. Recoveries of Known Quantities of Arsenic

Arsenic Added to Synthetic Urine Salts (*5*) Equivalent to 50 Ml. of Urine *Micrograms*	Arsenic Found *Micrograms*
0	Nil
2	2.1
5	4.7
10	10.3
50	50.0
100	99
200	196

Table III. Comparison of Results by Photometric and Polarographic Methods

Sample	Quantity *Grams*	Arsenic Found: Photometric Method (*8*) *Micrograms*	Arsenic Found: Polarographic Method *Micrograms*
Rabbit tissues:			
Colon	5.2 (not dried)	2.5	2.5
Heart	4.2	2.7	1.3
Kidney	5.1	33	31
Liver	5.5	18	18.5
Lungs	4.1	3.5	4.0
Lungs	6.4	10.5	11.3
Uterus	4.8	2.4	2.1
Uterus	4.9	12.4	11.5
Blood (human)	8.2	2.8	2.6
	6.3	9.6	7.5
Feces (rabbit)	5 (not dried)	60	59
Feces (human)	12.5	137	134
Feces (human)	4	312	309
	Ml.		
Urine (rabbit)	25	1.8	1.2
	25	2.7	3.9
	25	3.6	4.2
	25	89	88
	25	166	163
Vomitus (human)	25	52	52
	25	60	58
	19	100	101
	10	154	142
	Grams		
Spinal fluid (human)	2.7	1.6	2.0

Summary

A polarographic method is described for the microdetermination of arsenic in biological material. The arsenic is evolved from the prepared sample as arsine, which is absorbed in mercuric chloride solution; the mercury arsenides are changed to arsenious oxide and mercurous chloride by heating with the excess mercuric chloride; the mercury is precipitated by reduction with hydroxylamine; and the resulting solution is polarized in hydrochloric acid. The method is rapid and covers a range from 1 microgram to 1 mg. of arsenic. With quantities of arsenic below 10 micrograms, the error is 1 microgram; above 10 micrograms, the error is 3 to 5 per cent. No interfering substances have been encountered.

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Apparatus for Precision Calibration of Pipets, Volumetric Flasks, and Burets

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THE apparatus described here was designed for rapid calibration of pipets and flasks well within usual tolerances—e. g., one part per thousand for volumes of 10 ml. or more, and within 0.01 ml. for those less than 10 ml. In practice it appears to be subject to errors usually less than one tenth as great. A pipet of 0.5-ml. capacity may be measured to about 0.001-ml. approximation of calibration by other accepted means (*3*).

Apparatus

Figure 1 gives a schematic diagram of the essential elements of the calibration apparatus. Convenient dimensions are given below.

Control Vessel. A double Y-tube is rigidly constructed from six standard-taper stopcocks (preferably Pyrex) of about 2-mm. bore, with connecting tubes of approximately 4-mm. bore and 7-mm. outside diameter. The Y-angles are about 60°. Vertically ascending from a 7-cm. delivery tip are stopcocks *D*, *C*, and *A* with intervals of 6 and 10 cm., respectively. The stem above *A* extends 5 cm. to a neatly fire-polished butt at *Q*. Midway between *A* and *C* is joined the branch ascending at 30° from horizontal. About 6 cm. along this is stopcock *E* and 8 cm. farther is *F* with another butt end after about 3 or 4 cm. Midway between *E* and *F* is a vertically ascending branch about 14 cm. in length with stopcock *B* about 4 or 5 cm. above the Y-joint. The top of this branch is a butt at *G* like that above *A*.

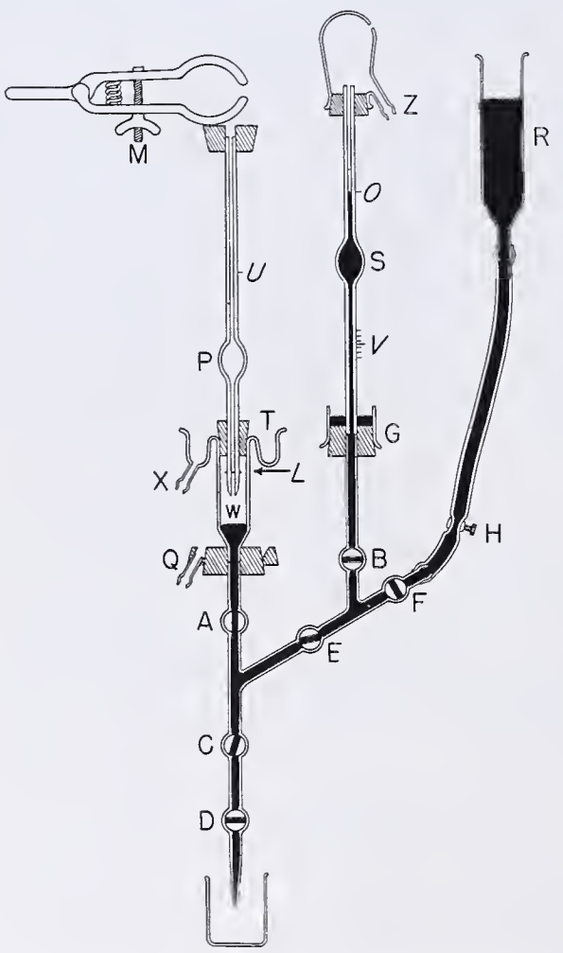

Figure 1. Apparatus for Pipet Calibration by Refilling or Mercury Weighing

Each of these is fitted with an inverted No. 8 stopper bored with a slightly smaller hole for tight fitting around the 7-mm. glass tubing, on which it is mounted with the glass butts halfway through in each case (*Q* and *G*). The other halves of the borings supply joints to apparatus placed vertically above. The stopper at *G* is surrounded by a short glass cylinder, about 4 cm. high and 4 cm. in outside diameter, to form a well. The stopper at *Q* is surrounded by a moat with a drain to a waste bottle. The moat may be made by use of a rubber ring with chamfered inner edge and the drain supplied by a boring fitted with a small glass tube. The apparatus is mounted in clamps on a firm upright rod—e. g., a ring stand clamped to the bench with a C-clamp. Between clamp jaws and apparatus are placed rubber stoppers suitably bored and slit on one side, one between *C* and the Y-joint and another 3 or 4 cm. above *B*.

Mercury Charge and Reservoir. To the butt beyond *F* is attached rubber tubing, leading to reservoir *R* at suitable height, with a Hoffman clamp, *H* (or another stopcock), just beyond the butt near *F*. Either through this lead, or a temporary substitute, vacuum is applied at *F* and also at *B* and *A* as required to fill the apparatus with mercury from the tip below *D* to and through stopcocks *A*, *B*, and *F* with careful exclusion of air. Then reservoir *R* is filled with mercury and placed in position, and air is expelled from the connections to *F* by slightly whirling part of the rubber tube.

Standard Pipet. *A* and *D* are left closed and *C* and *E* open (partly, at least) while mercury is allowed to run through *F* and *B* partly to fill well *G*. Through this mercury, the surface of which is cleaned by aid of a small glass vacuum attachment, if necessary, a standard pipet, *S*, is inserted and joined (glass to glass) through the rubber stopper at *G*. The pipet is provided with capillary arms above and below its bulb, adapted, if necessary, at the lower end to make a good fit (about 7 mm. in outside diameter).

For convenience of reading, the arm capillary volume has been made about 0.005 ml. per cm. for a 0.5-ml. standard pipet and 0.03 ml. per cm. for 3- or 5-ml. standards. The volume of mercury that is delivered from an initial mark, *O*, above the bulb to a point below is read on a scale, *V*, calibrated by weight of mercury delivered from the tip through open *E*, *C*, and *D* with *A* and *F* closed and controlled at *B*. The tip is touched to a level mercury surface at the beginning, the discharged mercury caught in a tared vessel, and the tip touched to the level surface of mercury in it at the end of delivery. At the top of pipet *S* may be attached an overflow safety vessel, *Z* (of well-known type), leading to a catch bottle. However, careful adjustment of clamp *H* and the level of *R* make this unnecessary. *S* is clamped lightly at the bulb to the main support rod.

Water Chamber. A vessel, *W*, on the main vertical stem above *A* is attached through the rubber stopper at *Q*, previously flooded with a few drops of water to cover the hole in the stopper so as to exclude air from the joint. *W* is adapted to fit this joint below, about 7 or 8 mm. in outside diameter at the base, to allow formation of a mercury-water interface in a large-diameter tube (25 mm. or more in inside diameter); and to admit attachment of pipet *P* through a rubber stopper at the top of *W*. *W* is provided with a surrounding moat, *T*, at the top, conveniently made all of glass with a drain, *X*, leading to a waste bottle. Alternatively the moat and drain might be made with a rubber ring as at *Q*, or with a rubber stopper around *W* and a glass attachment like that at *Z* but without the dome top.

Attachments for Pipet to Be Calibrated. The tip of pipet *P* must be above the mercury-water interface throughout all calibration operations on it. Any parts required to be visible for reading or observation of flow should be well above or below the rubber stopper at *T*. The bulb of a small pipet, if near the tip, may be placed below *T*. Air is carefully excluded by flooding *W* with water just before insertion of stopper *T*, the overflow passing away through *X*. In calibration between marks, *P* will have an upper mark, *U*, and a lower mark, *L*. *L* may be placed, as convenient, above or below *T*. In calibration to drain and touch, *L* may be a temporary mark—e. g., a fine rubber ring cut from tub-

ing—placed where the meniscus stands after completing drainage with the tip against the wet wall of a receiving vessel (*3*), or touching the tip of the drained pipet to a level water surface and then slowly withdrawing it, as prescribed by convention. The former is usually preferred, but the latter was used in experiments described below.

Vessel *W* is clamped firmly to the main support rod (with care not to interfere with readings). It is convenient to place a rubber stopper at the top of pipet *P* to be calibrated, and adjust a clamp from the main support so that its movable jaw presses gently upon the top. This furnishes a micrometer screw type of adjustment, *M*, acting on the remote stopper at *T* as a tambour to effect minor adjustment of the air-water interface in the pipet at the upper mark, *U*, and later at the lower mark, *L*, or at the actual tip as required in blow-out or to-contain types of calibration. Slight adjustments at the lower points are usually admissible in calibrations, if the pipet is simply refilled with a definite volume of water after drainage, approximately as in use.

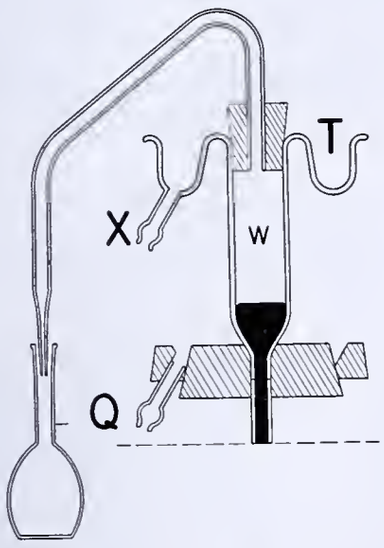

FIGURE 2. ADAPTATION WITH DELIVERY TUBE FOR CALIBRATION OF VOLUMETRIC FLASKS OR OTHER VESSELS

Stopcock *C* is adjusted in preliminary trial to control flow rate conventionally, so as to approximate conditions of drainage of pipet *P* expected in actual use. *E* is used chiefly to control flow rate from *S* to *P*. The other stopcocks, *F*, *B*, *A*, and *D* are usually either closed or wide open in use. Accordingly, it is convenient to distinguish *C* and *E* from the others by a red rubber cover, made from tubing with a little hole cut in the side and slipped over the stopcock handle.

Procedure

Have in place the required standard pipet, *S*, and suitable vessel, *W*. Take care to eliminate any entrapped air. Usually keep *C* and *E* partly open, and never have *A*, *B*, *F*, and *D* all closed longer than necessary to deliberate operation. Have *H* adjusted to give steady flow so as not to entrap air in the standard pipet when refilling (from *V* to *O*) with *B* and *F* wide open and *A* and *D* closed.

Then, with *B* closed and *A* open, adjust mercury in *W* through *F* or *D*, so that it will extend up to the wider cylindrical portion but not high enough to reach pipet *P* in later operations—i. e., the water in *W* between the top of the mercury level and the pipet bottom should have a volume slightly exceeding that to be contained in the pipet. The rubber stopper for use with *M* may be attached at the pipet top before inserting the pipet into *W*. If the pipet has a stopcock, keep it open throughout.

Flood *W* with water. Immediately insert *P* and the stopper at *T*; or insert the stopper only, run water up to flood the hole, and insert the pipet tip through the drop of water and the stopper, if only the tip is to enter *W*. The latter is convenient when several pipets may be calibrated without removal of the stopper at *T;* the former is required when the bulb is to be placed within *W* or when a temporary marker (rubber ring) is used near the tip as in drain and touch calibration. Adjust the clamp at *M* to give a slight tension.

Operate *F* to fill pipet *P* up to *U* (approximately). Close *F*, open *D*, and adjust *C* to give the required outflow rate. Repeat the filling and drainage till the adjustment is satisfactory. If air has been admitted to *W*, remove the pipet and stopper at *T* and replace, after flooding *W* as previously. A small amount of air in *W* might not interfere appreciably with preliminary adjustment of *C*, but might affect later operations considerably.

Close *A*, open *B*, and fill standard pipet *S* to *O*. To facilitate this, *E* may be set in advance so as to restrict subsequent downward flow and then mercury run from *F* into *S* till above *O*, *F* closed, and *D* opened to run mercury slowly down to *O*. Close *B*, then *D*, and open *A*. Adjust water level in *P* to *U* (by *F*, *D*, and *M* as necessary). Open *D* wide, and drain pipet *P* to the lower mark as required. Adjust by *M*, if necessary. Open *B*, control flow rate by *E*, and run back to *U* (or down to the prescribed *V* in the standard, or both in succession) using *B* as shut-off. Read *V* on *S*, or set new mark *U* on *P* if the previous mark is found in repeated trial to yield a delivered volume outside tolerable limits.

If required as a final check calibration, the weight and temperature of mercury delivered from the tip below *D* may be used, provided that the drainage is made by control at *D* alone, with *M* not used except at *U*.

In calibration of volumetric flasks or tubes, the procedure is essentially the same except that an inverted U-delivery tube with a fine tip (illustrated in Figure 2) is used at *T* instead of *P;* and, after delivery, water is added to the system, as required, by dipping the tip of this tube in water and running mercury out through *D*. If air bubbles form in the delivery tube, they may be removed by running more air into it (opening *D*) to contact the entrapped air and following by reversal of flow (closing *D*, opening *F*) to run the air out. Just before the start and at the finish of the measured delivery, the tip is touched to a water surface, first in the beaker used for filling and, finally, within the vessel being calibrated.

Experimental Results

A standard 0.5-ml. pipet was made with marks in close approximation on the *V*-scale to 0.5 ml. and 0.005 and 0.010 ml. above and below. Recalibration at the 0.5-ml. mark by weight of mercury delivered (as described above) gave the *V* estimates, 0.49924, 0.49974, 0.49921; mean $V \cong 0.4994$. The difference of 0.01 ml. in volume delivered corresponded approximately to 20 mm. on the *V*-scale.

As an illustrative application, a 0.5-ml. Ostwald pipet was calibrated in various ways. Its natural outflow time was between 6 and 7 seconds. In drain and touch delivery (touching as previously described after 5 seconds more) calibration from the original mark by weight of water delivered gave the volume estimates, 0.4915, 0.4913, 0.4917, 0.4903; and $\overline{V} \cong 0.4912$. The position of the meniscus in the tip after touching was noted (about 29 mm. from the end) and a fine red rubber ring set as lower mark *L* in that position. In the volumetric apparatus against the standard pipet with the indicated outflow times in seconds (*D. T.*), the following values of *V* were obtained: $V = 0.4915, 0.4915, 0.4915$, *D. T.* = 5.8 to 6.6; $V = 0.4930, 0.4930, 0.4935$, *D. T.* = 21.0 to 21.4; $V = 0.4940, 0.4940, 0.4940$, *D. T.* = 55.4 to 55.8. Thus, at natural drainage rate $V \cong 0.4915$ volumetrically and $V \cong 0.4912$ gravimetrically, a difference of 0.0003 ml. This is remarkable in view of the rapid outflow rate. Volumetric readings were made to the estimated nearest 0.0005 ml. (tenth of smallest scale division on *S*—i. e., to the nearest millimeter). Thus, it would seem that even greater precision might be attainable with closer reading, but the precision was already far greater than sought for the purpose.

The same pipet was calibrated also to deliver from another initial mark, *U*, to the tip, simulating blow-out and to-contain calibration according as the drainage time was approximately that of free delivery or relatively very great. The results are given in Table I. For drainage time (*D. T.*) of 134 seconds or more, there appears close approximation in the 13 observations to their mean value, 0.49885 ml. Recalibration of the dry pipet with mercury, using the meniscus correction (*3*), gave the volume estimates, 0.4983, 0.4987, and 0.4993 ml. with a mean *V* of 0.49877 ml. Not only are the volumetric

Table I. Volumetric Measure of Delivery to Tip in Given Drainage Times

	Drainage Time Range, Seconds						
	1.6–1.8	7.4–8.0	22–23	65–66	134–138	420–445	500–552
	Ml. × 10^{-4}						
	4885	4950	4975	4990	4985	4985	5000
	4895	4965	4970		4985	4985	4995
		4960	4980		4985	4990	
		4955			4985	4990	
		4960			4985		
					4990		
					4990		
Mean	4890	4958	4975	4990	4986	4988	4998

Table II. Volumetric and Gravimetric Calibrations of 3-Ml. Pipet

	Observer W. R. T.		Observer C. E. F.		Observer P. M. L.	
	V	*V'*	*V*	*V'*	*V*	*V'*
	Ml.	*Ml.*	*Ml.*	*Ml.*	*Ml.*	*Ml.*
	3.003	3.0010	3.001	3.0000	3.002	3.0014
	3.002	3.0014	3.003	3.0020	3.002	3.0018
	3.003	3.0019	3.002	3.0017	3.002	3.0014
Mean	3.0027	3.0014	3.0020	3.0012	3.0020	3.0015

and gravimetric means in close approximation to each other, but only 2 of 13 individual observed volumetric values differ from 0.4988 ml. by more than 0.0005 ml. and the greatest deviation is only 0.0012 ml.

Gravimetric calibration to blow-out after free drainage gave the volume estimates, 0.4928, 0.4932, 0.4918, 0.4930, with a mean of 0.4927. This differs by 0.0031 ml. from the volumetric mean for *D. T.* between 7.4 and 8.0 seconds (Table I). Obviously, with such short drainage time, considerably more variation in delivery is to be expected than in the cases approximating complete drainage more closely, and such an influence is evident by comparison with the first column of Table I where *D. T.* is approximately 2 seconds.

Although the same size of capillary tubing could be used for larger standard pipets, actually 0.03 ml. per cm. was the volume difference on the scales of the 3- and 5-ml. *S*-pipets used. Accordingly, a comparison of results by different observers was made to indicate reproducibility of calibration of a stopcock pipet for delivery of 3 ml. between marks. As a control on the volumetric estimates (*V*), the mercury delivered each time was caught and weighed in a tared vessel to give a corresponding gravimetric estimate (*V'*). The results are given in Table II.

The apparatus may readily be adapted, as indicated in Figure 3, by use of a modified vessel, *W*, so that flow from the vessel to be calibrated goes directly to the standard pipet, *S*. This is useful in calibration of instruments such as burets and Van Slyke-Neill chambers for manometric gas analysis.

Calibration of Microburet. As illustrated in Figure 3, a modified vessel, *W*, was made as follows:

From a 7-mm. outside diameter adapted tip to fit *Q*, a 15-mm. outside diameter tube rose to 34 cm. above, joined to a bulb about 50-mm. outside diamter and 4-cm. length with tapered ends about 3 or 4 cm. above and below, joining an 8-mm. outside diameter delivery tube, arched at the top and turned down leading to a level approximating that of stopcock *D*, then turned upward again and adapted to a cup, *T*, similar to that in the vessel described above although a trough was not actually used. The modified vessel, *W*, was joined at *Q* as previously described, clamped just below the bulb and around the cup below *T* about 15 cm. to the left of *C*. Mercury was run up almost to its top. Then water was added at cup *T*, and run into the bulb by withdrawing mercury through *D*. At first some air was trapped in the bulb and top of the delivery tube, but this was removed by repeating the raising and lowering of the mercury level, and supplying water at *T* as required.

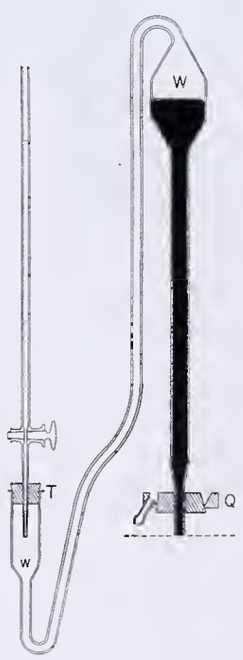

Figure 3. Modified Vessel *W* for Calibration by Direct Flow, All One Piece from *Q* to *T*

In the manner previously employed, the tip of a 10-ml. microburet was inserted through a stopper at *T*, and the buret clamped near the top in a vertical position. Using a standard pipet, *S*, but with *O* below and *V*-scale above, successive withdrawals were made from the microburet to readings as given in Table III with the estimated cumulative volumes (sum of successive readings on the standard). Just before each withdrawal, the buret setting was carefully checked to guard against any drift, such as might result from a temperature change during an interval of delay. However, there was seldom any need for resetting. The delivery speed was about 1 ml. in 40 seconds, controlled by *E*. A set of observations at about 0.5-ml. intervals was made and at about 5-ml. intervals by each of two observers. The results are given in Table III, with gravimetric data subsequently obtained by weight of mercury delivered in the manner described for check calibrations in the case of the 3-ml. pipet (*V'*).

Table III. Estimated Cumulative Volume from Zero Reading to Given Reading

Reading	V_1 (W. R. T.)	V_2 (P. M. L.)	V_1/V_2
Ml.	*Ml.*	*Ml.*	*Ml.*
0.5	0.5020	0.5015	1.0010
1.0	0.9990	1.0025	0.9965
1.5	1.5000	1.5035	0.9977
2.0	1.9985	2.0015	0.9985
2.5	2.5055	2.5065	0.9996
3.0	3.0115	3.0110	1.0002
3.5	3.5120	3.5135	0.9996
4.0	4.0135	4.0105	1.0007
4.5	4.5200	4.5155	1.0010
5.0	5.0250	5.0185	1.0013
5.5	5.5250	5.5195	1.0010
6.0	6.0280	6.0180	1.0017
6.5	6.5245	6.5150	1.0015
7.0	7.0300	7.0190	1.0016
7.5	7.5335	7.5190	1.0019
8.0	8.0345	8.0235	1.0014
8.5	8.5410	8.5270	1.0016
9.0	9.0435	9.0290	1.0016
9.5	9.5465	9.5325	1.0015
10.0	10.0490	10.0350	1.0014
5.0	5.019	5.017	1.0004
10.0	10.040	10.033	1.0007
5.0	5.015[a]	5.016[a]	
10.0	10.032[a]	10.034[a]	

[a] Gravimetric observations, *V'*, made subsequently by W. R. T.

Discussion

The present system of calibration by equal volume displacement differs primarily from others previously developed (*1*, *2*, *4*) in that the measurement is made in the standard pipet with a liquid (mercury) which does not wet the glass. Thus is avoided a source of drainage variation which otherwise may become progressively worse with use. Obviously, another liquid forming a similar layer above mercury may be used if required instead of water. The volume range employed thus far has been from 0.5 to 10 ml. Great variations in pressure on the glass by the mercury might interfere with precision in large volume calibration; and, perhaps, use of volumes exceeding 100 ml. might give unsatisfactory results. In the case of the apparatus constructed by the author, addition of a gas pressure equivalent to about 48 cm. of mercury in the standard pipet, with *B* closed, caused a depression of about 0.002 ml. on the *V*-scale. Slight influences of this nature are compensated automatically by the suggested

method of calibration of the standard pipet system, and a check upon variability of such influence is covered by replicate observations.

Only those variations in temperature that occur during the usually short period required for actual measured volume displacements affect the precision. If the volume coefficient of thermal expansion is the same for the glass of the standard pipet as for that of the apparatus being calibrated, and their temperatures are equal, correction to a prescribed standard temperature is automatically made. Otherwise, a correction for a difference in thermal expansion coefficient may be introduced in the usual manner.

The system should be most useful where many pipets, small flasks, or tubes are to be calibrated. Manufacturers of such apparatus should thus be aided in meeting tolerances prescribed by the National Bureau of Standards for certification, with a consequent saving by a lower frequency of rejection. Furthermore, improvement in precision of setting initial marks on apparatus may permit actual reduction of some such tolerances without increase in cost.

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Riboflavin Analysis of Cereals

Application of the Microbiological Method

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AMONG the rapid methods proposed for the estimation of riboflavin, the microbiological and fluorometric appear to be the most promising (*2*, *7*). Both have been shown to give comparable results on a wide variety of biological materials (*6*) and to agree satisfactorily with values obtained by animal assays (*1*, *3*, *8*, *10*, *11*). Neither method, however, is universally applicable without modifications designed to remove interfering impurities. The microbiological procedure may be adversely affected by extraneous solids (*8*) or other nonflavin growth-influencing factors (*4*), while the fluorometric procedure is accurate only if pigments and nonflavin fluorescent substances are compensated for or removed (*5*, *6*, *9*).

For the past two years the authors have used the microbiological method for the examination of cereals and cereal products, and more recently have applied the fluorometric technique to this same study. Numerous instances have been found where the results from the two methods did not agree and these findings have led to a study of the factors which are responsible for this lack of agreement.

Microbiological Studies

While in the microbiological method the direct assay of the solid, finely ground cereals has an advantage in the possibility that incomplete extraction may thereby be eliminated, the presence of the suspended solids can lead to unreliable results (*8*). When different levels are used in the microbiological assay there is frequently a marked tendency toward appreciably lower values as the weight of the sample is increased. This introduces uncertainties as to the accuracy of the average of the several levels (Table I).

In extracts prepared by autoclaving with water and centrifuging, this tendency is decreased although not entirely removed. If uniformity is taken as a criterion of reliability, the values for the aqueous extracts would be more acceptable. However, the assays thus obtained are materially lower than those from the direct assay and at once the question of extraction efficiency is introduced. Either extraction is incomplete or some nonflavin growth factor is removed. But if the latter is present and is responsible for the higher values in the direct assay, why is there a falling off in the assay values as the weight of the sample is increased?

If one attempts to improve on the extraction conditions by treating the sample with takadiastase prior to removing the undissolved solids, there is a further change in the assay results. The absence of uniformity at the different levels is almost entirely removed, but the riboflavin values are still lower! For this reason it is believed that completeness of extraction is a minor problem compared to the behavior of the organism under the different assay conditions.

In order to determine whether the residue remaining after extraction contained any appreciable quantity of riboflavin, it was subjected to assay. The solid had to be used, since further extraction yields extracts very dilute in riboflavin. The results indicated that refined cereal residues contained 6 to 8 per cent of their original flavin, while cereal grains contained up to 20 per cent. However, these assays were as erratic as the direct assays on the original solid samples and no decision could be made as to whether the results were due to riboflavin in the residues or to the presence of extraneous solids in the assay medium. The latter can cause fictitiously high values which could readily account for the "apparent" riboflavin content of the residues.

A partial answer to these problems was found in the examination of cereals to which pure riboflavin had been added.

Table I. Effect of Weight of Sample on Direct Microbiological Assay

Cereal Product	Weight of Sample	Riboflavin
	Gram	*μg./g.*
Whole wheat flour	0.053	2.33
	0.068	2.20
	0.083	2.13
	0.098	1.86
		Av. 2.13
Patent flour	0.21	0.49
	0.27	0.41
	0.33	0.36
	0.39	0.32
		Av. 0.37

Table II. Recovery of Pure Riboflavin Added to Patent Flour

(Microbiological assay of aqueous extracts)

Sample	Riboflavin Found	Recovery
	μg./g.	%
Patent flour	0.50	..
Patent flour + 0.55 μg. per gram	1.15	118
Patent flour + 1.1 μg. per gram	1.95	132
Patent flour + 2.2 μg. per gram	3.55	138
Patent flour + 4.4 μg. per gram	6.50	137

Aqueous extracts of patent flours containing various quantities of flavin were assayed. The results are shown in Table II.

It is obvious that some factor other than flavin is influencing the growth of the organism. Strangely enough, this influence is greater as the actual amount of flour in the assay is decreased. Higher recoveries are obtained with the larger quantities of added riboflavin where the actual sample weights are less.

The fact that recovery values decrease toward the theoretical 100 per cent as the weight of the sample increases leads to the suspicion that the flour contributes some inhibitor which prevents the true evaluation of the riboflavin content. The observation that below a certain sample weight recoveries become constant, suggests that this inhibitor effect can be diluted until it is no longer measurable. This reasoning indicates that the true flavin content of the flour alone may be appreciably higher than the 0.5 microgram per gram actually observed.

However, when corrections for this inhibitor effect are made in order to estimate the flavin content of the flour, the values are widely divergent, ranging from 0.7 to 2.1 micrograms per gram. Such a simple accounting for interfering factors cannot be applied.

Table III. Apparent Riboflavin Content of Flour Derived by Deducting Added Riboflavin

Amount Added	Total Found	Apparent in Flour	Difference
μg./g.	μg./g.	μg./g.	μg./g.
0.55	1.15	0.60	..
1.10	1.95	0.85	0.25
2.20	3.55	1.35	0.50
4.40	6.50	2.10	0.75

Another approach to the problem can be made by assuming that the added riboflavin is quantitatively recovered and that the values representing the difference between the total flavin found and that added is a measure of the growth factors or "apparent flavin" in the flour. Such calculations are shown in Table III.

The regular increases in the amounts of added riboflavin are accompanied by uniform differences in the apparent flavin contents. If it is assumed that the difference of 0.25 microgram per gram prevails between the flour alone and that containing the lowest amount of added riboflavin, a value of 0.35 microgram per gram would be approximately the true value for the flour. As will be shown, this figure closely agrees with analyses carried out by other procedures. Thus, rather than being too low the microbiological values are about 30 per cent too high for patent flour when aqueous extracts are assayed. This suggests that the flour contains a nonflavin growth-stimulating factor and leads to the postulate that both inhibiting and stimulating factors are involved. Relative concentrations of these materials may decide the total influence on the assay and thus account for the variable results obtained at different assay levels—i. e., high values which decrease with increasing sample weights.

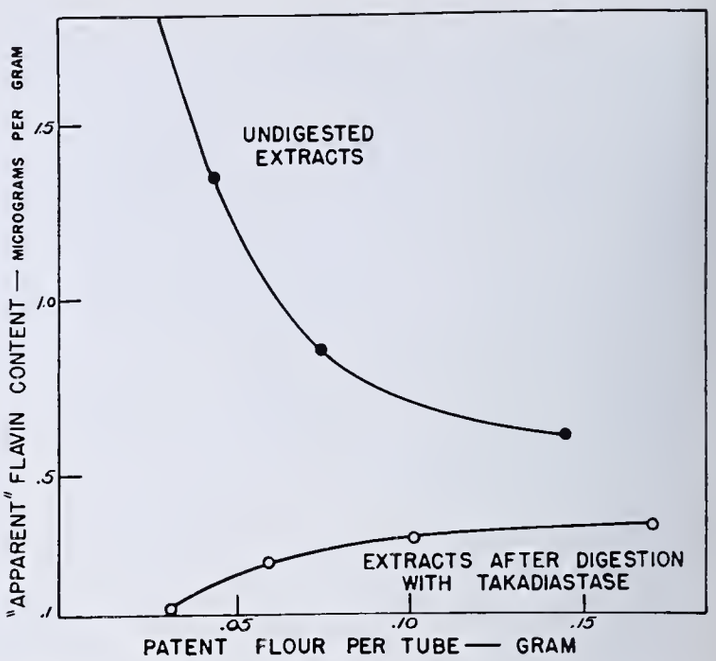

Figure 1. Riboflavin Assays of Aqueous Extracts before and after Digestion with Takadiastase (Patent Flour)

Since more uniform values are obtained when extracts are digested with takadiastase, the same flour blends were examined by treating aqueous extracts with this enzyme. The entirely different picture which resulted is shown in Table IV.

The effectiveness of the takadiastase is at once apparent. Recoveries no longer exceed the theoretical but are relatively constant and quantitative within limits of experimental error.

If the amount of added riboflavin is deducted from the total, the apparent values for the flour are now approximately the same for each recovery level. While the relative difference between 0.35 and 0.12 is considerable, at the highest levels of added flavin it is actually only about 5 per cent of the total. The contrast between the assays of aqueous and digested extracts is shown graphically in Figure 1.

The inhibiting effect which accompanies the increasing amounts of flour in the undigested extracts is absent in those which have been digested with takadiastase. The stimulating effect which produces high values for the apparent flavin content of the undigested extracts, particularly at the lower concentrations, is also shown.

Similar recovery experiments have been carried out on whole wheat flour and similar observations have been made. Table V compares the microbiological values obtained on aqueous and digested extracts.

As in the case of the patent flours, the apparent values obtained from the aqueous extracts are much higher than those from extracts which have been digested with takadiastase, and the apparent values for the aqueous extracts are less constant than those obtained after enzymatic digestion.

Table IV. Recovery of Pure Riboflavin Added to Patent Flour

(Microbiological assay of aqueous extracts treated with takadiastase)

Added	Total Found	Apparent in Flour	Recovery[a]
μg./g.	μg./g.	μg./g.	%
0.55	0.90	0.35	100
1.1	1.42	0.32	97
2.2	2.44	0.25	95
4.4	4.52	0.12	93

[a] Assumed that flour alone contains 0.35 microgram per gram.

Table V. Microbiological Assays of Aqueous and Takadiastase-Digested Extracts of Whole Wheat Flours Containing Added Riboflavin

Sample	Aqueous Extracts	Apparent in Flour	Digested Extracts	Apparent in Flour
	μg./g.	μg./g.	μg./g.	μg./g.
Whole wheat flour	1.9	1.9	1.05	1.05
Whole wheat + 0.5 μg. per g.	2.7	2.2	1.55	1.05
Whole wheat + 1.0 μg. per g.	3.3	2.3	2.20	1.20
Whole wheat + 1.5 μg. per g.	3.9	2.4	2.65	1.15
Whole wheat + 2.0 μg. per g.	4.5	2.5	3.15	1.15
		Av. 2.26		1.12

Table VI. Recovery of Riboflavin Added to Wheat Starch

(Determined microbiologically)

Sample	Aqueous Extracts	Takadiastase-Treated Extracts
	μg./g.	μg./g.
Wheat starch	Trace	Trace
Wheat starch + 2 μg. per g.	4.05	2.5

They show a regular increase as did the patent flours but the magnitude is less since the actual quantities of flour assayed cover a smaller range. These relations are shown in Figure 2.

Just what mechanism is involved in these observations is not easily deduced from the facts now known. Diastasis of the starch appears to be one factor, since studies with purified starch prepared from wheat flour gave similar results. The data in Table VI show a recovery experiment on such material.

While the digestion did not bring the found and calculated values into entire agreement, it did markedly lower the stimulating effect of the undigested starch.

The other major component of flour, the gluten protein, was also examined for its effect on riboflavin recovery. Treatment with takadiastase did not change the results, and recoveries with and without digestion were theoretical within the limits of experimental error. Thus, the discrepancies observed in the microbiological assay of aqueous extracts of cereal products appear to be due at least in part to the starchy constituents or closely associated substances.

The actual nature of these is not known. Their effect on the growth of the organism may be either chemical or physical, or both. That colloidal phenomena may be involved is suggested by the physical appearance of the assay media. Cloudiness decreases as we go from direct assay to aqueous extracts and finally to extracts digested with the enzyme. Supporting this idea is the observation that the cloudier extracts prepared by centrifuging gave higher values than those clarified by filtration. While the possibility is not ruled out that this difference may be due to adsorption of flavin on the filter paper, the magnitude of the difference is much greater than any adsorption which has been observed with pure solutions.

When fuller's earth is added to solutions of pure riboflavin and then removed by centrifugation and assayed as the solid, the microbiological method quantitatively measures the flavin content of the original solution. When aqueous extracts of cereals are similarly treated the values are much lower than those found by assay of the extracts themselves. It was at first believed that these observations indicated incomplete adsorption of the riboflavin, but in view of later observations it is probable that the lower values resulted from separation of the flavin from nonflavin growth factors.

Experiments have also been tried with the synthetic adsorbent, Florisil, or Supersorb as it was formerly termed. Passage of flavin solutions through columns of this material has been shown to remove the vitamin quantitatively (*3*, *5*). Extracts of cereals were passed over Florisil and the unadsorbed fraction was examined microbiologically. The results are shown in Table VII.

The fact that the 5-ml. quantity of extract gave no growth of the organism in the absence of added riboflavin is evidence that adsorption from the aqueous extract has been complete. On the other hand, in the presence of added riboflavin, a definite growth stimulation is observed. This must be partly due to some nonflavin factor, since the found riboflavin considerably exceeds the amount which was added. That this may be a colloidal response is not excluded, since no essential clarification of the extract is effected by the treatment with Florisil.

The same experiments were carried out on whole wheat flour with entirely similar results.

Table VII. Microbiological Assay of Aqueous Extracts of Patent Flour Following Adsorption on Florisil

Volume of Extract	Riboflavin Added	Riboflavin Found
Ml.	*μg./tube*	*μg./tube*
1.3	0.15	0.253
1.6	0.15	0.248
2.0	0.15	0.255
2.4	0.15	0.258
5.0	None	0.000

Fluorometric Studies

The recent development of improved fluorometric procedures for riboflavin has enabled us better to evaluate the observations made by the microbiological studies. Mention has been made of the efficiency of Florisil for adsorbing flavin from cereal extracts in confirmation of the report by Conner and Straub (*3*). Accordingly, the aqueous and digested extracts examined by the microbiological method were treated with this adsorbent and the eluates examined fluorometrically. Table VIII shows the results.

The rather good agreement between the values for the two types of extracts is in sharp contrast to the results obtained microbiologically. Instead of finding much higher values by aqueous extraction, we actually observe values slightly below those obtained by takadiastase digestion. This lends confirmation to the belief that the microbiological assay of aqueous extracts gives excessively high results due to the presence of nonflavin growth factors and that enzymatic digestion removes most of this interference. The apparent values for the

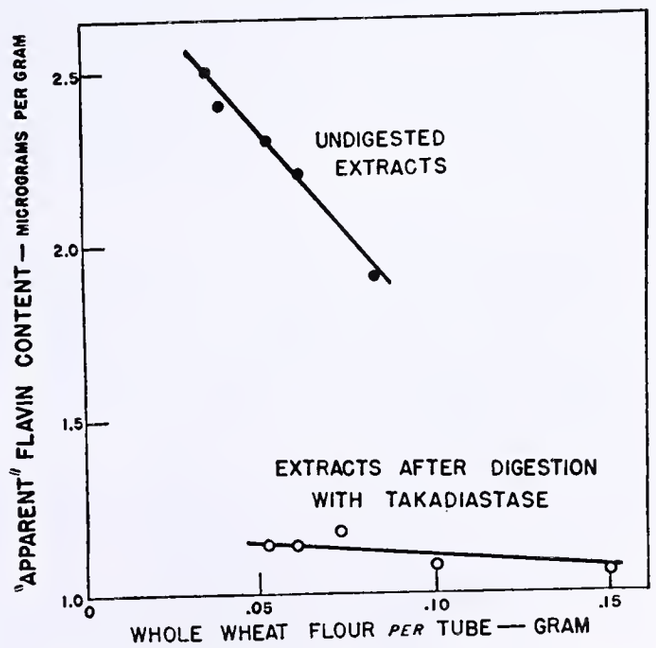

Figure 4. Riboflavin Assays of Aqueous Extracts before and after Digestion with Takadiastase (Whole Wheat Flour)

Table VIII. Recovery of Riboflavin Added to Patent Flour

(Determined fluorometrically)

Sample	Aqueous Extracts	Digested Extracts	Apparent in Flour
	μg./g.	μg./g.	μg./g.
Flour + 0.55 μg. per g.	0.80	0.85	0.30
Flour + 1.1 μg. per g.	1.35	1.40	0.30
Flour + 2.2 μg. per g.	2.40	2.60	0.40
Flour + 4.4 μg. per g.	4.35	4.75	0.35

flour are constant and in good agreement with the 0.35 microgram per gram obtained by "correcting" the microbiological assays of aqueous extracts. This value is also practically the same as that obtained microbiologically from extracts prepared by the diastatic treatment and is believed best to represent the actual riboflavin content of the flour sample.

The same study has also been applied to whole wheat flour (Table IX).

The large difference between the two methods is seen in the values obtained for the aqueous extracts, where the microbiological values were nearly twice those shown in Table IX. The fluorometric examination of the digested extracts gave values somewhat higher than those for the aqueous extracts, indicating that the enzymatic hydrolysis slightly improved the extraction. From these values the apparent riboflavin content of the flour alone has been calculated. The average of 1.03 is in excellent agreement with the 1.05 found by microbiological assay of the digested extract of the same flour, but much below the 1.9 found when the aqueous extract was assayed.

On the basis of the observations reported in this paper it is apparent that the method used in preparing extracts of cereals or cereal products is vital to the success of the microbiological assay. Digestion of the samples with takadiastase is one means of eliminating factors which adversely affect the results. It also improves the extraction of riboflavin and in the case of patent and whole wheat flours it brings into close agreement the results obtained by the microbiological and fluorometric procedures.

There are, however, other cereal products which give somewhat different values by the two methods. Here the discrepancies are apparently due to other factors than those encountered in refined and whole wheat flours. Bran and wheat germ give lower values by the fluorometric method. To some extent these appear to be due to the presence of pigments which are not easily removed by adsorption and oxidation, and, in the case of wheat germ, to nonflavin growth factors other than those removed by takadiastase. Studies to determine the merits of the two methods for such products are now being made.

Experimental

The digested extracts of patent and whole wheat flours were prepared by autoclaving 10-gram and 5-gram samples, respectively, with 90 ml. of distilled water. After cooling to about 50° C., 5 ml. of 6 per cent takadiastase were added and the suspensions allowed to stand for one-half hour. The volumes were then made up to 100 ml. with distilled water and clarified by centrifuging. Aqueous extracts were prepared in the same manner with the omission of the takadiastase.

Microbiological assays of the extracts were carried out by the method of Snell and Strong (*8*).

Fluorometric analyses employed the same extracts and were carried out by a procedure which incorporated modifications of the methods described by Hodson and Norris (*6*) and Conner and Straub (*3*).

A 20-ml. aliquot of the extract was passed through a column of Florisil, and the adsorbent was washed with 5 to 10 ml. of distilled water, and then dried in a current of air. The riboflavin was eluted with a solution of 20 per cent pyridine in 2 per cent acetic acid until the eluate, collected in a graduated cylinder, amounted to 20 ml.

Fourteen milliliters of the well-mixed eluate were pipetted into the cuvette of a Pfaltz & Bauer fluorophotometer and the fluorescence was determined (*A*). Prior to this measurement the intensity of the fluorophotometer light source was adjusted by means of the iris diaphragm to give a 25-scale division deflection of the galvanometer when the cuvette contained an 0.1μg. per ml. solution of sodium fluorescein. Under this condition a solution of pure riboflavin (0.2μg. per ml.) gave a galvanometer deflection of 27 scale divisions. Filters used were a 511–038 combination for the incident light and No. 351 for the fluorescent light.

After determining the fluorescence of the eluate (*A*), 1 ml. of solution containing 1.5μg. of pure riboflavin in 20 per cent pyridine–2 per cent acetic acid was added, and the resulting fluorescence was measured (*B*). A 10- to 20-mg. quantity of solid sodium hydrosulfite was then stirred in to reduce the riboflavin and the fluorescence again observed (*C*).

The riboflavin content of the sample was calculated from the equation

$$\frac{A - 1.07\,C}{B - 0.934\,A} \times 0.1 \times \frac{100}{\text{wt. of sample}}$$

where *A*, *B*, and *C* are the galvanometer deflections in scale divisions, and the numerical values 1.07 and 0.934 are correction factors which compensate for dilution with the added solution of pure riboflavin.

Notes. All the analytical operations were carried out in a darkened room to avoid losses of riboflavin from excessive light.

The sodium fluorescein used was the standard product supplied by the Eastman Kodak Company. Different preparations of this compound may vary in their fluorescence properties, thus requiring concentrations other than that of the solution noted above. The actual concentration should be adjusted to yield a fluorescence intensity similar to that of the pure riboflavin solution.

The takadiastase should not contain sufficient amounts of riboflavin to effect the assay results appreciably. The Parke-Davis product employed in these studies did not contribute significant quantities.

The autoclaving of the cereal with distilled water may result in riboflavin losses if the hydrogen-ion concentration is too low. Under such conditions extraction with dilute acid may be necessary. In the instance of the flours used in the present studies, the pH ranged between 5.5 and 6.0. The quantitative recovery of added riboflavin demonstrated that no measurable extraction losses occurred.

Table IX. Recovery of Riboflavin Added to Whole Wheat Flour

(Determined fluorometrically)

Sample	Aqueous Extracts	Digested Extracts	Apparent in Flour
	μg./g.	μg./g.	μg./g.
Whole wheat flour	0.80	0.95	0.95
Whole wheat + 0.5 μg. per g.	1.35	1.60	1.10
Whole wheat + 1.0 μg. per g.	1.85	2.00	1.00
Whole wheat + 1.5 μg. per g.	2.30	2.55	1.05
Whole wheat + 2.0 μg. per g.	2.80	3.05	1.05
			Av. 1.03

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Extraction of Metals from Aqueous Solutions with Dithizone

LEAD

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The results of a series of studies on the effect of pH, diverse anions, and amount of reagent used in lead determinations with dithizone are reported. It was found that favorable pH ranges for extraction vary considerably with different masking anions, whereas only limited change occurs with variation in amount of excess reagent.

DITHIZONE (diphenylthiocarbazone) is an important reagent for the separation and determination of traces of metals. It has become established in the A. O. A. C. methods for the determination of lead, mercury, and zinc (*1*), while other metals reported as being adaptable to dithizone analysis include bismuth, copper, cadmium, silver, tin, cobalt, nickel, palladium, and thallium. Fischer has published a wealth of data concerning the use of the reagent from both qualitative and quantitative viewpoints. His review articles (*6*, *7*) cover an extensive survey of the progress made with dithizone, and include references to much of the important literature on the subject.

The principal disadvantage of dithizone is its nonspecificity. This problem has been solved partially for particular cases by a number of investigators, but has yet to receive a more thorough and comprehensive examination from a fundamental standpoint. A more selective extraction of metals in the presence of interfering cations has been accomplished in some instances by controlling the pH of the aqueous solution, using masking reagents, or varying the solvents for extracting the dithizonates. Wichmann, in a recent review article (*11*), has given an excellent discussion of these practices; furthermore, he has established a plea for a more critical study of the dithizone system with respect to the equilibrium curves for the various metals, obtained by plotting percentage of extraction against pH value. Clifford and Wichmann (*5*) have initiated this task with a preliminary study of the distribution of lead and its complex in solutions of varying pH.

It is the purpose of this work to repeat the experiments of these men, adhering to a more strict pH measurement, and to augment their results by studying the extractability of lead under varying experimental conditions, including pH, diverse anions, and amount of reagent used. It is hoped that through these and similar investigations, the selectivity of dithizone may be materially increased.

Apparatus

GLASSWARE. Pyrex glassware was used throughout. Since dithizone is a very sensitive reagent, extreme precautions were taken when cleaning the equipment. Two 50-ml. Pyrex burets, employed for measuring the volume of dithizone, were calibrated by weighing.

STILLS. It was necessary to redistill many of the C. P. chemicals used in the lead determinations. For this purpose an all-Pyrex still, adapted from a 1-liter distilling flask, was used. For redistilling water, an elaborate all-Pyrex still, built especially for the purpose, was employed. For effecting a preliminary rectification of chloroform in the recovery process, a 90-cm. (3-foot) Pyrex Vigreux column was found useful.

pH METER. For measuring pH values a glass electrode (quinhydrone in 0.1 *M* hydrochloric acid) was used. The reference electrode was the calomel-saturated potassium chloride type. A "universal" potentiometer assembly described by Mellon (*10*) was utilized.

PHOTOMETER. An Aminco neutral wedge filter photometer was found to give highly satisfactory results in the final determinations. The instrument is designed to give scale readings as a linear function of concentration for solutions following Beer's law. Aminco, style D absorption cells, 0.9995-cm. and 1.9920-cm., respectively, with parallel fused ends, were used. An all-glass, "Pb", color filter (Aminco), having an effective wave length of 510 millimicrons was used in all cases.

COMPARATOR. A Bausch & Lomb Duboscq color comparator was employed in a trial of the balancing method of colorimetry.

SHAKER. In the lead determinations a mechanical shaking apparatus was found to be thoroughly satisfactory as a means of establishing a standard shaking procedure, as well as a time saver. The machine allows direct addition of reagents to the funnels without removing the funnels. It is so constructed as to give rotary, end-over-end motion to the funnels. A 3-minute shaking period was allowed in each case, and was found sufficient.

Reagents

ACETIC ACID, C. P., redistilled, diluted to 0.50 *M*.

AMMONIA SOLUTION (CONCENTRATED), C. P., redistilled into cold redistilled water.

AMMONIA SOLUTION (DILUTE). Concentrated solution diluted to 0.50 *M*.

AMMONIA-CYANIDE SOLUTION. Equivalent of 150 ml. of 15 *M* ammonia solution and 200 ml. of 10 per cent potassium cyanide solution diluted to 1 liter.

AMMONIUM ACETATE SOLUTION, 10 per cent weight by volume from purified salt. Solution was purified by extraction with dithizone solution.

AMMONIUM TARTRATE SOLUTION. A 0.50 *M* solution of reagent grade salt.

CHLOROFORM, U. S. P., redistilled, stabilized with 1 per cent, by volume, absolute ethanol (latter redistilled over solid potassium hydroxide). Chloroform was recovered by method of Biddle (*2*). Considerable difficulty was experienced with chloroform that had been recovered a number of times. Solutions of dithizonates containing this multi-recovered chloroform became cloudy on standing a few minutes after extraction. This cloudiness, which was probably due to an emulsion formation, necessarily vitiated any subsequent photometer readings; however, it was found possible to effect a temporary clarification of the solution by filtering through paper. Apparently the new phase appeared as part of the ternary system, chloroform-alcohol-water. The cause was undoubtedly the accumulation of a fairly high percentage of alcohol during purification, resulting in increased water absorbability. Since the chloroform that had become thus contaminated was rectified with difficulty, it was found expedient to introduce little or no additional alcohol as preservative after the first reclamation process.

Furthermore, in the purification process, it is advisable to reflux chloroform that hints of phosgene formation, for 2 to 3 hours prior to the final distillation. In such case, when phosgene has formed, alcohol addition is recommended to prevent further oxidation.

CITRIC ACID SOLUTION, prepared from C. P. crystals according to method of Clifford and Wichmann (*5*).

DITHIZONE. The solid was obtained from the Eastman Kodak Company. It was found to be of high purity, but it was repurified by the A. O. A. C. method (*1*) as an added precaution.

DITHIZONE STOCK SOLUTION. A 0.1250-gram portion of the purified solid was dissolved in redistilled chloroform and diluted to 500.0 ml.

DITHIZONE STANDARD SOLUTION. The stock was diluted to give 12.5 micrograms of dithizone per ml.

HYDROCHLORIC ACID. C. P. acid was redistilled and diluted to 1 *M*.

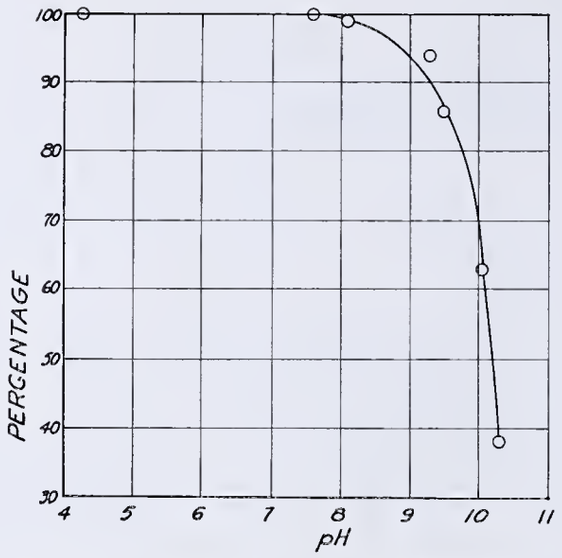

FIGURE 1. EFFECT OF pH UPON RETENTION OF DITHIZONE BY CHLOROFORM LAYER

LEAD NITRATE STOCK SOLUTION. C. P. lead nitrate which had been recrystallized five times and analyzed for lead was used to prepare 500 ml. of solution containing 0.500 gram of lead. A few drops of nitric acid were included.

LEAD NITRATE STANDARD SOLUTION. A 10.00-ml. portion of the stock solution was diluted to 1000 ml. to contain 10 micrograms of lead per ml. The solution strength was checked by the titrimetric and mixed-color dithizone methods of analysis.

NITRIC ACID (CONCENTRATED). C. P. reagent distilled.

NITRIC ACID (DILUTE). Concentrated nitric acid diluted to 0.1 *M*.

POTASSIUM CYANIDE SOLUTION. Reagent grade salt used to make a solution 10 per cent weight by volume.

TARTARIC ACID SOLUTION. A 0.10 *M* solution prepared from analytical reagent solid.

WATER. Redistilled over sulfuric acid from an all-Pyrex still. Stored in an all-Pyrex delivery apparatus.

Preliminary Study in Developing Procedure

SELECTION OF METHOD. A rather extensive study of three general methods of final lead determination was undertaken in order to select the most appropriate procedure for this type of work. Of the methods attempted—titrimetric; "one color", using a Duboscq comparator; and "mixed color", using a filter photometer—the last named was found to be greatly superior to the others. The one-color procedure was abandoned because of the low order of reproducibility attainable and because it proved to be time-consuming; certain other disadvantages were also prominent. The titrimetric method, although of value in certain standardized dithizone determinations, was poorly adaptable to this study.

DITHIZONE PARTITION. Figure 1 shows, in a semiquantitative manner, the partition of dithizone between the chloroform and aqueous layers for varying pH. It is entirely evident that suitable correction must be made for transfer of dithizone to the aqueous layer at pH values above 8 when the mixed-color technique is employed. At values above 10 the partition coefficient rapidly approaches values increasingly in favor of the aqueous phase. Evidently dithizone extractions at higher pH values than 10 are subject to considerable modification before becoming valuable in quantitative determination.

DETERMINATION OF VOLUME OF DITHIZONE NECESSARY. Some trouble was encountered in preparing dithizone solutions of exactly known concentration for use as standards. In the first place, chloroform that had been recovered frequently seemed to cause fading of a solution, evidently through oxidation by phosgene (cf. *4*). Also, since no statement of analysis could be obtained for the dithizone solid used, there was no assurance that the material was sufficiently pure for direct preparation of standards by weight; nevertheless, standard tests (*5*) failed to reveal more than a trace of impurity. In spite of this fact, it was deemed advisable to subject the dithizone to the A. O. A. C. purification method (*1*). A stock solution was prepared from the resulting product by dissolving a carefully weighed amount in fresh, redistilled chloroform. A standard solution, made up immediately thereafter from the stock, gave a reading of 16.4 on the photometer. This value was then assumed to be correct for a solution containing 0.0125 mg. of dithizone per ml. After standing for several days, the standard dithizone slowly lost strength, as evidenced by a gradual decline to a constant photometer reading of 15.4. Since the solution follows Beer's law (*9*), and since the absorption attributive to a small amount of dithizone oxidation product is negligible, it was thought reasonably accurate to use 16.4/15.4 times the volume of dithizone originally calculated to be a 25 or 50 per cent excess. Thus, in the results recorded herewith, the volumes used were all determined on this basis.

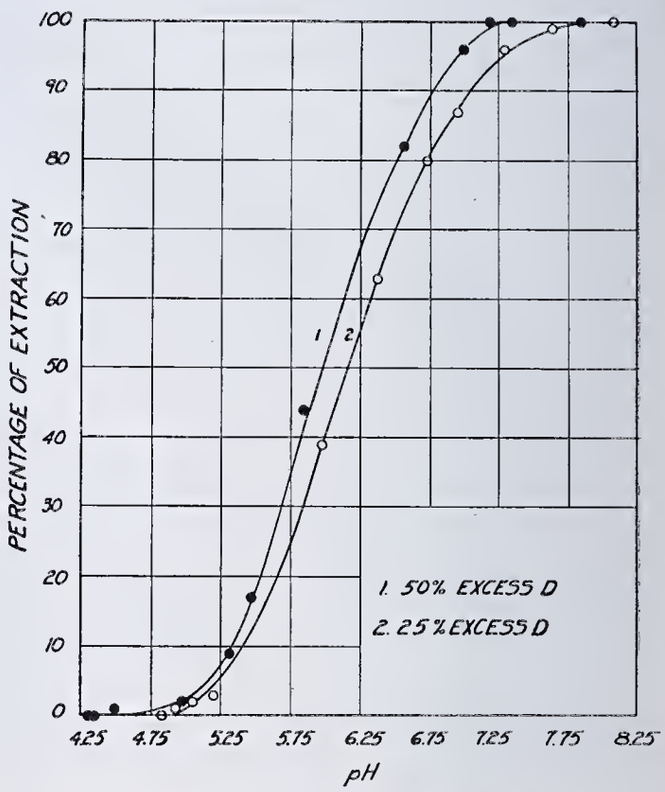

FIGURE 2. EFFECT OF pH AND EXCESS DITHIZONE UPON LEAD EXTRACTION FROM ACETATE SOLUTIONS

STANDARDIZATION. It was found advisable to construct calibration curves for each new dithizone standard prepared, using a constant volume of dithizone solution for amounts of lead ranging from zero concentration to 100 micrograms in 50-ml. samples. These solutions were extracted at previously determined optimum pH values, and in necessary cases, appropriate corrections were applied for distribution of dithizone to the aqueous layer. The resulting photometer readings were plotted against the amount of lead introduced. This plot is a straight-line function. Since the reciprocal of the slope of the straight line has the units $\frac{\gamma\ \text{Pb}}{\text{photometer unit}}$ it is desirable to use this reciprocal as a "lead factor". When the lead factor is multiplied by the photometer reading ob-

tained in the final measurement, the number of micrograms of lead extracted is obtained. In the case of 100-microgram samples, as used in these studies, percentage of extraction is obtained directly.

Blank Determinations. Since the reagent is sensitive to extremely small amounts of constituent, blank determinations are essential in all cases, even though meticulous care may have been used in purification of reagents. It was found experimentally that the best way to determine blank corrections for this type of determination was to run a series of blank analyses for each set of determinations at various pH intervals over the applicable range. The blanks did not reach zero with suitably low pH values, as might have been expected, but the variation was very nearly a linear function of pH value in all cases. Thus, from a few blank determinations it was possible to interpolate for any desired pH value in making corrections in photometer readings.

Correction of Photometer Readings. The percentage of lead extracted from a solution is given by the product of the standardization factor and a corrected photometer reading. This corrected reading was obtained by adding the correction for loss of dithizone in aqueous solution to the original reading on the extracted chloroform solution, and subtracting the appropriate blank.

Determination of pH. The measurements of pH in this work were made with a glass electrode, corrected for temperature variations. The order of reproducibility was ±0.02 pH unit. Clark and Lubs buffers (*3*), prepared from recrystallized potassium dihydrogen phosphate, boric acid, potassium chloride, and C. P. sodium hydroxide, were used in calibrating the instrument.

Lead Extraction

Cyanide-Ammonia-Citrate Curves. The procedure for determining percentage of extraction of lead from cyanide-ammonia-citrate solution was adapted from that of Clifford and Wichmann (*3*). A 10-ml. portion of the ammonia-cyanide solution, 10.00 ml. of standard lead nitrate, and sufficient citric acid solution to bring the pH to the desired value were measured into a 250-ml. separatory funnel by means of burets. The mixture was diluted to 50 ml. with water. A definite volume of standard dithizone solution was added and the funnels were shaken. After allowing the funnels to stand for a few minutes, separation was effected, and 10 ml. of the aqueous layer were drawn off for pH determination. The 4/5 aliquot remaining was acidified with about 15 ml. of 1 *N* hydrochloric acid, and re-extracted with pure chloroform for the purpose of correcting for dithizone that may have been lost to the aqueous layer at higher pH values. At pH values of 8 and lower this step was proved to be superfluous.

A curve determined experimentally by plotting pH values obtained against volume of citric acid solution used, in 10 ml. of ammonia-cyanide solution, proved helpful in deciding the volume of the former solution necessary to approximate desired pH values.

Cyanide-Ammonia–Hydrochloric Acid Curves. The curves for these solutions were obtained by the same technique as the above, 1 *N* hydrochloric acid merely being substituted for the citric acid solution. Difficulties in adjusting pH for certain ranges were encountered, due to the poor buffering action of this solution. Below pH values of about 7.5, only an extremely small amount of acid caused a pronounced drop in pH. It was found advisable to use an approximate volume of acid, shake the mixture, and then note the color of the chloroform phase. By subsequently adding ammonia-cyanide solution or hydrochloric acid solution dropwise, followed by shaking, it was possible to adjust the solutions to obtain a fairly good range of pH values, using the dithizone complex itself as an indicator.

Acetate-Ammonia Curves. The acetate solutions adapted themselves readily to this type of determination. Ten milliliters of the ammonium acetate solution, 10.00 ml. of standard lead solution, and a calculated volume of either ammonia or acetic acid solution were added to separatory funnels and diluted to a total volume of 50 ml.

A theoretical curve of pH plotted against volume of acetic acid or ammonia, as the case might be, was constructed to predetermine amounts of the latter to use. This curve was adaptable with very good success and no difficulty was had in approximating very closely desired pH values. The rate of reaction of the lead with dithizone was considerably slower in acetate solutions, but aside from this fact no peculiarities were noted.

Tartrate-Ammonia Curves. The procedure for tartrate solutions was identical to that for acetate, except that tartaric acid was employed for lowering the pH; 10 ml. of the ammonium tartrate solution were used in each case.

Discussion of Results

A practically negligible difference was obtained for the curves showing extraction with 50 per cent excess dithizone as compared to those representing extraction with 25 per cent excess, for both tartrate solutions and cyanide-ammonia–hydrochloric acid solutions. On the other hand, a shifting of the upper portion of the curve is observed for the acetate solutions, but almost no change for lower pH values (see Figure 2).

By reference to Figure 3 it is observed that for cyanide-ammonia-citrate solutions, the curve for 82 per cent excess dithizone assumes a position nearly 0.5 pH unit to the left of the curve for 37 per cent excess. This is in accord with the expectations of Clifford and Wichmann, but only a limited change can be effected by this procedure, and even then it is not generally applicable to all solutions. The curve for 13 per cent excess dithizone demonstrates the necessity for using considerably more than an equivalent amount of dithizone in a quantitative determination. Between 25 and 50 per cent is advised. Less than 25 per cent may result in incomplete extraction unless more than one extraction is made. More than 50 per cent excess tends to decrease precision through a shorter range in photometer readings.

Figure 4 demonstrates the effect of various complexing anions on the extraction of lead. The function of citric or tartaric acid in the solution to be extracted is to prevent the metals from precipitating as the hydroxide or phosphate (*5*, *8*). It is advisable, therefore, not to use citric acid if some other dodge may be employed. Tartrates, provided they

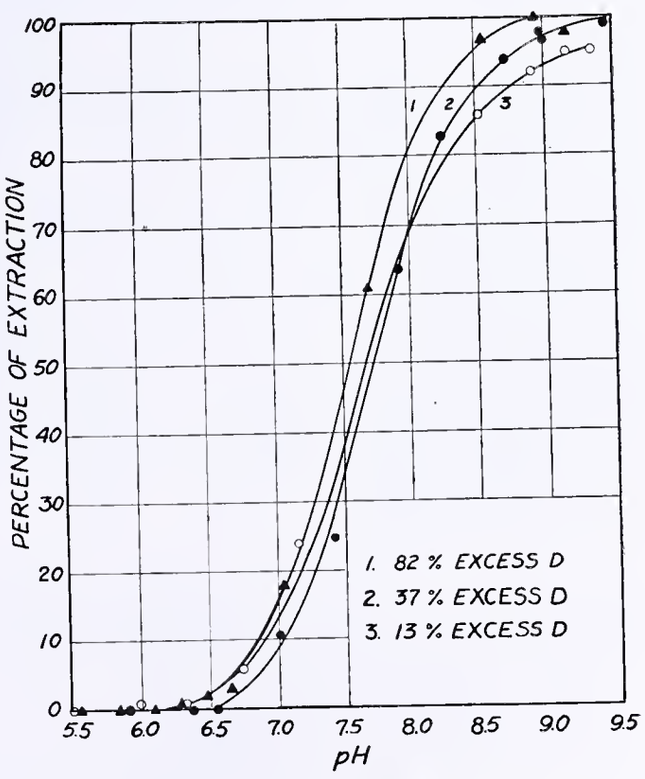

Figure 3. Effect of pH and Excess Dithizone upon Lead Extraction from Cyanide-Citrate Solutions

serve the purpose of citrates, are satisfactory as regards extraction at favorable pH.

Ammonia-cyanide solutions acidified with hydrochloric acid are readily extracted and show the greatest pH range for complete extraction; however, on account of the toxic nature of the hydrogen cyanide formed in overacidification, this latter procedure is not generally recommended.

The use of acetate ion as a complex former is seen not to interfere markedly in the dithizone extraction. Furthermore, it has a decided advantage in that pH is very easily regulated to nearly any desired value. It is suggested that the pH of ammonia-cyanide solutions might be regulated by the use of acetic acid, although these mixtures have not been tried in this laboratory.

High pH values are to be avoided whenever possible in lead determinations because of the partitioning of dithizone to the aqueous phase. In special cases, however, when particular interferences occur, recourse to higher pH may become obligatory.

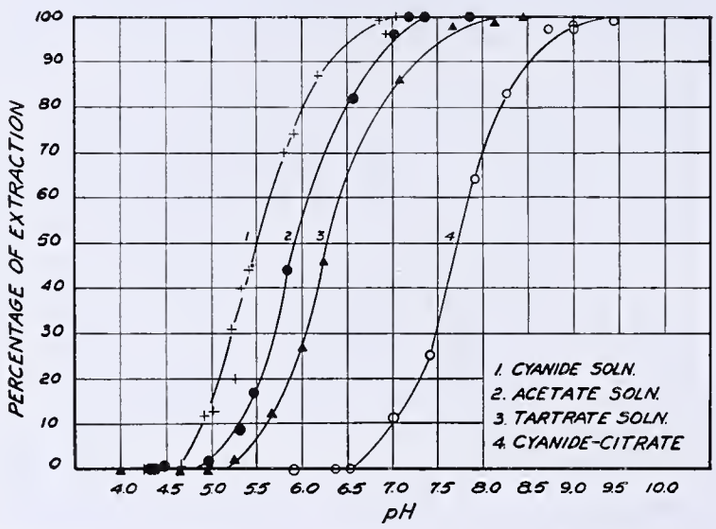

FIGURE 4. EFFECT OF ANIONS UPON LEAD EXTRACTIONS, USING 50 PER CENT EXCESS DITHIZONE

The extraction curves for lead are particularly valuable at and near the points where they contact the 100 and 0 per cent boundaries. This fact may be illustrated aptly by a comparison of curves 1 and 4 in Figure 4. Thus, it is seen that for cyanide-ammonia-citrate solutions a pH value of about 9.5 or greater is needed for complete extraction, whereas, in the case of the cyanide-ammonia–hydrochloric acid solutions, extraction is complete at values greater than 7.0. On the other hand, lead is negligibly extracted below pH 6.5 in the former case, but a value of 4.5 or lower must be attained to realize the same phenomenon in the latter instance. Theoretically, one should be able to calculate from these curves, distribution coefficients for any pH value of a particular solution. Actually this proves to be of little value, inasmuch as the increment in percentage of extraction is comparatively great for a small pH change.

Summary

In comparing three colorimetric methods—balancing, titrimetric, and photometric—the last named was found to be most generally applicable in this study.

At pH values above 8, dithizone is partitioned appreciably between the chloroform and aqueous phases.

In the presence of certain anions, over a given pH range, an increase in excess of dithizone causes a limited increase in percentage of lead extracted.

The anions, cyanide, citrate, acetate, and tartrate, were studied with regard to their effect on the extractability of lead by dithizone.

The use of citrates in extraction solutions is found to be inadvisable.

Improvements in the method of reclaiming chloroform residues are recommended.

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PRESENTED before the Division of Analytical and Micro Chemistry at the 102nd Meeting of the AMERICAN CHEMICAL SOCIETY, Atlantic City, N. J.

Application of Infrared Radiation to Spot-Testing

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THE spot plate is a very useful device in microqualitative analysis, in that it often eliminates special, more expensive apparatus and requires no great skill in microtechnique. Nevertheless, many micro tests have not been considered applicable to direct spot-plate work, because the methods depend upon warming, evaporation, or fusion. Thus, many of the tests given by Feigl (*1*) involve the use of microcrucibles, microburners, microcentrifuge cones, and similar apparatus, as well as appropriate steam baths or water baths.

The author has found, however, that an appreciable number of these tests may be performed directly on the spot plate by using an infrared lamp as a source of heat.

The infrared lamp, preferably with a reflector, is mounted on a ring stand several inches from the base. Heat may then be applied to a mixture on a spot plate by placing the spot plate on the base, directly under the lamp. The heat may be controlled by either of two methods:

1. Regulation of distance between lamp and spot plate. This is accomplished either by mounting the lamp in a fixed position and placing the spot plate on an adjustable ring, or by mounting the lamp so that its height is adjustable and placing the spot plate on the base of the stand. In either case, the adjustable part may be moved into different positions and the approximate maximum temperature noted. Positions for several temperatures may thus be empirically found and indicated on the stand. The heat, of course, will vary inversely as the square of the distance between the spot plate and the lamp.
2. Regulation of voltage.

Among the many micro tests that have been adapted as spot tests by the author are tests for chlorates, ketohexoses, malates, aluminum, Fehling's test, Millon's test, Benedict's test, etc.

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A Simple Method for B_1 Determination

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OWING to the high cost of equipment available for B_1 determinations, the author has developed a comparatively simple and rapid method which involves the use of the catox apparatus (*2*). The principle is the stimulation of yeast by B_1 as described by Schultz, Atkin, and Frey (*3*). The method gives reliable results with very small amounts of material; 0.01 microgram of B_1 can be determined with fair accuracy.

Description of Apparatus

The reaction is carried out in an apparatus similar to the one used for oxidase determinations (*1*), and differs from the other apparatus mainly in two respects: It is used for measurements of increased pressure and the calibration of the manometer indicates directly the weight of carbon dioxide produced.

The apparatus is shown in Figure 1. Compartment 1 contains 1 cc. of nutrient solution (*3*) and 1 cc. of the vitamin-containing extract or thiamin solution of known concentration. Compartment 3 contains 1 cc. of 0.4 per cent yeast suspension (free from added vitamins). Compartment 2 is used only when the effect of other substances on the reaction is to be studied. 4 fulfills a function in catalase determinations and is eliminated for the purpose here described. 5, the manometer, is graduated so that every division is equal to 100 micrograms of carbon dioxide, when apparatus is charged as indicated. 6 is a ground joint provided with corresponding vent holes, 7, to allow for equalization of pressure when manometer is at right angles to the body of the apparatus. When manometer is filled with mercury, the apparatus can be closed by rotating the manometer through 90°. It is then as shown in illustration. 8,8′ is a rubber band which, with the aid of the glass hooks shown, ensures tightness of the apparatus during the reaction.

Method

Six experiments are usually run concurrently. In some of these standard thiamin solutions and in some the unknown, with and without sulfite correction, were used (Table II).

The apparatus is clamped on a shaking machine mounted in a constant-temperature chamber maintained at 30° C. The air vents are adjusted to allow for equalization of internal and external pressure.

After temperature has been maintained at 30° C. for 25 minutes, the apparatus is closed as described, making use of a special trap door in the constant-temperature chamber. The shaking machine is set in motion and readings are made at the end of 90, 120, 150, and 180 minutes. The readings of the unknowns are interpreted in terms of B_1 concentration by interpolation from the readings obtained with the standards. The author's shaking machine has a stroke of 6 cm. and a period of two excursions per second.

A check sample of flour furnished by the American Association of Cereal Chemists was reported as having a B_1 content of 2.9 I. U. per gram (average of three other laboratories). One vitamin-testing laboratory in New York City obtained 2.76 I. U. per gram (average of 3) for the same sample. Results obtained by method here described were 2.75 and 2.83 I. U. per gram.

Table I shows results of a test of hard candy.

Constant-Temperature Chamber

While the temperature should be in the vicinity of 30° C., a constant temperature chamber is not essential if all experi-

TABLE I. HARD CANDY

(Laboratory No. 13,046)

Apparatus	1	2	3
Contents of compartment A	0.5 cc. of 0.25% soln. of sample 1 cc. of nutrient solution	0.20 cc. of thiamin soln. (0.1γ per cc.) 0.30 cc. of H_2O 1 cc. of nutrient soln.	0.40 cc. of thiamin soln. (0.1 γ per cc.) 0.10 cc. of H_2O 1 cc. of nutrient soln.
Contents of compartment B	1 cc. of 0.4% yeast suspension	1 cc. of 0.4% yeast suspension	1 cc. of 0.4% yeast suspension
Temperature, ° C.	31.0	31.0	31.0
Reading at end of 180 minutes	17.9	16.5	19.4

B_1 in 0.5 cc. of 0.25% solution (0.00125 gram of sample) = $0.02 + \frac{0.020}{19.4 - 16.5} \times (17.9 - 16.5) = 0.0297$ microgram

B_1 in 1 gram of sample = 23.76 micrograms

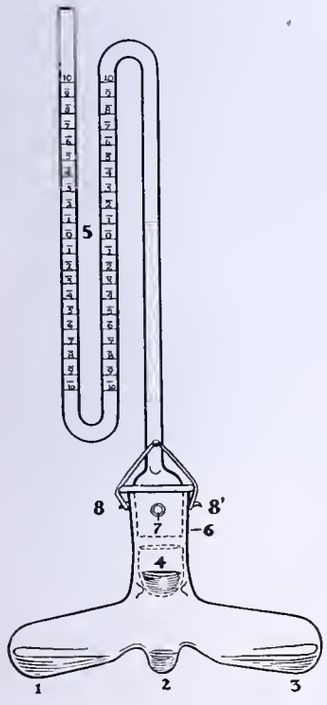

FIGURE 1

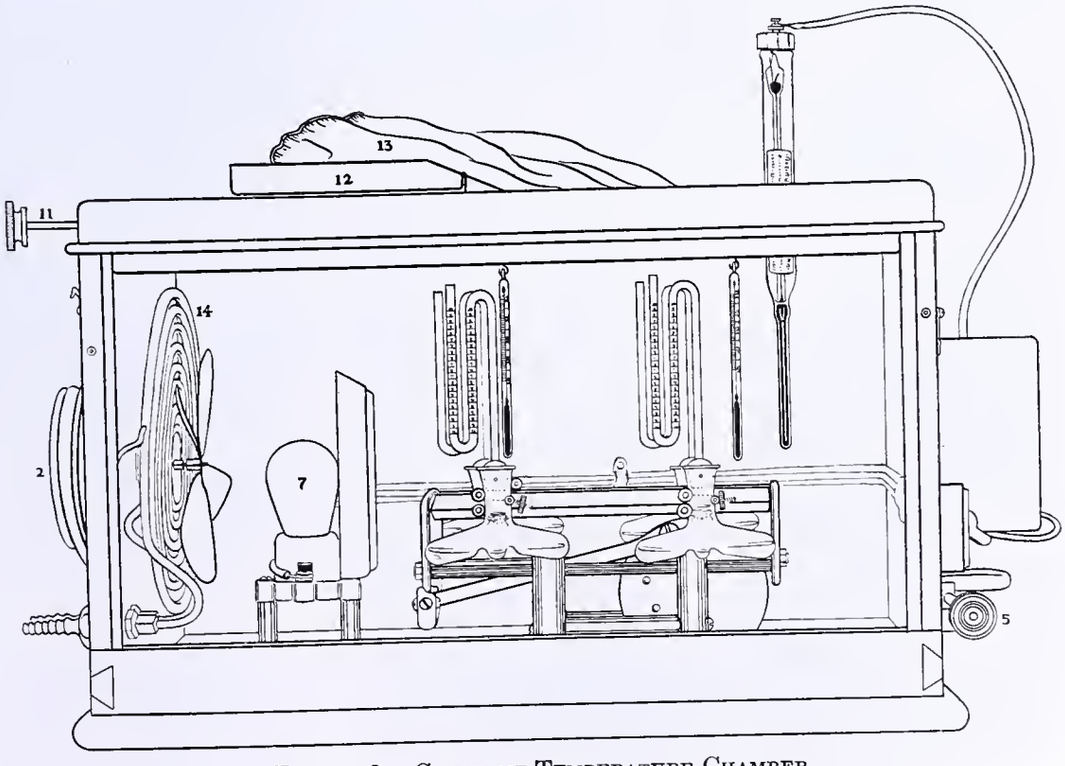

FIGURE 2. CONSTANT-TEMPERATURE CHAMBER

Table II. Readings at the End of 3 Hours

No Thiamin	cc. of Unknown Extract (0.1%)	0.005 γ B_1	0.015 γ B_1	0.025 γ B_1	0.035 γ B_1
4.7	8.5	6.4	7.3	8.4	9.2

Accordingly, the 0.1 per cent extract contains 0.026 microgram per cc., or 26 micrograms per gram of material.

ments are carried on simultaneously under identical conditions.

The constant-temperature chamber used is very convenient for this purpose and is shown in Figure 2. In general, its operation is obvious from the illustration. 12 is a trap door which, when opened, exposes sleeve 13. After arm is slipped into this sleeve, a slide also closing opening and operated by lever 11 admits the arm to turn the manometers. The front is a removable panel with double glass and a black slide to eliminate light. Where light effect is suspected, a black lamp, 7, is used. The motor is mounted in the rear and its shaft operates the fan through pulley 2. It also operates the shaking machine through a reducing gear. Shaking is started and stopped through a clutch arrangement operated by knob 5. 14 is a cooling coil making maintenance of box at 30° C. possible at higher outside temperatures.

The author will be glad to assist investigators in securing the equipment required in connection with the method.

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Micro-Kjeldahl Determination of Nitrogen

A New Indicator and an Improved Rapid Method

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WINKLER (*12*) proposed in 1913 the direct titration of ammonia, absorbed in boric acid solution, by mineral acid for the Kjeldahl determination of nitrogen. A thorough study was later made by Markley and Hann (*3*), who recommended it as a standard method. It has three advantages over the regular back titration method: only one standard solution is needed, there is no danger of spoiling a determination because of insufficient standard acid for the absorption of the ammonia, and in case the content of the receiving flask is sucked back to the distilling flask, through an error of operation, distillation can be resumed without bad effect.

Table I. pH and Color of 2 Per Cent Boric Acid

2% Boric Acid	Mixed Indicator	0.01 *N* HCl	0.01 *N* NH_4OH	Color	pH
Ml.	*Ml.*	*Ml.*	*Ml.*		
5	0.05	...	...	Bluish purple	4.52
5	0.05	0.02	...	Pink	4.26
5	0.05	...	0.02	Bluish green	4.90

Several investigators have tried to adapt Winkler's direct titration method to the micro scale, using as indicator a mixture of methyl red and tetrabromophenol blue (*10*), methyl red and methylene blue (*1*, *9*), or methyl red alone (*4*, *11*). The end point of the titration is located by matching color with a standard, because the indicators show only a gradual transition of color. The amount of boric acid used and the volume of solution in titration have to be carefully controlled (*4*). Consequently, the operation becomes more complicated and time-consuming than Pregl's procedure for micro-Kjeldahl analysis (*8*).

In connection with some work in which a large number of micro-Kjeldahl determinations were required, the writers made an extensive study of the indicators suitable for the titration of ammonia in boric acid, as well as the minimum time required for the micro-Kjeldahl distillation. It was found that bromocresol green (tetrabromo-*m*-cresolsulfonphthalein) compares favorably with the indicators mentioned above. This indicator changes from blue color in ammonia solution to greenish yellow at the end point. While the two colors, blue and greenish yellow, are not easily distinguishable, the transition is rather sharp. A still better indicator has been found, however, in a mixture composed of 5 parts of bromocresol green and 1 part of methyl red, which gives a bluish-purple color in 2 per cent boric acid solution, changing to bluish green in the presence of a trace of ammonia and to pink with a trace of mineral acid. The intensity of color does not vary appreciably with the amount of indicator added. The transition at the end point is very sharp and distinct, a color standard being entirely superfluous.

Pregl (*8*) prescribed 6 minutes for the micro-Kjeldahl distillation. Niederl and Niederl (*5*) reduced the time to 4 minutes, while Hallett (*2*) remarked that 5 minutes are required for a complete distillation. In a review article, Parnas (*6*) indicated that 1.5 to 2.5 minutes are sufficient. The writers found that distillation for 2 minutes with collection of 10 to 15 ml. of condensate recovered completely 1 mg. of nitrogen, the feasible upper limit of micro-Kjeldahl analysis. They also found that a Pyrex flask may be used as the receiver without being previously steamed out, and that a mixture of selenium, copper sulfate, and potassium sulfate may be used as catalyst for the digestion in preference to hydrogen peroxide.

Taking advantage of these observations, the writers have developed a method for micro-Kjeldahl analysis in which the operation is simplified and the time required is considerably shortened. This method has been used in this laboratory and other laboratories, by analysts as well as students, for more than a year with satisfactory results.

Sensitivity of Mixed Indicator

In Table I are listed the pH values obtained by the addition of 2×10^{-4} millimole of hydrochloric acid or ammonium hydroxide to 2 per cent boric acid solution, together with the observed color of the mixed indicator for each pH value, determined by means of a glass electrode.

Distillation Experiments

A solution of ammonium sulfate containing an equivalent of 0.125 mg. of nitrogen per ml. was prepared by dissolving C. P. ammonium sulfate in distilled water. The concentration of the solution was determined by both nitrogen and sulfur analysis.

Measured volumes of the ammonium sulfate solution were delivered from a microburet into a series of micro-Kjeldahl digestion flasks. To each flask was added 0.5 ml. of concentrated sulfuric acid. The content was diluted or concentrated, as the case may be, to about 2 ml. Ammonia was then recovered by distillation in the micro-Kjeldahl distilling apparatus under different conditions as outlined in Table II, which gives the complete group of test distillations. No results have been omitted because of inaccuracy. In the first series (*a*), Pregl's procedure (*8*) was followed, using methyl red as indicator. In the other series (*b* to *f*), 0.05 ml. of the mixed indicator was added to the Pyrex receiving flask, no matter whether it contained 5 ml. of 4 per cent or 2 per cent boric acid.

Table II. Recovery of Ammonia

		Nitrogen Found					
			Direct-Titration Method				
			With 5 ml. of 4% boric acid			With 5 ml. of 2% boric acid	
		(*a*)	(*b*)	(*c*)	(*d*)	(*e*)	(*f*)
$(NH_4)_2SO_4$	Nitrogen Present	Pregl's back-titration method	Steamed out, distilled 3 min., drained 3 min.	Not steamed out, distilled 3 min., drained 3 min.	Steamed out, distilled 1 min., drained 1 min.	Steamed out, distilled 3 min., drained 3 min.	Not steamed out, distilled 1 min., drained 1 min.
Ml.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
8.00	1.000	0.998	1.004	....	....	....	1.000
4.00	0.500	0.497	0.500	0.500	0.500	0.498	0.505
3.00	0.375	0.379	0.378	0.377	0.375	0.379	0.375
2.00	0.250	0.255	0.251	0.246	0.249	0.252	0.246
1.00	0.125	0.124	0.125	0.129	0.124	0.129	0.126
0.50	0.062	0.062	0.063	0.059	0.063	0.062	0.059

These experiments seem to indicate that ammonia recovery is not affected by the method of titration, whether back (*a*) or direct (*b* to *f*); concentration of boric acid (2 per cent or 4 per cent); time of distillation between 2 (*d*, *f*) and 6 minutes (*b*, *c*, *e*); nor by steaming out the receiving flask. The most simple and rapid procedure (*f*) was therefore chosen as the standard method for micro-Kjeldahl analysis.

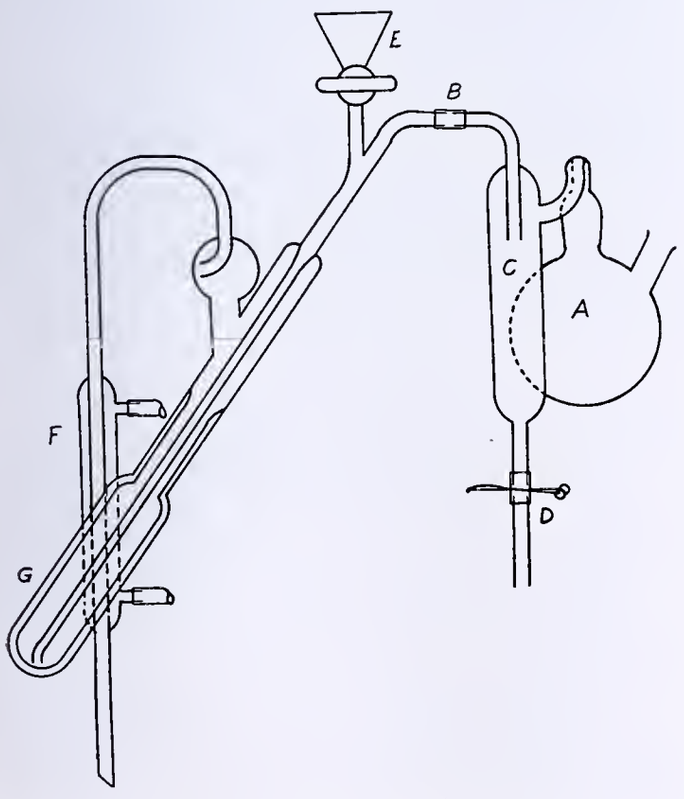

Figure 1. Distillation Apparatus

Analysis of Known Compounds

In order to check the accuracy of the new method, a number of known compounds were analyzed with the procedure described in detail below. The results are shown in Table III.

Apparatus

The apparatus used in the microanalytical laboratory of the University of Chicago is described because it is simple, compact, and easy to make. Other types of apparatus for micro-Kjeldahl analysis may be used without changing the procedure.

Digestion Flasks. The digestion flasks are made from ordinary 15-cm. (6-inch) Pyrex test tubes with the bottom blown out to form a bulb of about 25-mm. diameter and 6-ml. capacity.

Digestion Stand (Figure 1). A digestion stand for 6 flasks is made from a Transite board, 10 × 35 cm., with 6 holes of 22-mm. diameter drilled in it. The board is set on a metal frame with four legs 10 cm. high. A heavy copper wire supports the necks of the digestion flasks. The burner is made from a Bunsen burner with its tube replaced by a copper tubing 35 cm. long. Six tiny holes, about 1 mm. in diameter, are drilled along the copper tubing, 2.5 cm. below the holes of the Transite. The first hole should be about 8 cm. away from the air screw of the burner. A fume duct (*5*) is not necessary when the digestion is carried out in the hood.

Distillation Apparatus (Figure 2). This is a modification of the Parnas-Wagner apparatus (*7*) with the rubber connections eliminated. It is made of Pyrex glass and is built in two compact units joined glass-to-glass with short rubber tubing, *B*. This renders the apparatus less rigid, and reduces the danger of breakage due to bumping when water boils in the steam generator, *A*. The whole apparatus is conveniently clamped onto an iron stand and occupies a desk space of 30 × 40 cm. The steam generator is made from a 1-liter round-bottomed flask to which a side arm is attached for refilling. When it is two-thirds filled with distilled water before distillation is begun, enough steam will be generated for 8 to 12 determinations.

Reagents

Mixed Indicator. Prepare 0.1 per cent bromocresol green and 0.1 per cent methyl red (both indicators purchased from Eastman Kodak Co.) solutions in 95 per cent alcohol separately.

Table III. Analysis Results

Compound	Nitrogen Calculated %	Nitrogen Found %
Benzamide	11.56	11.49
Phthalimide	9.53	9.59
Acetanilide	10.38	10.23
Urea	46.65	46.54
Urotropin	39.99	40.28
Oxamide	31.82	32.08
p-Chloroaniline	10.98	11.01
Tetramethyldiaminobenzophenone	10.45	10.52
p-Bromoacetanilide	6.55	6.63
Benzanilide	7.11	7.07
Glycine	18.66	18.74
Alanine	15.73	15.75
p-Toluidine	13.08	12.91
p-Tolylurea	18.66	18.65
p-Bromoaniline	8.15	8.21
Uric acid	33.34	33.53
$C_6H_5.NH.SO_2.(CH_2)_2CO_2CH_3$	5.76	5.72
p-Anisidine	11.38	11.45

Mix 10 ml. of the bromocresol green with 2 ml. of the methyl red solution in a bottle provided with a dropper drawn out into a fine capillary. The dropper delivers about 0.05 ml. per 4 drops.

Boric Acid 2 Per Cent. Dissolve 10 grams of boric acid (crystal) in 500 ml. of boiling distilled water. After cooling, transfer the solution into a glass-stoppered bottle. It keeps indefinitely.

Hydrochloric Acid 0.01 *N*. Dilute standard 0.1 *N* hydrochloric acid quantitatively. Check the concentration of the final solution against pure sodium carbonate or by chlorine analysis.

Sodium Hydroxide 30 Per Cent. Dissolve 150 grams of sodium hydroxide pellets in 350 ml. of distilled water. Store the solution in a bottle closed with a rubber stopper.

Catalysts. Powdered selenium; pulverized mixture of potassium sulfate (1 part) and copper sulfate pentahydrate (3 parts). Only a small quantity is needed.

Procedure

Digestion. Weigh out a 2- to 5-mg. sample, containing 0.2 to 1 mg. of nitrogen, and transfer it into the bottom of the digestion flask. Use a long-handled charging tube for solid samples. For semisolids and heavy oil, use a porcelain microboat (Coors, size 00000) which is slid into the digestion flask with the sample. Add about 3 mg. of powdered selenium and 5 mg. of copper sulfate–potassium sulfate mixture, followed by 1 ml. of concentrated sulfuric acid. When a large volume of the sample is taken, as in the case of biological fluids and dilute solutions, it is better to acidify the sample (after introduction into the digestion flask) with a drop of sulfuric acid, and concentrate the volume to less than 1 ml. before adding the catalyst and 1 ml. of concentrated sulfuric acid. Place the digestion flask on the digestion stand and boil the digestion mixture gently with a flame about 2 cm. high. The reaction is usually complete in 10 minutes. After cooling, add 2 ml. of distilled water, mix, and again cool.

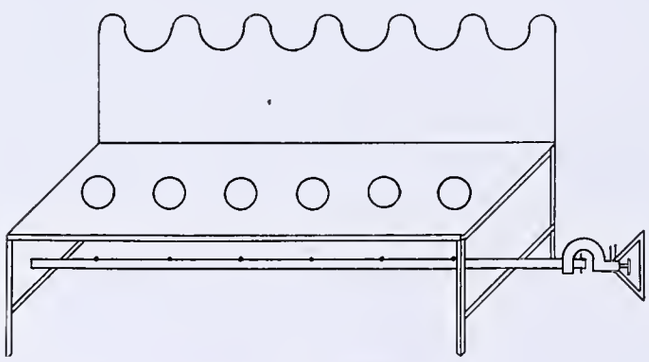

Figure 2. Digestion Rack

Distillation and Titration. A 25-ml. graduated cylinder for 30 per cent sodium hydroxide, a 5-ml. pipet for boric acid, and several 50-ml. Pyrex Erlenmeyer flasks are needed. Place a beaker under the condenser (*F*, Figure 2). Boil the distilled water in the steam generator, *A*, with a flame from the Bunsen burner about 10 cm. high, stopcock *E* and pinch clamp *D* being closed. Cold water is running in the condenser, from which about 5 ml. of distillate should collect per minute. Remove the burner, whereupon the condensate in the distilling flask, *G*, is sucked back into the steam trap, *C*. Fill funnel *E* with distilled water and open the stopcock momentarily to drain the water into *G*. Replace the burner under the steam generator for about 20 seconds and remove it again. Meanwhile rinse a 50-ml. Pyrex Erlenmeyer flask with distilled water. Introduce 5 ml. (need not be accurately measured) of 2 per cent boric acid and 4 drops of the mixed indicator into the Erlenmeyer flask. Fill the microburet with 0.01 *N* hydrochloric acid to the zero mark. By this time the distilling flask, *G*, is empty.

Replace burner under the steam generator, and open pinch clamp *D* to remove liquid from the steam trap, *C*. Leave the pinch clamp on the glass tubing through which the steam escapes. Replace the beaker under the condenser with the Erlenmeyer flask containing boric acid, and support the flask in an oblique position, so that the tip of the condenser is completely immersed in the liquid. Smear a trace of vaseline on the lip of the digestion flask to prevent the liquid from dribbling down outside. Hold the digestion flask in one hand, open the stopcock *E* with the other hand, and pour the digestion mixture through the introduction funnel into *G*. Rinse the digestion flask twice with about 2 ml. of distilled water, then introduce 8 ml. of 30 per cent sodium hydroxide and close stopcock *E*. Replace the pinch clamp *D* on the rubber tubing, whereupon steam enters *G*, stirs up the digestion mixture and sodium hydroxide, and liberates ammonia which escapes with steam through the condenser into the boric acid solution.

The boric acid changes from bluish purple to bluish green as soon as it comes into contact with ammonia. The change, which is very sharp, takes place between 20 to 60 seconds after the pinch clamp is closed and usually coincides with the time when the first drop of condensate reaches the Erlenmeyer flask. One minute after the boric acid has changed color, lower the Erlenmeyer flask so that the condenser tip is 1 cm. above the liquid. Wash the end of the condenser with a little distilled water. Continue distillation for another minute, then remove the burner. Bring the Erlenmeyer flask to the titration stand and titrate until the blue color disappears. (If preferred, the titration may be continued until a faint pink tinge appears; 0.02 ml. is then subtracted from the buret reading. There is no danger of missing the end point, because after the pink tinge appears, the intensity of pink color increases tremendously with a trace more 0.01 *N* hydrochloric acid. The titration may be done in daylight or artificial light.) The distilling flask *G* is now again empty. *E* is washed with distilled water as described above, and distillation of the next sample follows. Distillation and titration require from 5 to 8 minutes.

Conclusion

This paper presents a simple and rapid method for micro-Kjeldahl analysis. The sample is digested with sulfuric acid in the presence of selenium, copper sulfate, and potassium sulfate. The ammonia is distilled into 2 per cent boric acid solution and titrated directly with standard 0.01 *N* hydrochloric acid, using a mixed indicator which consists of 1 part of methyl red and 5 parts of bromocresol green. After digestion of the samples, which is usually complete in about 10 minutes, an operator can easily run 8 to 10 distillations and titrations in an hour.

The present method eliminates the standard alkali and conserves the standard acid. The mixed indicator gives a sharp and clear-cut end point. Its characteristic of giving distinctly different colors to 2 per cent boric acid containing a trace of ammonia or mineral acid has certain merits: (1) a receiving flask contaminated with acid or base is immediately discovered when the boric acid and mixed indicator are introduced, and (2) in the course of distillation, if the boric acid does not change from bluish purple to bluish green with the arrival of the first drop of condensate, the operator is informed that either the sample is nitrogen-free or the digestion mixture has not been sufficiently neutralized with sodium hydroxide.

The writers also recommend the mixed indicator for macro- and semimicro-Kjeldahl analysis using the boric acid absorption method, because it gives a better end point than any other indicator reported in the literature for this purpose.

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Amperometric Titration of Copper with Benzoinoxime

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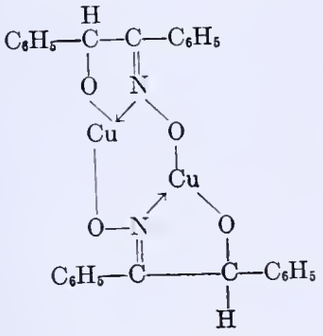
ALPHA-BENZOINOXIME (cupron) was first recommended as a specific organic reagent for copper in an ammoniacal solution by Feigl (*2*) in 1923. Since then the reaction with copper has been studied, not only in regard to its analytical use, but also from the viewpoint of the chemistry of complex compounds. The reason for the latter investigations was the surprising stability of the salt in ammoniacal solutions, although the copper in the established formulas does not seem to have its normal coordination number of four. Feigl (*3*) explains this behavior by assuming chelation between the copper and the benzene rings. Investigations done jointly with Dubský (*1*) suggested the possibility of the formation of a basic "diol" salt; addition of ammonia was also observed. But before any other explanation is proposed, molecular weight determinations or x-ray diffraction work should be used to exclude the possibility of a dimeric structure:



With copper, the reaction is specific only in an alkaline (ammoniacal) solution. In acid solutions other metals are also precipitated; thus benzoinoxime was used for the determination of molybdenum by Knowles (*4*). In the work reported here only the copper reaction was investigated.

Apparatus and Reagents Used

A compensating type of polarometer with a Leeds & Northrup Type K potentiometer was used, the cell having an external saturated calomel electrode as reference electrode. Oxygen was expelled from the supporting solutions by purified nitrogen. The general procedure of titration was that described in previous papers (*5*).

The 0.01 *M* copper sulfate stock solution was prepared from recrystallized C. P. copper sulfate, and to prevent basic salt formation a drop of 0.1 *M* sulfuric acid was added to 250 ml. The 0.01 *M* stock solution of benzoinoxime was prepared from the Eastman Kodak chemical by dissolving in 50 per cent ethyl alcohol. The supporting solutions were made up from Baker's C. P. chemicals in redistilled water.

Current-Voltage Curves of Copper and Benzoinoxime

It was found, in accordance with the observations of Stackelberg and Freyhold (*7*), that the cupritetrammino ion is reduced in a supporting solution of 0.1 *M* ammonium chloride and ammonium hydroxide in a double wave with a half-wave potential of approximately −0.17 volt for the reaction $Cu^{++} \rightarrow Cu^{+}$ and −0.40 volt for the reaction $Cu^{+} \rightarrow Cu$.

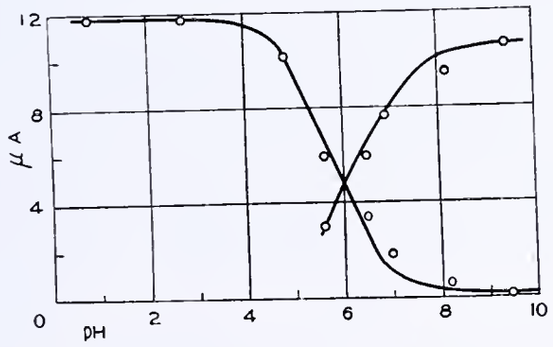


FIGURE 2. WAVE HEIGHT CHANGE FOR APPROXIMATELY 10^{-3} *M* BENZOINOXIME WITH pH OF SUPPORTING SOLUTION

Both half-wave potentials are shifted with increasing or decreasing ammonia concentration to more negative or positive values—about 0.1 volt per pNH_4OH. The waves were without maxima if a few drops of a 10 per cent gelatin solution were added.

The current-voltage curves of benzoinoxime were taken in buffer solutions of different pH—acetic acid–sodium acetate, boric acid–sodium hydroxide, and ammonium chloride–ammonium hydroxide. The pH was measured with a Beckman glass electrode pH meter. For a given concentration of the oxime in the acid region, a wave was observed having a half-wave potential varying with the pH of the solution as $E_{1/2} = -(0.71 + 0.6\ pH)$ volt. There was also found to be a pronounced dependence of the wave height on the pH, as indicated in Figure 1. If we plot the diffusion current per millimole per liter for different values of the pH, we obtain a sigmoid type of curve (Figure 2). From Figures 1 and 2 we can also see that when the first wave is disappearing, another wave at a more negative potential appears. The second wave can be observed only after pH 5 is exceeded, because of the interference with the hydrogen wave. When the first wave is diminishing (between pH 4 and 8) maxima are formed which make the determination of the diffusion current of the first wave uncertain. The figures also indicate that the sum of the two waves is almost constant. The half-wave potential of

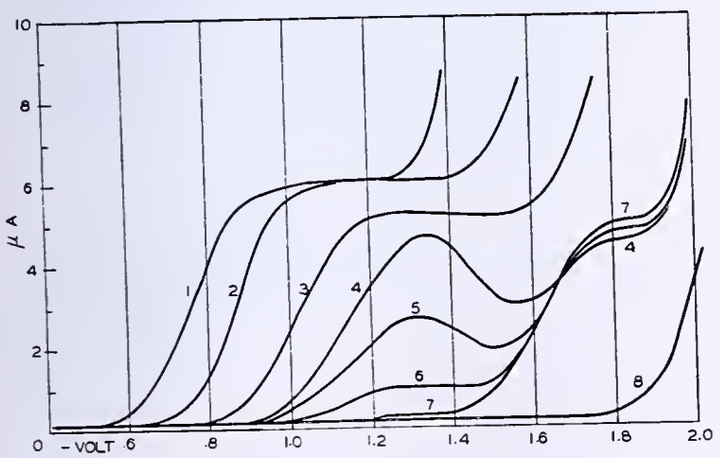


FIGURE 1. CURRENT-VOLTAGE CURVES

Approximately 5×10^{-4} *M* α-benzoinoxime in buffer solutions of indicated pH

1. pH = 0.8
2. pH = 2.7
3. pH = 4.8
4. pH = 5.6
5. pH = 6.5
6. pH = 7.0
7. pH = 8.2

Temperature 22–24° C., Saturated Calomel Electrode t = 2.9 seconds, m = 1.64 mg. sec.$^{-1}$ Hg

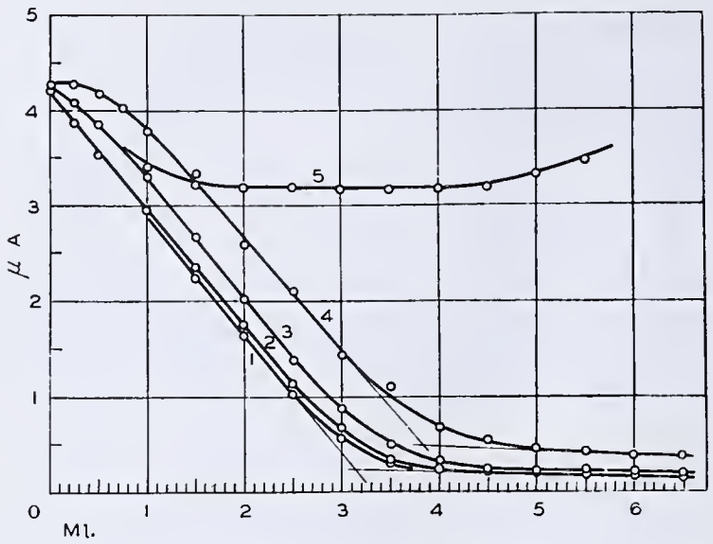


FIGURE 3. TITRATION CURVES

As in Figure 4 at an applied potential of −1.0 volt

Concentration of ammonia:
1. 0.01 *M* 3. 0.1 *M* 5. None
2. 0.05 *M* 4. 0.5 *M*

the second wave is around −1.63 volt and is not markedly influenced by the pH of the solution.

This result leads to the conclusion that we have a mobile equilibrium between an acid-stable and an alkaline-stable form of benzoinoxime, each form being reduced at a different potential. Both forms are present in equal concentrations at pH 6.0. Preliminary investigations showed that acetaldoxime and acetophenonoxime behaved very similarly, and therefore that the change is probably connected with the oxime group. A possible explanation would be a tautomeric equilibrium such as oxime ⇌ nitroso, or more probably an equilibrium between the ion and the molecule:

$$\rangle C = N - O^- + H^+ \rightleftharpoons \rangle C = N - OH$$

The neutral molecule would be favored by the increasing acidity of the solution and reduced at more positive potentials. The latter reaction gives

$$\frac{\left[\rangle C = N - O^-\right]\left[H^+\right]}{\left[\rangle C = NOH\right]} = Ka$$

If A is the total amount of oxime present, and x the amount ionized at any pH, we obtain

$$x = \frac{A}{1 + \frac{[H^+]}{Ka}}$$

At $x = \frac{A}{2}$ we have the condition that $[H^+] = Ka$, and we find $Ka \doteq 10^{-6}$ as the dissociation constant of benzoinoxime.

Titration of Copper with Cupron

From the current-voltage curves of copper and the reagent in an ammoniacal solution, it can be deduced that in the presence of a maximum suppressor at applied potentials between −0.8 and −1.4 volt, L-shaped titration curves would be obtained, whereas at potentials higher (to about −1.9 volt) the V-type titration curves should result. This was actually observed.

Titrations made in supporting solutions of 0.1 *M* ammonium chloride with variable amounts of ammonia from 0.001 to 0.5 *M* are shown for an applied potential of −1.0 volt in Figure 3 and for an applied potential of −1.7 volt in Figure 4. With increasing ammonia concentration the titration curves deviate strongly from straight lines, so that a determination of the end point by the tangent method is made with considerable difficulty and large errors result. This result led to a theoretical investigation with Stevenson (*6*), where the shapes of the ideal titration curves were calculated for substances with different solubilities. It was shown that the curves in a precipitation reaction $A^{n+} + B^{n-} \rightleftharpoons AB$ should consist of a straight line and a hyperbola, depending on $\frac{S}{A_0}$ where S = solubility product and A_0 the initial amount of substance present. From the shapes of the curves in the copper-cupron titration, we can conclude that the solubility of the copper salt increases strongly with increasing concentration of ammonia. The same behavior was found when the titration was carried out in a supporting solution of 0.1 *M* potassium chloride with different concentrations of ammonia. During the titrations it was observed that in 0.5 *M* ammonia the precipitate formed was light green and crystalline, as contrasted with the much darker green precipitates in lower concentrations of ammonia. If the titration was carried out in ammonium chloride or potassium chloride alone, no precipitate was formed and the titration curve was very flat, as shown in curve 5, Figure 3. The titration curve was similar at an applied potential of −1.7 volt. These curves might indicate formation of a soluble complex of copper with the reagent.

To use the tangent method for the end-point determination of copper in solutions less than 10^{-3} *M*, the ammonium hydroxide concentration has to be low; 0.02 *M* solutions were used. The titer of the reagent was taken as the average of five titrations under the same conditions. The values obtained agreed within 1 per cent. Large graphs were used to correspond to the accuracy of the buret (5 ml. with 0.01-ml. divisions) and potentiometer readings.

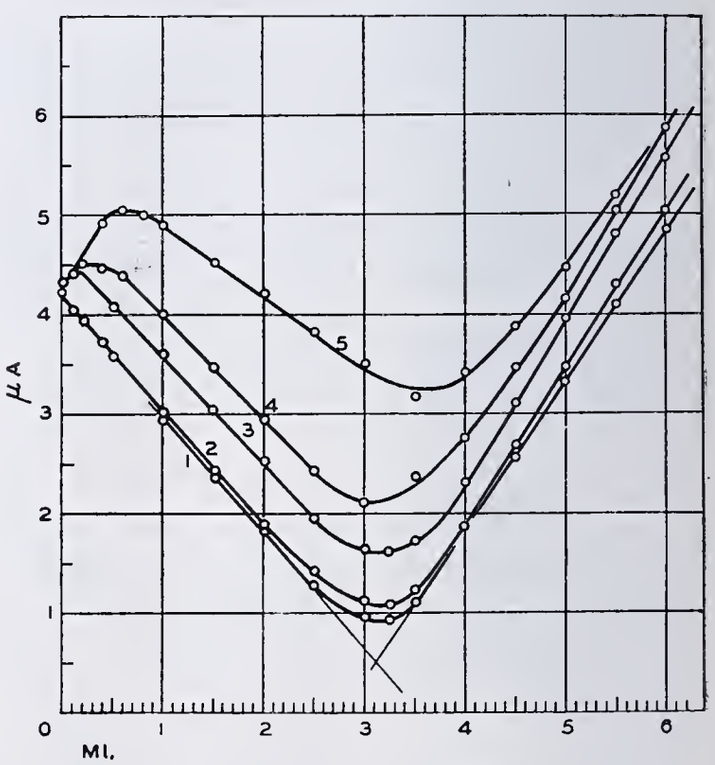


FIGURE 4. TITRATION CURVES

1.907 mg. of copper in 45 ml. of supporting solution of 0.1 *M* ammonium chloride with varying concentration of ammonia in presence of 4 drops of 10% gelatin with approximately 0.01 *M* benzoinoxime solution in 50% alcohol. Applied potential −1.7 volt

Concentration of ammonia:
1. 0.01 *M* 3. 0.1 *M* 5. 0.5 *M*
2. 0.05 *M* 4. 0.2 *M*

Temperature, 23° C., t = 3.0 seconds, m = 1.64 mg. sec.$^{-1}$ Hg

In Table I are given a few results obtained with different amounts of copper in 40 ml. of 0.1 *M* ammonium chloride and 0.02 *M* ammonia with 5 drops of 10 per cent gelatin, obtained at an applied potential of −1.7 volt.

TABLE I. DETERMINATION OF COPPER

Used	Found	Error
Mg.	*Mg.*	*Mg.*
0.636	0.646	+0.010
1.271	1.290	+0.019
1.907	1.910	+0.003
2.543	2.538	−0.005

The possibility of using 0.001 *M* benzoinoxime for titration of 0.05 to 0.5 mg. of copper in 20 ml. of 0.1 *M* ammonium chloride and 0.01 *M* ammonia was studied, but the points obtained were so scattered that only approximate lines could be drawn. The errors were sometimes more than 10 per cent.

Influence of Other Ions

The interference of other metal ions on the copper titration was investigated briefly. Ferric ions present in about double the amount of copper and precipitated as hydroxide gave results a few per cent too low in 0.02 *M* ammonia, but when the concentration of ammonia was increased to 0.1 *M*, the results were only about 1 per cent too low. In the presence of precipitated lead, the results were somewhat higher. Zinc present in five times the concentration of copper gave values 6 per cent too high in 0.1 *M* ammonia, but if present in smaller concentration than the copper, the results were only 0.5 to 2 per cent too high. With small amounts of zinc, the V shape of titration curve could also be used. Nickel interferes strongly.

Summary

α-Benzoinoxime was investigated as a possible reagent for amperometric (polarometric) titration of copper.

It was found that the reagent is reduced at the mercury dropping electrode in waves, with half-wave potentials and wave heights which depend on the pH of the supporting solution. It is suggested that the wave height dependence is a result of a mobile ionization equilibrium. It was also observed that the shapes of the titration curves depend strongly on the concentration of ammonia, because of the increasing solubility of the precipitate. For 10^{-3} *M* copper solutions, concentrations of ammonia less than 5×10^{-2} are favorable. The results obtained are accurate to within about ±1 per cent. The influence of a few other metals was studied.

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Distribution of Salt in Butter

A Volumetric Micromethod

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THE macromethods for the determination of salt in butter are adequate for obtaining the average salt content of a churning, but from the bacteriological standpoint such general information is inadequate. Since butter is not a homogeneous mass and organisms are not uniformly distributed through it, the bacteriological changes in a lot of butter are the combined result of the changes in numerous small portions. Accordingly, in studying the effect of salt distribution on bacterial action in butter, salt contents of as small portions as possible are desired, and for this reason a microprocedure for the determination of salt in butter was developed. The method can also be applied to various other products.

Apparatus and Reagents

MICROSCOPE. A Spencer, wide-field, low-power, binocular microscope, having a ×6 magnification, was used in picking the portions of butter.

MICROBALANCE. An Ainsworth microbalance (Type FDJ) was employed.

MICROSPOONS. The microspoons in which the butter samples were weighed and ashed were made by fusing pieces of platinum foil about 7 mm. in diameter to 24-gage platinum wires 2.5 cm. in length. The centers of the spoons were made slightly concave, and the wires were bent so that the spoons were level when hanging.

SPOT PLATE. The titrations were made in a white porcelain spot plate with depressions 20 mm. in diameter and 5 mm. deep.

CAPILLARY BURETS. The burets needed for this work are shown in Figure 1. They were made from pieces of capillary tubing, which was tested for uniformity of bore by introducing enough mercury to form a column about 2 cm. long and moving this mercury along the tube while measuring its length. Uniform pieces of tubing about 30 cm. long and 1 mm. in bore were selected. One end was drawn out to a fine tip. A piece of rubber tubing was attached to the upper end and, equipped with a plug and screw clamp, served to fill and discharge the buret. A celluloid ruler served as a scale.

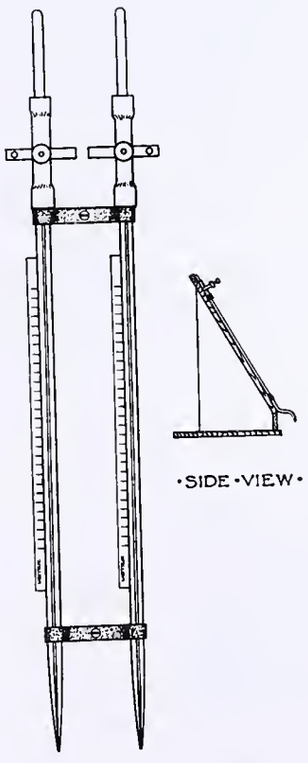

FIGURE 1. BURETS

Calibrating the burets was done by weighing various amounts of mercury on an ordinary analytical balance. The bores of the capillaries were sufficiently uniform so that a single calibration factor was determined for each buret, and used to convert the length of column of solution discharged to cubic centimeters.

For the titrations, a special technique must be used. The buret is filled by compressing the screw clamp, immersing the tip in a small tube of reagent, and unscrewing the clamp. It is filled a little above the top mark on the scale. The tip is wiped with a cloth or piece of filter paper and is then just touched to the surface of distilled water in another tube and the screw clamp is

Table I. Microtitrations on 0.01 *N* Sodium Chloride Solution with Approximately 0.01 *N* Silver Nitrate

Trial No.	Cm. Divisions on Buret Scale Corresponding to Volume of Solution: NaCl solution	$AgNO_3$ solution
1	14.0	14.1
2	14.0	14.2
3	14.0	14.1
4	14.0	14.1
5	14.0	14.1
6	14.0	14.2
7	13.0	13.1
8	13.0	13.1

Table II. Salt Distribution in Normal Commercial Butter

Churning No.	Incorporation of H_2O in Butter	NaCl, Macro-method	NaCl in Microsample									
			No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No .8	No. 9	No. 10
		%	%	%	%	%	%	%	%	%	%	%
1	Very good	2.53	2.40	2.21	2.27	2.48	2.53	2.26	2.48	2.46	2.65	2.68
2	Good	2.35	2.50	1.77	2.18	1.83	2.14	2.17	2.09	1.90	1.92	1.98
3	Fair	2.21	2.46	2.26	2.36	2.36	2.17	2.19	3.26	2.05	2.06	2.28
4	Very poor	1.74	1.24	1.48	2.16	1.69	4.32	1.86	2.82	1.99	1.82	2.16

compressed until the level in the buret is brought exactly to the mark. The movement of the liquid in the capillary is smooth and precise only when the tip is immersed.

Sodium Chloride Solution. A 0.01 *N* sodium chloride solution was prepared from reagent quality sodium chloride.

Silver Nitrate Solution. An approximately 0.01 *N* silver nitrate solution was prepared and standardized against the standard sodium chloride solution, using the microtitration that was later employed for the analyses. The silver nitrate solution was kept in a dark bottle covered with aluminum foil and the concentration was checked at frequent intervals.

Dichlorofluorescein Solution. One-tenth gram of dichlorofluorescein was dissolved in 60 ml. of ethyl alcohol (95 per cent) and the volume made up to 100 ml. with distilled water. One milliliter of this solution was then diluted with 9 ml. of ethyl alcohol.

Procedure

The butter to be studied was tempered and held at approximately 13° C. for sampling. This temperature was low enough so that the original body and texture of the butter were not changed and yet high enough to avoid any great tendency for moisture from the air to condense on the sample. To minimize the chances of weighing condensate from the air, a freshly exposed surface was used for each microsample, and the butter was broken instead of cut, so that the original texture at the freshly exposed surface was not changed.

An approximately 0.2-mg. (±0.05-mg.) portion of butter was picked under the microscope with a dissecting needle and placed in a weighed microspoon. The spoon containing the butter was hung on the pan of the microbalance and the weight determined to within 0.002 mg. No difficulty was experienced in weighing the microsamples except on warm, humid days; on such days moisture condensed on the cool sample, and then when the sample was placed on a relatively warm platinum spoon the temperature rather quickly increased and the moisture slowly evaporated.

The microspoon containing the sample of butter was hung on a Nichrome wire drawn across the top of a 15-ml. porcelain crucible. As the crucible was heated slowly with a microburner, the butter melted and spread in a thin film over the entire surface of the spoon; no spattering of the fat could be observed. After the butter had charred, the heat was increased, the crucible being heated just to redness until all the carbon had oxidized and only the ash and salt remained. With proper spacing of the spoon in the crucible, the heating could be completed in 5 minutes without loss of sodium chloride.

The ash and salt were transferred to the spot plate in which the titration was to be made by placing the spoon in a drop of water in a depression and covering the spoon with another drop of water. After several minutes the salt solution was washed from the spoon with 3 or 4 drops of water followed by 3 or 4 drops of 95 per cent ethyl alcohol. The addition of alcohol sharpened the end point and facilitated removal of the salt solution. The volume of the solution was approximately 0.5 ml. and was not allowed to become much greater than this, since the color change in the titration was more distinct in a relatively concentrated solution.

The color change at the equivalence point is due to the adsorption of the indicator on the surface of the precipitate. Accordingly, increasing the surface of the precipitate also increases the visibility of the color. The surface of the silver chloride precipitate was increased in the determination by adding from a capillary buret a volume of 0.01 *N* sodium chloride solution equivalent to 10 to 12 cm. on the buret scale. The addition of sodium chloride also corrected any error due to the color change at the end point

A small drop of dichlorofluorescein was added with a capillary pipet, and the solution was titrated with silver nitrate. The solution was stirred continuously during the titration, and the first permanent color change was taken as the end point. The titrations were made in a dark room under a fluorescent light.

The amount of silver nitrate in excess of the amount necessary to react with the added sodium chloride was equivalent to the sodium chloride from the butter. From the results of the titration and the weight of the sample, the percentage of salt in the sample was calculated.

In some samples of butter large water droplets were present on freshly cut surfaces. The salt contents of these individual droplets were determined as follows: A portion of each droplet was removed with a very small, weighed capillary tube, the ends of the tube were sealed with a small flame, and the tube was reweighed. The tube was crushed in a few drops of water in the depression of a spot plate and the chloride titrated. The salt concentration of the solution was high enough so that no additional sodium chloride was necessary.

Accuracy of Titration

The accuracy of the titration could not be determined by analyses on duplicate samples, since butter is not a homogeneous mass and duplicate microsamples could not be obtained. However, the accuracy was checked by titrating a 0.01 *N* sodium chloride solution, using the microprocedure, and expressing the volumes of the solutions as centimeter divisions on the buret scale.

Table I gives the results of eight titrations of the 0.01 *N* sodium chloride solution. The titration values checked to within 0.1 cm. on the buret scale; this represents a volume of 0.0004355 ml. of an approximately 0.01 *N* silver nitrate solution. Since the water-alcohol solution of the ash and salt was clear, it is probable that the titrations made on the butter samples were as accurate as those made on the salt solution.

Analyses of Butter

The distribution of salt was studied in normal commercial butter from a number of churnings. With each churning ten microsamples were picked from a 15-gram portion of butter. The data on four representative churnings are given in Table II. These churnings ranged from thoroughly worked to very poorly worked butter, as judged by the size and number of moisture droplets appearing on freshly cut surfaces of the cold butter. The analyses show that the salt contents of microportions of butter vary considerably, even in thoroughly worked butter. In poorly worked butter the variations are much greater.

Summary

A reasonable degree of accuracy undoubtedly can be obtained in determining the sodium chloride content of an approximately 0.2-mg. sample of butter. However, the salt content of such a microsample is not representative of a churning of butter. The value of the method lies in the study of the salt distribution in butter, and it is particularly useful in the study of the effect of salt distribution on bacterial action.

The method should find application in the study of nonuniformity in other products. For homogeneous materials it is a rapid and a precise method for chloride analysis.

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Colorimetric Determination of Phosphorus in Iron Ore

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IRON interferes indirectly with the colorimetric determination of phosphorus. A sample run by the regular procedure but without addition of the final reagent is colorless to the eye, and regardless of the percentage of iron in the sample the transmittance at 450 millimicrons in a Coleman spectrophotometer is the same (distilled water is used as a blank). Curve 2, Figure 1, shows the spectral distribution of such a sample. Curve 1, Figure 1, shows the spectral distribution of a regular sample containing the yellow phosphorus complex $[(NH_4)_3PO_4.NH_4VO_3.16MoO_3$ according to Mission (*1*)].

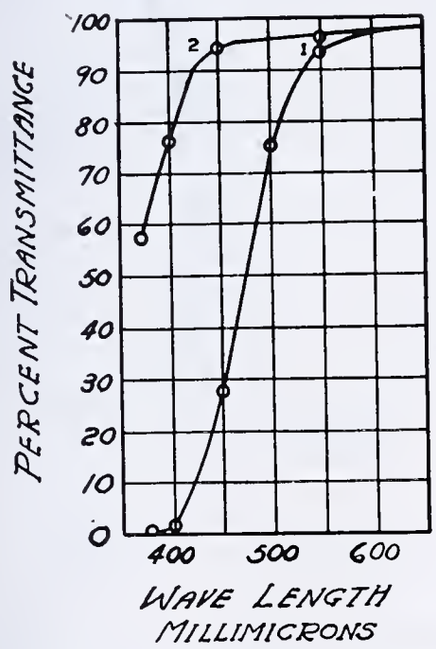

FIGURE 1. TRANSMITTANCE CURVES
1. Color developed
2. Color undeveloped

The proper width of the monochromatic light band (30 millimicrons) is also indicated on this curve, which shows that a narrower slit, 5 or 10 millimicrons, would not be applicable in eliminating fixed iron interference, and is impractical because of the decreased energy output from the spectrophotometer (less light falls on the photoelectric cell) and the critical wave-length setting. Figure 1 also indicates that a larger slit is out of the question. Thus a wave length of 450 millimicrons is employed with a width of 30 millimicrons.

Although the iron interference is constant for varying percentages of iron when the color complex is undeveloped, a marked deviation from the correct phosphorus content is noted when the color is developed and the iron value falls below the 50 per cent mark. In a lean ore or rock (iron below 50 per cent) this phenomenon indicates a low value for the phosphorus content. Figure 2 shows the deviation in the phosphorus value as a function of iron per cent.

For an ordinary iron ore the interference is negligible. If the amount of iron in a sample of lean ore or rock is known, the per cent of phosphorus can be obtained from Figure 2 by adding the correction from the plot to the apparent colorimetric value.

The ammonium phosphovanadomolybdate solution is appreciably sensitive to temperature changes, and, for the most precise analysis, samples must be run at constant temperature. In this work 27° C. is arbitrarily selected as a convenient temperature for seasonal work.

Distilled water does not change appreciably in transmission with temperature changes. Therefore, it can be substituted for the theoretically correct iron blank thus eliminating the necessity of keeping the blank at any definite temperature; moreover, distilled water does not vary in transmission over a period of time whereas a colored, highly acid iron blank must be constantly checked against a standard. The use of a dis-

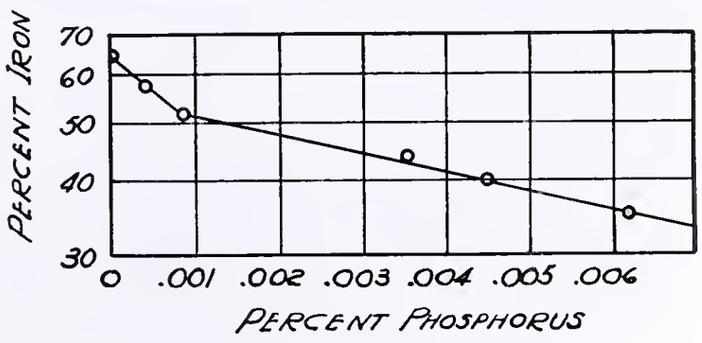

FIGURE 2. IRON INTERFERENCE WITH COLORIMETRIC PHOSPHORUS

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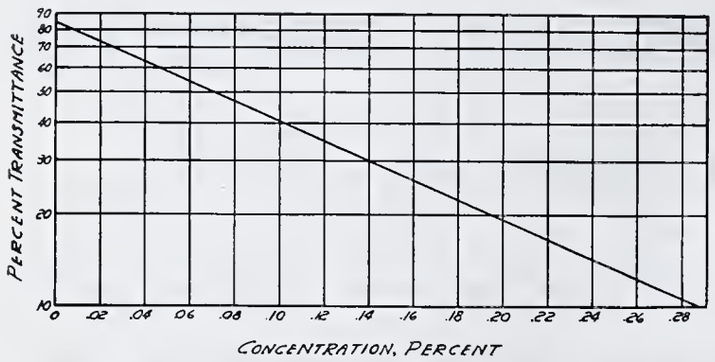

FIGURE 3. PER CENT TRANSMITTANCE *vs.* PER CENT PHOSPHORUS

Distilled water used as a blank. Color compared at 27° C.

tilled water blank changes the concentration *vs.* transmittance curve (Figure 3).

For increased accuracy and economy of time the original colorimetric method for phosphorus (*2*) has been slightly modified. A 1-gram sample is used instead of the original 0.5 gram to allow the determination of silica and manganese on the same sample.

Procedure

Weigh out a 1-gram sample of iron ore into a 150-ml. beaker, add approximately 10 ml. of concentrated hydrochloric acid, and heat on a hot plate until the sample is in solution, adding more hydrochloric acid if necessary.

When the ore is in solution add 0.5 ml. of concentrated nitric acid, evaporate the solution to dryness, and carefully bake. Allow the beaker to cool somewhat, add 15.0 ml. of 70 to 72 per cent perchloric acid, and fume on a hot plate until the dark brown ferric solution has changed to a light straw color. The color change requires from 3 to 6 minutes, depending on the temperature of the hot plate. Extreme care should be taken to see that no organic matter contacts the hot fuming perchloric acid.

Allow the sample to cool slightly, add 10.0 ml. of ammonium vanadate solution, place the sample back on the hot plate, and bring it to a boil.

TABLE I. DETERMINATION OF PHOSPHORUS

Sample No.	Phosphorus	
	Volumetric	Colorimetric
	%	%
888 GG	0.058	0.058
889 GG	0.066	0.064
7106 GB	0.082	0.081
7107 GB	0.089	0.088
7108 GB	0.093	0.092
866 MB	0.104	0.104
867 MB	0.105	0.105
868 MB	0.129	0.128
869 MB	0.128	0.127
870 MB	0.082	0.082
871 MB	0.099	0.099

Remove the sample from the hot plate and wash the cover glass with a little distilled water; filter into a 100-ml. volumetric flask. Scrub the beaker and wash the contents onto a filter paper. Wash the filter paper five times with distilled water. The insoluble residue on the paper may be used for a silica determination.

Fill the volumetric flask to the mark with distilled water, and mix by repeatedly inverting the stoppered flask. Measure out 50.0 ml. to use for a manganese determination if required.

To the remaining aliquot in the flask add 7.5 ml. of ammonium molybdate solution. Invert and shake the flask to mix the reagents and dissolve the precipitate that first forms. Place the sample in a water bath maintained at 27.0° C. When the sample is at bath temperature measure the transmittance at 450 millimicrons in a Coleman spectrophotometer. Read the per cent of phosphorus from the concentration *vs.* transmittance curve (Figure 3).

Table I shows a comparison of the colorimetric and volumetric methods for determining phosphorus.

Summary

The colorimetric determination of phosphorus in iron ores by the phosphovanadomolybdate method gives results in good agreement with the usual volumetric method. Iron gives a definite measurable interference which is negligible if the ore is above 50 per cent iron. Temperature must be controlled for the most accurate work.

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A Filtering Device for Viscosity Tubes

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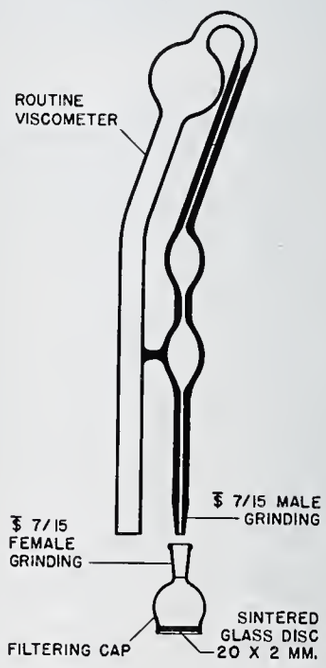
CONSIDERABLE difficulty is often encountered in preparing an uncontaminated sample of oil or other liquid for a viscosity determination. A. S. T. M. Designation D-445-39T requires that the sample be filtered through sintered glass or a fine wire screen into a small beaker or bottle. It is somewhat difficult to keep all the apparatus clean and free from solid particles or lint and, unless great care is exercised, the sample may pick up lint from the air before being transferred to the viscometer pipet.



The filtering device illustrated is a much more convenient and rapid means for ensuring an uncontaminated sample for viscosity measurement. It consists of a small interchangeable filtering cap made by sealing a sintered-glass disk 20 mm. in diameter and 2 mm. thick in a glass tube fitted with a standard $ 7/15 female joint. A male joint is ground on the end of the capillary arm of the viscometer. With the cap in place the pipet is inverted and immersed in the beaker or wide-mouthed bottle containing the sample, and filled by suction in the usual manner.

Two porosities of sintered-glass disks, medium or coarse, depending on the viscosity of the sample, may be used. The filtering cap holds about 6 to 7 cc. of sample.

Cleaning is accomplished by drawing petroleum ether or benzene through the cap in order to retain any foreign particles on the outside and drying in a stream of air or in an oven.

Colorimetric Determination of Nickel with Ammonia

A Spectrophotometric Study

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ALTHOUGH it has long been known that nickelous salts in the presence of excess ammonia give a blue to violet colored complex, until recently little has been done toward the development of a colorimetric method for the determination of nickel based upon this reaction. Two methods have been proposed for its determination in steel.

Fieber (*4*) determined it in the filtrate after filtration of the iron precipitate produced by ammonium hydroxide, while Ayres and Smith (*1*) separated the nickel as the dimethylglyoxime salt, decomposed the precipitate with nitric acid, and determined the nickel in the resulting solution. They have stated that as little as 5 p. p. m. of nickel can be detected with certainty when the Yoe (*16*) photoelectric colorimeter is used. The region of highest sensitivity, however, lies between 500 and 1500 p. p. m. The error may amount to as much as about 5 per cent.

The purpose of the work described in this paper was to make a critical study of this method by means of the photoelectric recording spectrophotometer (*10*), with particular attention to the effect of diverse ions upon the color system. Similar studies of other colorimetric methods have recently been made with this instrument (*2, 3, 5, 6, 8, 9, 11, 12, 13*).

Apparatus and Solutions

All spectrophotometric measurements in the present work were made at Purdue University with the self-recording instrument used by the writer in previous investigations (*8, 9*).

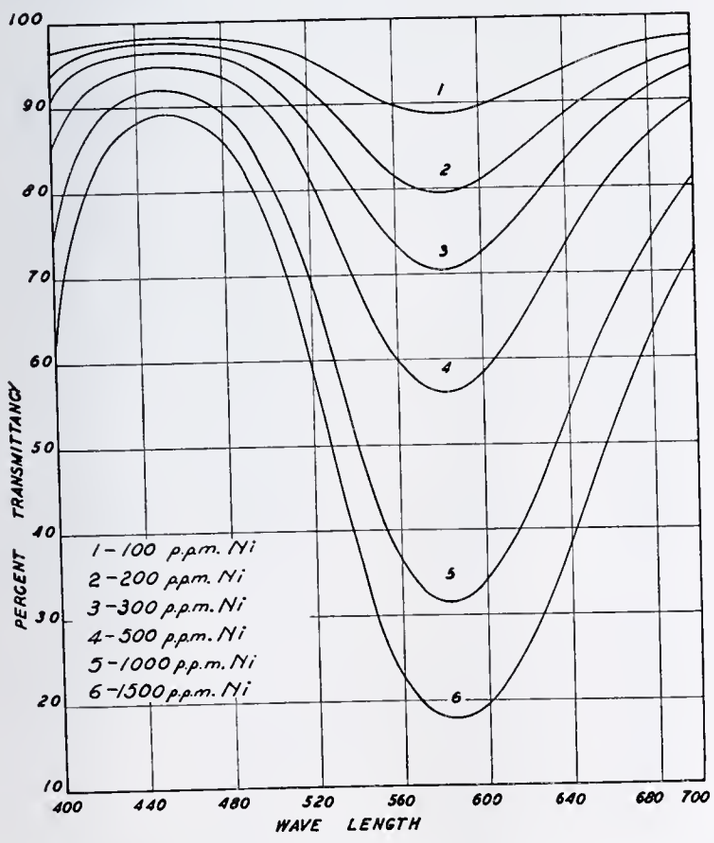

FIGURE 1. TRANSMITTANCY CURVES FOR NICKEL-AMMONIA
100, 200, 300, 500, 1000, and 1500 p. p. m. of nickel in 1.5 *M* ammonium hydroxide. 4.976-cm. cell

A standard stock solution of nickelous nitrate, each milliliter of which contained 10 mg. of nickel, was made by dissolving 20.0133 grams of metallic nickel, of 99.93 per cent purity, in dilute nitric acid and accurately diluting with redistilled water to 2 liters.

Ammonium hydroxide solutions of various concentrations, such as 0.5, 1.5, 3, 4.5, and 6 *M*, were made by suitable dilution of the concentrated solution of specific gravity 0.90.

Standard solutions of the diverse ions were prepared from the chloride, nitrate, or sulfate salts of the cations and from the sodium, potassium, or ammonium salts of the anions. Redistilled water was used in all cases. Each milliliter contained 10 mg. of the ion in question.

To produce the color system 5 ml. of the standard stock solution of nickel in a 100-ml. volumetric flask were just neutralized with 15 *M* ammonium hydroxide, diluted to the mark with 1.5 *M* ammonium hydroxide (*1*), and thoroughly shaken.

The spectral transmission curves were determined for a solution thickness of 4.976 cm. and a spectral band width of 10 mμ. Compensation for the absorption of light by the glass cell and solvent was obtained by placing in the rear beam of light a similar cell filled with 1.5 *M* ammonium hydroxide.

That the color reaction may be reproduced to a high degree of precision is shown by the fact that thirty-one solutions of the nickel-ammonia complex, each containing 500 p. p. m. of nickel and prepared by the above procedure, gave transmittancies at 582 mμ (the peak of the absorption band), the average deviation of which from the mean was 0.15 per cent.

The transmittancy curves for varying amounts of nickel are shown in Figure 1.

Conformity to Beer's Law

Proof that Beer's law is followed by the color system is shown by the straight line which resulted when the logarithms of the observed transmittancies at 582 mμ for six solutions, containing from 100 to 1500 p. p. m. of nickel, were plotted against the respective concentrations. Ayres and Smith (*1*) using the Yoe colorimeter (*16*) reported that the system follows Beer's law up to 600 p. p. m. of nickel.

Effect of Ammonium Hydroxide

Yoe and Crumpler (*16*) have pointed out that ammonia solutions show an appreciable absorption of light in the visual region. It has been shown (*9, 15*) that the concentration of ammonium hydroxide has a pronounced effect upon the color of the copper-ammonia system. A similar result should be expected for the nickel-ammonia system, which to the eye shows a change in hue from blue to violet as the concentration of ammonia increases.

The spectral transmission curves produced by a series of five solutions, each containing 500 p. p. m. of nickel, but made by use of 0.5, 1.5, 3, 4.5, and 6 *M* ammonium hydroxide, were compared. In each case the corresponding concentration of ammonium hydroxide was used in the rear cell. The curves (Figure 2) show that the hue gradually changes as the concentration of the ammonium hydroxide varies. Both the maximum absorption and the wave length of maximum absorption decrease as the concentration increases. Therefore, it is necessary that the concentration chosen for the determination be used throughout in making all the unknown as well as standard solutions which are to be used for comparison. In

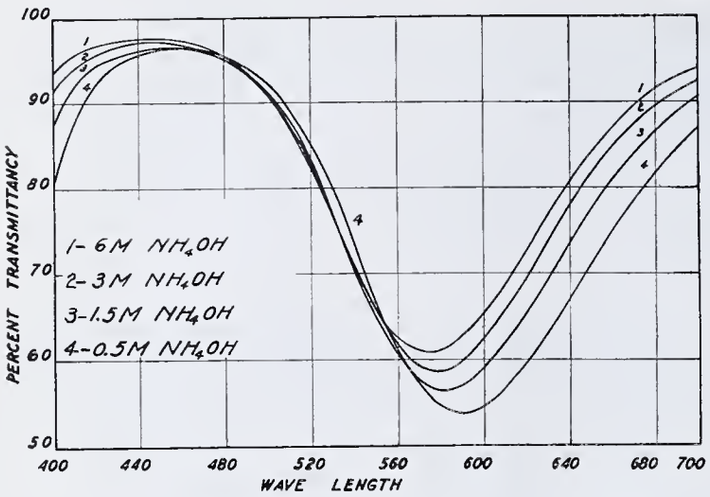

FIGURE 2. EFFECT OF CONCENTRATION OF AMMONIUM HYDROXIDE

500 p. p. m. of nickel in 0.5, 1.5, 3, and 6 *M* ammonium hydroxide. 4.976-cm. cell

the present work the 1.5 *M* solution used by Ayres and Smith (*1*) was selected. Because of the high concentration of ammonia, no attempt was made to determine pH values.

Stability of the Color

Six solutions, containing 100, 200, 300, 500, 1000, and 1500 p. p. m. of nickel, respectively, in 1.5 *M* ammonium hydroxide, were stored in glass-stoppered Pyrex bottles and allowed to stand in diffuse light. Curves made at intervals for these solutions gave no evidence of fading or other change in color over a period of 4 weeks. Time was not available for a longer testing period. Apparently the color is stable indefinitely. Such marked stability makes possible the use of a series of permanent standards, which must, however, be kept tightly stoppered to prevent loss of ammonia. Any action of the ammonia on the glass to produce turbidity is reduced to a minimum by using Pyrex containers.

Ayres and Smith (*1*) found no change in the color after 150 hours, but did not make tests over a longer time.

Effect of Anions

In the ion interference studies the curve produced by the standard solution containing 500 p. p. m. of nickel in 1.5 *M* ammonium hydroxide was compared with the curve produced by a similar nickel solution containing in addition a known weight of the diverse ion. From the transmittancies at 582 mμ of the standard solution and of each of the other solutions and from the known nickel concentration of the standard solution, the apparent concentration of nickel in each of the solutions containing diverse ions was calculated by the aid of a special color slide rule. The difference between this value and the actual concentration multiplied by 100 and divided by the actual concentration gave the percentage error. The calculation is based upon the formula

$$T_2 = T_1^{\frac{c_2}{c_1}}$$

where T_1 represents the transmittancy, expressed as a decimal, for the solution of concentration c_1 and T_2 the transmittancy for the solution of concentration c_2. In accordance with the practice followed in three former studies by the writer (*8*, *9*) and by other workers at Purdue University (*2*, *3*, *5*, *6*, *11*, *12*, *13*) a 2 per cent error was set as the maximum allowable for negligible interference. Although visual methods of color comparison often have a precision of not less than 5 per cent, it was thought advisable to set a lower figure to provide for other possible factors.

When there is a change in hue the curve does not have the same general shape as the standard curve and the point of maximum absorption occurs at a different wave length.

The interfering anions may be divided into four general classes: those, such as cyanide, which form complexes with the nickel ion without a change in hue; those, such as citrate, pyrophosphate, and salicylate, which form complexes with a change in hue; those, such as dichromate, which cause a change in hue because of their own color; and those, such as chlorostannate, chlorostannite, silicate, tungstate, and vanadate, which cause turbidity because of their own hydrolysis or the precipitation of nickel salts.

In Figure 3, curves 2 and 3 show the effects of 500 and 100 p. p. m. of chromium as dichromate ion, curve 4 the effect of 300 p. p. m. of cyanide ion, and curve 5 the effect of 500 p. p. m. of oxalate ion.

The effects of the common anions and their approximate limiting concentrations are listed in Table I.

Effect of Cations

Cobaltous and cupric ions are the only common cations which really interfere. They form soluble, colored ammonia complexes, causing a decided change in hue. In Figure 4, curves 2 and 3 show the effects of 50 and 25 p. p. m. of cobaltous ion and curves 4 and 5 the effects of 50 and 25 p. p. m. of cupric ion. Silver, cadmium, and zinc ions form colorless ammonia complexes which do not interfere. Aluminum, antimonous, barium, beryllium, bismuth, chromic, ferric, ferrous, lead, magnesium (in the absence of ammonium chloride), manganous, mercuric, mercurous, strontium, thorium, uranyl, and zirconium ions precipitate as hydroxides or basic salts in the alkaline solution. These precipitates can

TABLE I. EFFECT OF DIVERSE ANIONS

(50 mg. of nickel in 100 ml. of solution)

Ion	Concentration *P. p. m.*	Apparent Change in Nickel Concentration %	Approximate Limiting Concentration *P. p. m.*
Acetate	500	Negligible	...
Arsenate	500 (As)	Negligible	...
Arsenite	500 (As)	Negligible	...
Benzoate	500	Negligible	...
Borate	500 (B_2O_3)	Negligible	...
Bromide	500	Negligible	...
Carbonate	500	Change in hue	
	100	Negligible	150
Chlorate	500	Negligible	...
Chloride	500	Negligible	...
Chlorostannate	500 (Sn)	Precipitates	
	300 (Sn)	+1.9	300
Chlorostannite	100 (Sn)	Precipitates	0
Citrate	100	Change in hue	0
Cyanide	300	−17.8	
	100	− 6.4	0
Dichromate	100 (Cr)	Change in hue	0
Fluoride	500	Negligible	...
Formate	500	Negligible	...
Iodide	500	Negligible	...
Molybdate	500 (Mo)	Negligible	...
Nitrate	500	Negligible	...
Nitrite	500	Negligible	...
Orthophosphate	500 (P_2O_5)	Negligible	...
Oxalate	500	Change in hue	
	100	+7.7	0
Perchlorate	500	Negligible	...
Pyrophosphate	100	−3.0	50
Salicylate	500	Change in hue	
	100	+1.6	100
Silicate	100 (SiO_2)	Precipitates	0
Sulfate	500	Negligible	...
Sulfite	500	Negligible	...
Tartrate	500	+2.0	500
Thiocyanate	500	Negligible	...
Thiosulfate	500	Turbidity	
	200	+2.2	150
Tungstate	500	Turbidity	
	300	+3.0	
	100	Negligible	150
Vanadate	20 (V)	Precipitates	0

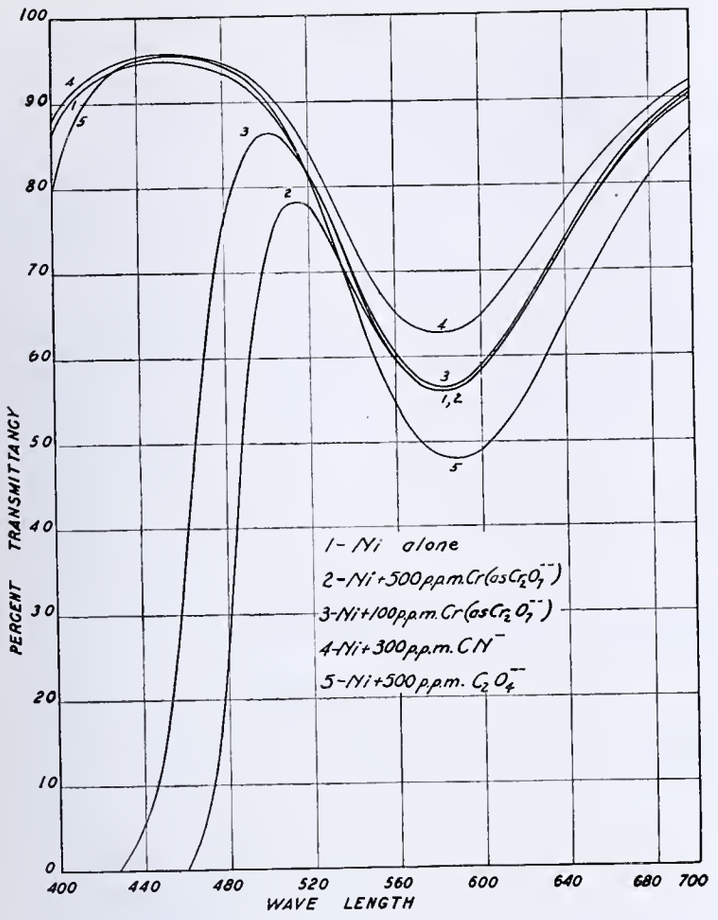

FIGURE 3. EFFECT OF ANIONS

500 p. p. m. of nickel with diverse anion in 1.5 *M* ammonium hydroxide. 4.976-cm. cell

be removed by filtration, as was done by Fieber (*4*), but Ayres and Smith (*1*) chose instead to separate the nickel by precipitating it from slightly ammoniacal solution in the presence of tartrate with dimethylglyoxime. This separation is satisfactory for steel, but if the method were to be applied to general testing where any of the ions listed above might be present, some of them would undoubtedly be precipitated in the alkaline solution. The writer recommends for miscellaneous materials relatively low in iron, in the absence of cobalt and copper, a procedure similar to the one used in the ammonia method for copper (*7*, *14*), wherein the precipitate produced by ammonium hydroxide is filtered and the determination is made on the filtrate.

Although ammonium chloride has been shown (*9*, *15*) to intensify the color of the copper-ammonia system, 1000 p. p. m. of ammonium ion as chloride have no effect upon the color of the nickel-ammonia system.

In Table II are listed the effects and approximate limiting concentrations of the common cations which do not cause precipitation.

Summary

A spectrophotometric study shows that the ammonia method for the colorimetric determination of nickel is satisfactory although not highly sensitive. The region of highest sensitivity lies between 500 and 1500 p. p. m. of nickel. While the precision is not high, the average of several determinations agrees well with the results obtained by the gravimetric dimethylglyoxime method.

The Ayres and Smith method need not be restricted to the determination of nickel in steel. However, for miscellaneous materials relatively low in iron it is recommended that any precipitate produced by ammonium hydroxide in the absence of tartrate be filtered and the nickel determined in the filtrate without resorting to the dimethylglyoxime separation.

The concentration of the ammonium hydroxide used for dilution must be carefully controlled because of the ability of the ammonia solution to absorb light. Since the hue varies with the concentration, the same concentration must be used for the standards as for the unknown solutions. A 1.5 *M* solution is recommended.

The color system follows Beer's law.

The color is stable in diffuse light for at least 4 weeks and undoubtedly for a much longer time. The use of a series of permanent standards is therefore possible.

The color reaction may be reproduced to a high degree of precision.

A study of the effect of sixty common ions shows that only a few, especially cobaltous, cupric, cyanide, and dichromate, seriously interfere with the color and that many of the cations

TABLE II. EFFECT OF DIVERSE CATIONS

(50 mg. of nickel in 100 ml. of solution)

Ion	Concentration P. p. m.	Apparent Change in Nickel Concentration	Approximate Limiting Concentration P. p. m.
Ammonium	500	Negligible	...
Cadmium	500	Negligible	...
Calcium	500	Negligible	...
Cobaltous	25	Change in hue	0
Cupric	25	Change in hue	0
Lithium	500	Negligible	...
Magnesium	200	Negligible[a]	...
Potassium	500	Negligible	...
Silver	500	Negligible	...
Sodium	500	Negligible	...
Zinc	500	Negligible	...

[a] If ammonium chloride is present.

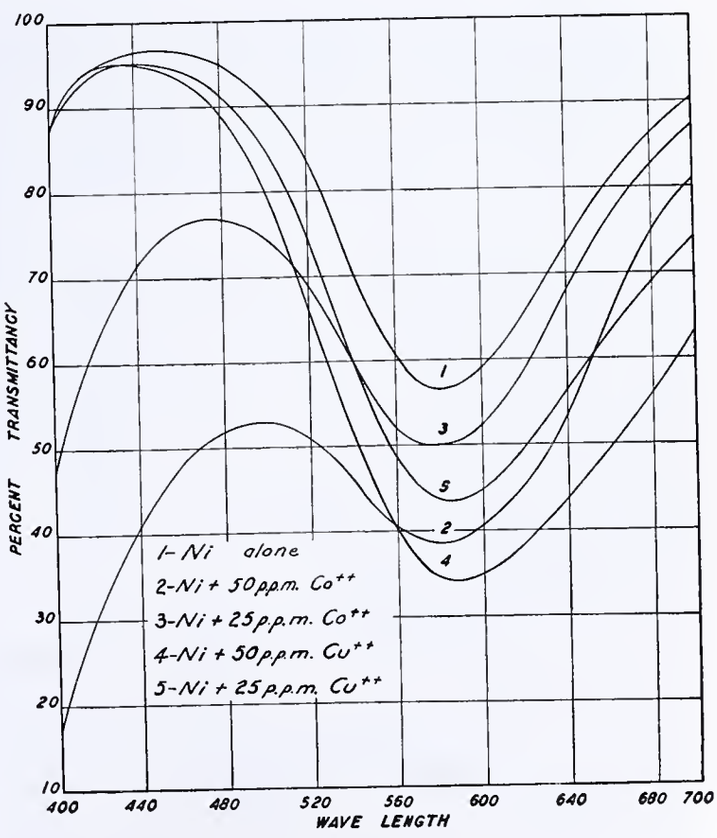

FIGURE 4. EFFECT OF CATIONS

500 p. p. m. of nickel with diverse cation in 1.5 *M* ammonium hydroxide. 4.976-cm. cell

cause precipitation or turbidity, but in the course of the determination this latter group would be removed.

Acknowledgments

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Analysis of Cellulose Derivatives

Analysis of Cellulose Mixed Esters by the Partition Method

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Several methods have been proposed for the determination of the combined acids in mixed esters of cellulose, most of them based on some physical property of the acids. The differential partition (or distribution) of the acids between immiscible solvents has proved to be a satisfactory and practical means for determining the composition of mixtures of acids. This principle was utilized by Behrens and the manipulation was improved and simplified by Werkman. It has now been applied to cellulose mixed ester analysis, and butyl acetate has been found to be a particularly suitable extractant for mixtures of acetic, propionic, and butyric acids.

The analysis of a cellulose mixed ester by the partition method involves the following steps: determination of total combined acids by some suitable method, saponification and isolation of these combined acids, determination of the partition coefficients between butyl acetate and water of the acid mixture and also separate measurement of the partition coefficients of each acid present, calculation of the molar ratios of the acids using simultaneous equations, and calculation of the weight per cent of the acids, or combined acyl, from the molar ratios and the total acid content of the ester. Precision, accuracy, and limits of applicability are given. Modifications of the procedure are described for certain higher acyl esters and for nonvolatile acid esters.

VARIOUS mixed esters of cellulose and particularly the cellulose acetate propionates and acetate butyrates have proved successful commercially because of the ability to vary their properties to meet different requirements by varying their compositions. The analysis of these esters presents the usual difficulties encountered in analyzing neighboring members of a homologous series of organic compounds. Since there is little difference in chemical properties between such members, it is necessary to make use of some physical property as the basis for an analytical method.

The older method for analyzing mixtures of such acids was that of Duclaux (*6*), with various modifications and improvements (*7*, *14*, *28*, *32*), which is based on differences in the rates of distillation of the various acids. Behrens (*2*) proposed the use of the differential distribution (or partition) of the acids between immiscible solvents such as diethyl ether and water. This method has many advantages over that of Duclaux, the most important of which is that a greater numerical spread in values may be obtained. Behrens titrated the acid present in each phase and calculated his results algebraically using simultaneous equations. He showed that mixtures of as many as five acids could be analyzed with reasonable accuracy by measuring partitions under several different sets of conditions. The acids studied were acetic, propionic, butyric, valeric, caproic, succinic, lactic, glycolic, malic, citric, and tartaric.

Werkman successfully applied this principle to two-component mixtures using isopropyl ether (*29*), diethyl ether (*30*), and isoamyl ether (*31*). Calculations were made by graphical methods. Osburn and Werkman (*18*) extended the procedure to mixtures of acetic, propionic, and butyric acids and developed nomograms to simplify the calculation. Later (*19*) they modified the procedure to include formic acid and to indicate the presence of other acids such as lactic and pyruvic. Fuchs (*8*) has systematically studied the indirect methods of analysis, including application of the partition method to mixtures of the lower aliphatic acids.

Yackel, Staud, and Gray (*34*) applied the procedure of Werkman to cellulose esters by saponifying the esters by the modified Eberstadt method (*16*), removing the alcohol by evaporation, acidifying with phosphoric acid, and steam-distilling. The acid distillates were extracted with diethyl ether. Steam-distillation of propionic and butyric acids is not very satisfactory from an analytical standpoint, so Yackel, Kenyon, and Gray (*33*) modified this procedure by saponifying, removing the alcohol by evaporation, diluting, and exactly neutralizing the alkali with standard hydrochloric acid. The regenerated cellulose was then filtered off, and the filtrate was extracted with diethyl ether by the method of Werkman (*30*). The sodium chloride present was shown to have a negligible effect on the distribution coefficients of the acids.

Malm and Nadeau (*15*) improved these procedures by saponifying the cellulose ester without the use of alcohol and used vacuum-distillation for separating the organic acids from the regenerated cellulose. Normal propyl and butyl acetates were found to have many advantages over the ethers as extracting

agents, particularly when three-component acid mixtures are to be analyzed.

Several other methods have been proposed for application to cellulose mixed esters, but in general they do not appear to be so practical and accurate as the partition method. Yackel, Staud, and Gray (*34*) saponified the mixed esters, steam-distilled, neutralized, evaporated to dryness, dissolved the sodium salts, and measured the refractive index of the resulting solution and compared it with a curve prepared using knowns. They found the accuracy was not very great and were troubled by interfering impurities. Tanghe (*25*) applied the method of thermal analysis to mixtures of sodium salts of two acids isolated from cellulose mixed esters. The freezing or congealing points as determined from the cooling curves of melts gave some indication of composition for values less than 25 mole per cent propionyl. For higher propionyl contents, known amounts of pure sodium acetate were added to shift the composition into the more sensitive range. The sensitivity of the method, however, was not so high as was desired.

Kolossowsky (*11*), in a series of papers, has reported distribution ratios of the common organic acids between water and many organic solvents.

Methods proposed for the analysis of mixtures of acids of the fatty acid series and related acids include the following: Crowell (*4*) described a method for analyzing mixtures of acetic, propionic, and butyric acids based on the extraction with kerosene of an aqueous solution saturated with calcium chloride to remove butyric acid, and titration and weighing of barium salts to measure the amount of acetic and propionic acids. McNair (*13*) determined propionic acid in the presence of formic and acetic acids by oxidizing the former to oxalic acid with permanganate and determining this acid as the calcium salt. Sukhanovskiĭ and Roginskaya (*24*) analyzed mixtures of formic, acetic, propionic, and oleic acids by a method involving separate determination of formic acid. Acetic and propionic acids were measured as lead salts and by special extraction and fusion steps; oleic acid was then obtained by difference. Lecoq (*12*) steam-distilled acetic and butyric acids from lactic acid and measured the amounts of these volatile acids either from the differential solubilities of their barium salts in ethyl alcohol or by converting the mixed barium salts to barium sulfate. Vinogradov and Ostroumova (*27*) determined propionic and butyric acids in acetic acid by stepwise neutralization with hydrochloric acid of a solution of the sodium salts of the acids. After each neutralization, the mixtures were extracted with ether and the acids in the ether layers were converted to their sodium salts. Addition of ferric chloride resulted in precipitates or colors from which the amounts of propionic and butyric acids could be estimated.

Tasman (*26*) estimated formic, acetic, and propionic acids in bacteriological nutritive media by steam-distilling the acids, measuring formic acid separately by the reduction of mercuric chloride to mercurous chloride, and destroying formic acid in another portion of the distillate by oxidizing with acid dichromate. The resulting mixture of acetic and propionic acids was then distilled in fractions, the distillates were neutralized, concentrated, and combined, and after acidification a "half distillation" value was measured, from which the relative proportions of the acids were found by comparison with a standard curve. Kline (*10*) determined butyric acid in the presence of formic, acetic, propionic, and lactic acids by controlled oxidation to acetone using hydrogen peroxide. The acetone formed was measured by precipitating with an alkaline mercuric cyanide–silver nitrate mixture. Bekhtereva and Ierusalimskiĭ (*3*) analyzed mixed acids obtained in a butyl alcohol–acetone fermentation. Mixtures of acetic and butyric acids were analyzed by measuring formic acid by the reduction of mercuric chloride, butyric acid by the colorimetric method of Peterson and Fred (*20*), and acetic acid by difference.

Qualitative methods for the identification of organic acids of the homologous fatty acid series include the following: Agulhon (*1*) identified these acids by observing colors formed in solvent layers when ferric or cupric salt solutions of the acids were extracted with certain organic solvents. Dyer (*7*) modified and improved the method of Agulhon. Denigès (*5*) formed characteristic crystals by treating the acids with cholesterol and examining them microscopically. Musicant and Kaszuba (*17*) identified propionic acid in the presence of acetic and butyric acids by microscopic examination of their mercurous salts. Schicktanz, Steele, and Blaisdell (*21*) analyzed mixtures of formic, acetic, propionic, *n*- and isobutyric acids qualitatively and semiquantitatively by a method based on azeotropic distillations with benzene and toluene.

Procedure

The procedure given has been found satisfactory from a practical standpoint, and involves the following steps:

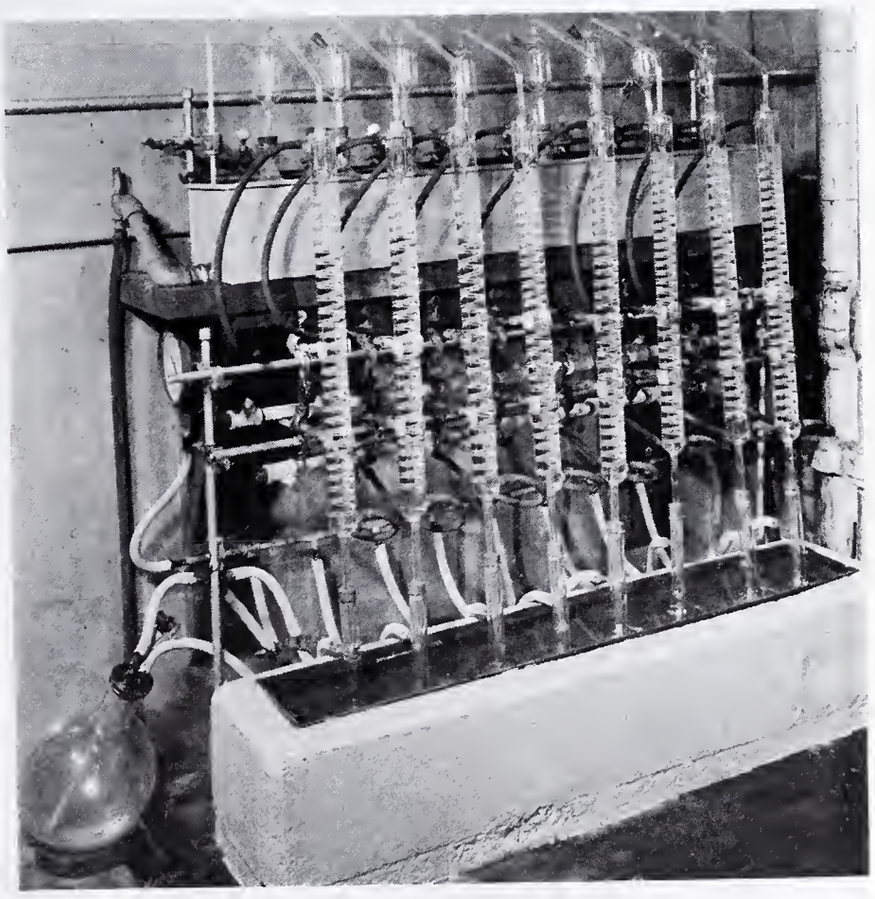
1. An analysis to determine the total combined acids present.
2. Saponification and isolation of the combined acids.
3. Determination of the partition coefficients between butyl acetate and water of the acid mixture and separate measurement of the partition coefficients of each acid present.
4. Calculation of the molar ratios of the acids using simultaneous equations.
5. Calculation of the weight per cent of the acids (or combined acyl) from the molar ratios and the total acid content of the ester.

Special Apparatus and Reagents. Constant-temperature bath or oven at 40° C.

Vacuum-distillation apparatus, each consisting of a 500-ml. round-bottomed flask fitted with a rubber stopper carrying a capillary inlet tube and Kjeldahl distillation head, which is in turn connected to a vertical condenser, the lower end of which reaches within 7.5 cm. (3 inches) of the bottom of a 500-ml. side-arm distillation flask used as the receiver. Efficient condensers, preferably of the spiral type, are recommended. The distillation flasks are heated in a water bath, and the receivers are cooled in a brine bath or with ice and salt at about 0° C.

Phosphoric acid solution, 1 molar.

Butyl acetate must be free from water to ensure good keeping quality and must be free from acidity to give accurate results. As much as 5 per cent of butyl alcohol can be tolerated. When commercial butyl acetate is used, it must be neutralized with sodium carbonate, dried over calcium chloride, and then carefully distilled. The distillate should be tested for freedom from acidity by mixing 50 ml. of butyl acetate with 50 ml. of carbon dioxide–free distilled water and titrating with 0.1 *N* sodium hydroxide solution. If a pure grade of butyl acetate is avail-



Vacuum Distillation Apparatus

able, it should be shaken with solid sodium carbonate and sodium sulfate. After settling and decanting, it is ready for use. This material may be analyzed for its water (*23*) and butyl alcohol (*22*) contents by the methods described by Smith and Bryant.

Pure samples of the acids in the ester to be analyzed.

Determination of Total Acyl Content. The total acyl content may be determined by one of the methods described by Genung and Mallatt (*9*). The modified Eberstadt method should be used if applicable; otherwise, the alcoholic alkali method should be employed. The alkali consumed is calculated as per cent acetyl (equivalent weight 43). The result is actually an "apparent acetyl" value, and is a measure of the total acyl content of the ester.

Isolation of a Mixture of Acids. Duplicate 3.0-gram samples (not especially dried) are heated with 100 ml. of 0.5 N sodium hydroxide solution in stoppered 500-ml. round-bottomed Pyrex flasks in a water bath or oven at 40° C. for 48 to 72 hours. Enough molar phosphoric acid solution (about 50 ml.) is added to each flask to form monosodium phosphate, which liberates the organic acids from their sodium salts. The flasks are connected to the Kjeldahl heads, the distillation apparatus is assembled, and the solutions are vacuum-distilled to dryness, allowing a small stream of air bubbles to enter if necessary to avoid bumping. Twenty-five milliliters of distilled water are added to the residue in each flask and the vacuum-distillation is again taken to dryness. This operation is repeated with second 25-ml. portions of distilled water. The conditions of the distillation must be such that no phosphate ion is carried over into the distillate.

In this operation, it is not necessary to work with quantitative accuracy, but it is necessary to obtain water solutions of the acids in the same ratio as they occur in the ester. The distillate is diluted to about 250 ml. with carbon dioxide–free distilled water which, in the majority of cases, automatically adjusts the acidity of the distillate to 0.08 to 0.12 N, the range desired for subsequent extractions.

Many modifications of the distillation apparatus might be used. The apparatus described, however, is simple, it may be assembled from ordinary laboratory equipment, and it has been proved by long use to be satisfactory and practical.

Determination of Molar Ratio of Acids. The following procedure is based on the distribution of the various acids and the unknown acid mixture between water and n-butyl acetate. The ratios are first determined for the individual acids using samples of known high purity. From these values and the partition ratio of the mixture, the molar ratio can be calculated.

The distribution ratios of the known acids are determined as follows. The case of acetic, propionic, and butyric acids is used in the following discussion:

The calculated amounts of the pure acids (1.43 ml. of acetic, 1.87 ml. of propionic, and 2.30 ml. of butyric acid) are each diluted to 250 ml. to give approximately 0.1 N solutions. A 25-ml. portion of each acid solution is titrated with 0.1 N sodium hydroxide, using phenolphthalein as indicator. The volume of alkali in each case is designated as M. Thirty milliliters of acid solution and 15 ml. of butyl acetate are transferred to a small separatory funnel, using accurate pipets or burets, the mixture is shaken thoroughly, and the two layers are allowed to separate. The conditions of shaking are not critical, but they must be standardized by each operator. It is recommended that the shaking be continued for 2 minutes at about 100 strokes per minute and that the funnel be held in such a way as to minimize heating by the hand. Several minutes' standing should be allowed for the layers to separate.

The lower layer is drawn off, and the upper layer is discarded or saved for recovery of the butyl acetate. A 25-ml. pipet is rinsed with 2 or 3 ml. of this aqueous solution and then 25 ml. are pipetted into a clean flask containing 25 or 50 ml. of carbon dioxide–free distilled water. This solution is titrated with 0.1 N sodium hydroxide solution as before, and the volume of alkali in each case is designated as M_1. Other 30-ml. portions of the acid solutions are extracted in the same manner, using in this case 60 ml. of butyl acetate, 25-ml. portions of the aqueous layers are titrated as before, and the volume of alkali used is designated as M_2.

The following ratios are then calculated for each acid:

$$\frac{M_1}{M} = k_1 \qquad \frac{M_2}{M} = k_2$$

The distribution ratios, or partition coefficients, thus determined represent the fraction of the acid remaining in the water layer under the conditions specified. Numerical values are thus obtained for the following partition coefficients:

k_1, acetic acid with 15 ml. of n-butyl acetate
k_2, propionic acid with 15 ml. of n-butyl acetate
k_3, butyric acid with 15 ml. of n-butyl acetate
k_4, acetic acid with 60 ml. of n-butyl acetate
k_5, propionic acid with 60 ml. of n-butyl acetate
k_6, butyric acid with 60 ml. of n-butyl acetate

These values must be determined by each operator for every new supply of butyl acetate. The numerical values will vary with the butyl alcohol content of the butyl acetate and with the individual technique of the operator.

Before continuing the analysis, the extraction technique and the partition coefficients should be checked by preparing and analyzing an acid mixture of known composition.

Convenient weights of the three acids are thoroughly mixed in a flask and 1.75 to 2 ml. of the mixture are diluted to 250 ml. to give an approximately 0.1 N solution, the exact molar composition of which is calculated from the weights of the acids. A 25-ml. portion is titrated, the titer is designated as M, and 30-ml. portions are extracted with 15 and 60 ml., respectively, of butyl acetate and are titrated using exactly the same technique previously employed.

The following percentage coefficients are then calculated:

$$\frac{M_1 \times 100}{M} = K_1 \qquad \frac{M_2 \times 100}{M} = K_2$$

In this case the K values are expressed as percentage partition coefficients rather than as ratios. The composition of the known acid mixture is then calculated from these data, using Equations 6, 7, and 1 as described below. If the result agrees satisfactorily with the known composition of the mixture, the coefficients and the equation are ready for use. If the agreement is poor, the individual partition coefficients should be rechecked and the mixture reanalyzed until satisfactory results are obtained. This may involve a considerable amount of work for a new operator, but when the extraction technique has been standardized, acceptable results can usually be obtained in the first attempt.

The distillates may then be analyzed by the procedure described above. 25-ml. portions are titrated, the volumes of alkali are designated as M, 30-ml. portions are extracted and titrated as before, and the titration from the 15-ml. extraction is designated as M_1 and from the 60-ml. extraction as M_2. The percentage partition coefficients, K_1 and K_2, are calculated as in the case of the known acid.

Calculations. *Case of a Mixture of Three Acids.* In order to evaluate three unknowns, three simultaneous

Table I. Partition Coefficients of Various Acids

Acid	k_1, 15 Ml. of Butyl Acetate, 30 Ml. of Water	k_2, 60 Ml. of Butyl Acetate, 30 Ml. of Water
Formic	0.885	0.660
Acetic	0.851	0.589
Propionic	0.592	0.263
n-Butyric	0.288	0.093
Isobutyric	0.280	0.090
n-Valeric	0.124	0.040
n-Caproic	0.036 (0.04 N solution used)	0.013
Crotonic	0.350	0.121
Methoxyacetic	0.920	0.752
Chloroacetic	0.532	0.233
Levulinic	0.869	0.624

Table II. Analyses of Acid Mixtures of Known Composition

Acid	Found	Taken	Found	Taken	Found	Taken	Found	Taken	Found	Taken
	Mole per cent									
Acetic	90.1	90.6	88.2	88.4	61.5	61.5	46.5	46.6	9.5	9.9
Propionic	5.1	4.8	4.1	4.6	29.3	29.2	32.9	33.0	46.7	46.0
Butyric	4.8	4.6	7.7	7.0	9.2	9.3	20.6	20.4	43.8	44.1

Acid	Found	Taken	Acid	Found	Taken
	Mole per cent			*Mole per cent*	
Formic	41.0	40.8	Acetic	77.1	76.8
Butyric	59.0	59.2	Caproic	22.9	23.2
Acetic	65.2	65.1	Acetic	21.7	21.6
Propionic	34.8	34.9	Chloroacetic	78.3	78.4
Acetic	37.1	37.2	Butyric	26.1	26.4
Butyric	62.9	62.8	Caproic	73.9	73.6
Acetic	77.0	76.8			
Crotonic	23.0	23.2			

algebraic equations involving the three unknown quantities are necessary. In the case of a ternary acid mixture, the sum of the mole percentages of the acids present represents the total acidity or 100 per cent. If A, P, and B represent mole percentages of acetic, propionic, and butyric acids, respectively, the first equation becomes:

$$A + P + B = 100 \tag{1}$$

The second and third equations are:

$$Ak_1 + Pk_2 + Bk_3 = K_1 \tag{2}$$

$$Ak_4 + Pk_5 + Bk_6 = K_2 \tag{3}$$

Note that k_1, k_2, k_3, k_4, k_5, and k_6 are known and refer to the pure individual acids, whereas K_1 and K_2 refer to the ternary mixture. By solving these equations for P, the following expressions may be derived:

$$P = \frac{\frac{K_1 - 100k_1}{k_3 - k_1} - \frac{K_2 - 100k_4}{k_6 - k_4}}{\frac{k_2 - k_1}{k_3 - k_1} - \frac{k_5 - k_4}{k_6 - k_4}} \tag{4}$$

$$B = \frac{K_2 - 100k_4 - P(k_5 - k_4)}{k_6 - k_4} \tag{5}$$

Typical values for the partition coefficients of the acids are:

$k_1 = 0.852$ $k_4 = 0.586$
$k_2 = 0.587$ $k_5 = 0.261$
$k_3 = 0.282$ $k_6 = 0.091$

When these values are substituted in Equations 4 and 5, they simplify to the following forms:

$$P = 10.5\,(58.6 - K_2) - 9.14\,(85.2 - K_1) \tag{6}$$

$$B = 2.02\,(58.6 - K_2) - 0.656P \tag{7}$$

The above constants and numerical values in the equations are typical, but the exact values should be determined by each analyst, using his own reagents and the best pure acids available. These constants must be checked from time to time, particularly when new reagent solutions are put in use.

The mole percentages of acid are converted to weight per cent acyl as follows:

$$A \times \text{per cent apparent acetyl} = \text{weight per cent acetyl} \tag{8}$$

$$P \times \text{per cent apparent acetyl} \times 57/43 = \text{weight per cent propionyl} \tag{9}$$

$$B \times \text{per cent apparent acetyl} \times 71/43 = \text{weight per cent butyryl} \tag{10}$$

Case of Two Acids. When it is known that only two acids are present, the manipulation and calculation may be simplified considerably. Only two simultaneous equations and only one set of partition coefficients are then required. In the case of a cellulose acetate butyrate, using the above terminology, the necessary equations are

$$A + B = 100 \tag{11}$$

$$Ak_1 + Bk_3 = K_1 \tag{12}$$

These equations, when solved, become

$$B = \frac{K_1 - 100\,k_1}{k_3 - k_1} \tag{13}$$

$$A = 100 - B \tag{14}$$

When the numerical values for k are substituted in Equation 13, it simplifies to the form:

$$B = 1.75\,(85.2 - K_1) \tag{15}$$

A similar equation can also be derived using the other set of constants:

$$B = \frac{K_2 - 100\,k_4}{k_6 - k_4} \tag{16}$$

Limits of Applicability

The procedure as described above is satisfactory for the analysis of cellulose acetate propionates and cellulose acetate butyrates having propionyl and/or butyryl contents less than about 35 to 40 per cent. The limit cannot be drawn more closely than this because the physical condition of the sample to be saponified is an important variable.

This principle can also be applied to the analysis of other volatile water-soluble acid esters of cellulose. The accuracy obtained depends upon the difference in the numerical values of the partition coefficients. Typical partition coefficients for a series of such acids are given in Table I. These values are comparable, for they were determined using one particular sample of butyl acetate which contained 0.04 per cent water and 1.81 per cent butyl alcohol, but such coefficients would vary with different operators and different reagents.

Analysis of Known Mixtures

When acid mixtures of known composition are analyzed, results such as those given in Table II are readily attainable.

Acid mixtures of known composition were distilled in the usual way to check the completeness of distillation, and analysis of the distillates gave the following results:

Acid	Found	Taken
	Mole per cent	
Acetic	56.9	57.0
Propionic	29.4	29.7
Butyric	13.7	13.3
Acetic	62.3	62.0
Propionic	28.3	28.6
Butyric	9.4	9.4

A known acid was also distilled and analyzed in the same way, except that 1.5 grams of cotton linters were present in the distillation flask during the distillation, which approximately duplicates the condition normally encountered.

Acid	Found	Taken
	Mole per cent	
Acetic	15.6	16.0
Propionic	30.6	30.6
Butyric	53.8	53.4

The effect of the saponification was measured by heating 1.5 grams of cotton linters and about 2 ml. of an acid mixture of known composition with 100 ml. of 0.5 N alkali at 40° C. for 3

days, then acidifying with phosphoric acid, distilling, and analyzing by the usual procedure. The results were as follows:

Acid	Found	Taken
	Mole per cent	
Acetic	15.6	16.0
Propionic	30.6	30.6
Butyric	53.8	53.4

These experiments show that no appreciable errors are introduced at any stage in the manipulation. This conclusion is supported by the fact that when cellulose acetate is analyzed, practically 100 mole per cent acetic acid is found. Qualitative tests on the distillates show negligible amounts or no phosphoric or formic acid to be present.

Precision and Accuracy

Examination of the data on known solutions indicates that accuracy within ±0.4 mole per cent is usually attained. In cellulose esters containing approximately 50 per cent acyl, the accuracy is thus about ±0.2 per cent acyl. The precision is better, and is usually within ±0.2 mole per cent or ±0.1 weight per cent acyl.

Procedure for Mixed Esters of High Propionyl or Butyryl Content

In the case of cellulose acetate propionates and acetate butyrates having more than 35 to 40 per cent propionyl and/or butyryl, the apparent acetyl content must be determined by the alcoholic alkali procedure (*9*) or an equivalent method, and the saponification and isolation of the mixed acids are carried out as follows:

Duplicate samples of approximately 3.0 grams (not especially dried) are weighed into 500-ml. round-bottomed flasks. Approximately 100 ml. of methyl or ethyl alcohol and 100 ml. of 0.5 *N* sodium hydroxide solution are added to each flask. The saponification is then conducted at room temperature for 48 to 72 hours, except in cases where this treatment is not vigorous enough. In some cases, it will be necessary to conduct the saponification at 40° C. for 48 to 72 hours. The flasks are then connected to distillation apparatus and the alcohol is removed by vacuum-distillation. After distilling to dryness, the vacuum is released, the condenser and receiver are rinsed down, and the distillate is discarded. The required amount of phosphoric acid and 100 ml. of distilled water are added to each flask, and the apparatus is reassembled. The distillation, extractions, and calculations are then carried on as described above. It is necessary to remove all the alcohol by the first distillation, or the partition constants and the results will be in error.

Small amounts of formic acid are formed when cellulose is heated in the presence of alkali. This effect can be reduced and the accuracy of the above procedure can be slightly improved when desired by filtering off the regenerated cellulose before removing the alcohol by vacuum-distillation.

This method is applicable to the analysis of volatile water-soluble acids which include acetic, propionic, butyric, and valeric acids. Caproic and higher acids cannot be analyzed by this exact procedure, because of their lower solubility in water and their poorer volatility. However, the procedure can be modified to include caproic by using more dilute solutions such as 0.04 *N*.

The precision and accuracy are slightly poorer when alcohol must be used, for the conditions of saponification are more vigorous, and the distillation to remove alcohol is made on the alkaline solution. As a result, measurable amounts of formic acid are formed, which cause the apparent acetic acid results to be high, and propionic or butyric results to be low. On the other hand, any alcohol which is not removed by the first distillation and is carried with the acids in the second distillation produces an error in the opposite direction. The accuracy is also affected by errors in the total acyl analysis. A precision of ±0.4 mole per cent or about ±0.2 weight per cent acyl is usually attained. The average accuracy is of the order of ±1 mole per cent or ±0.5 weight per cent acyl.

Procedure for Nonvolatile, Water-Soluble Acid Esters

The saponification is conducted as described above. If alcohol is used, it must first be removed by evaporating or distilling to dryness. Distilled water is added if necessary to restore the original volume, and then standard hydrochloric acid is added to neutralize exactly all the alkali present. The regenerated cellulose is removed by filtration, is washed well, and the volume of the filtrate is adjusted to 200 to 250 ml. This filtrate differs from the distillates obtained in the other procedures in that it contains sodium chloride equivalent to the amount of alkali originally taken. Partition ratios may be determined on this filtrate by the above-described procedure with the difference that the coefficients for the known acids must be determined using 0.1 *N* solutions of the acids containing the same sodium chloride concentration as the filtrates.

Partition coefficients of the known acids between butyl acetate and water are appreciably lowered by sodium chloride, whereas Yackel, Kenyon, and Gray (*33*) found that it has little effect on the coefficients for diethyl ether and water.

This method is particularly adapted to the analysis of esters of water-soluble volatile or nonvolatile acids. The accuracy obtainable depends upon the exactness of the neutralization of sodium hydroxide with hydrochloric acid and also upon the numerical difference between the distribution coefficients of the acids involved.

Table III. Effect of Butyl Alcohol Content on Partition Coefficients

	Butyl Acetate Sample						
	1	2	3	4	5	6	7
Butyl alcohol (*22*), %	0.87	1.30	1.90	2.09	2.42	5.10	8.80
Water (*23*), %	0.17	0.03	0.00	0.03	0.02	0.06	0.10
k_1	0.857	0.855	0.852	0.849	0.847	0.825	0.793
k_2	0.601	0.598	0.587	0.589	0.584	0.551	0.513
k_3	0.294	0.293	0.282	0.288	0.282	0.260	0.234
k_4	0.602	0.597	0.586	0.584	0.576	0.531	0.472
k_5	0.275	0.271	0.261	0.261	0.255	0.227	0.194
k_6	0.098	0.096	0.091	0.092	0.089	0.079	0.067

Effect of Variables

Effect of Butyl Alcohol Content of Butyl Acetate. The butyl alcohol content of the butyl acetate has an appreciable effect on the numerical values of the partition coefficients (k) for acetic, propionic, and butyric acids, as shown in Table III.

Other variables, such as the technique of the operator, may affect these numerical values, so it is not practical to use k values based on the butyl alcohol content as found by analysis.

Butyl acetate samples 1 and 7 have too high water contents to keep well, and sample 7 also has too much butyl alcohol to be satisfactory.

Effect of Temperature of Extraction. The temperature at which the extractions are carried out in connection with the determination of the partition coefficients has a small effect on the numerical values obtained. It is small enough, however, not to cause trouble if the partition coefficients for the known acids (k values) are measured at approximately the same room temperature at which the analysis is run. Accurate results on known acid mixtures have been obtained at all times and at all room temperatures ordinarily encountered.

Effect of Acid Concentration. Partition coefficients for the individual acids are measured using 0.1 *N* solutions, but the acidities of the distillates containing the unknown acid mixtures vary at least over the range 0.08 to 0.12 *N*. The effect of such variations was measured by analyzing a known acid mixture at concentrations about 0.060, 0.10, and

Table IV. Effect of Acid Concentration

Ternary Mixture		Acid Concentration 0.06 *N*	0.10 *N*	0.12 *N*
K_1		46.10	46.20	45.85
K_2		21.85	21.70	21.50
Acid	Taken	Found		
	Mole Per Cent			
Acetyl	16.0	17.2	16.5	16.6
Propionyl	30.6	29.8	30.5	29.4
Butyryl	53.4	53.0	53.0	54.0

Table V. Effect of Acid Concentration on Partition Coefficients

Acid	Coefficient	0.06 *N*	0.10 *N*	0.12 *N*
Acetic	k_1	0.846	0.844	0.844
	k_4	0.574	0.573	0.573
Propionic	k_2	0.583	0.579	0.579
	k_5	0.256	0.254	0.252
Butyric	k_3	0.285	0.278	0.273
	k_6	0.092	0.090	0.090

0.12 *N*, and the results are shown in Table IV. The best agreement between the observed analyses and the amounts taken was obviously obtained with the 0.1 *N* solution, and poorer results were found using the other solutions.

The six *k* values for acetic, propionic, and butyric acids were also measured at these same three concentrations (Table V).

It is apparent that the accuracy must suffer as the normality of the test solution deviates from the normality of the known acid solutions used in measuring the partition coefficients. For this reason, it is suggested that the normalities of the distillates be adjusted to within the range of 0.08 to 0.12.

Extractants Tested

Partition coefficients for acetic, propionic, and in some cases butyric acid were measured between water and each of the following organic solvents:

Ethylene dichloride
Propylene dichloride
Carbon tetrachloride

Ethyl ether
Isopropyl ether
Butyl ether
Amyl ether

Benzene
Cyclohexane
Decahydro naphthalene

n-Propyl acetate
n-Butyl acetate
Ethyl *n*-butyrate

Heptane
Pentane
Ligroin

n-Propyl and *n*-butyl acetates were found to give the greatest spread in numerical values for the three acids, and have been selected as the most satisfactory for this purpose.

Summary

The partition (or distribution) method has been found to be practical and satisfactory for the analysis of mixed esters of cellulose, and particularly for cellulose acetate propionates, acetate butyrates, and acetate propionate butyrates.

Details of manipulation are given together with limits of applicability, precision, and accuracy of the method. Necessary variations in the procedure are described for esters of higher acyl content and of nonvolatile acids.

Commercial cellulose acetate propionates and acetate butyrates can be analyzed with a precision of about ±0.1 weight per cent acyl and an accuracy of about ±0.2 weight per cent acyl.

The effects of some of the variables have been measured.

Acknowledgment

The authors gratefully acknowledge the assistance of J. W. Mench, H. M. Barden, J. V. Fleckenstein, M. L. Townsend, N. G. Baumer, and M. Van Graafeiland in carrying out the analyses and tests reported in this paper.

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Correction

In the article on "Determination of Particle Size Distribution" [Ind. Eng. Chem., Anal. Ed., **14**, 10 (1942)] several errors occur on page 12.

Column 1, paragraph 4, the last word in the line should be "liquid" instead of "solid".

Column 1, paragraph 8, P_2 in the numerator of the fraction should be ρ_2.

Column 2, Sample Calculation, in the expression for ρ_1 the numerical factor should be 1.0001.

Column 2, the explanation in small type "For 40μ at 20 cm.[a] t_{40} = 9.75/9 = 1.2 minutes" should be "For 40μ at 20 cm.[a] t_{40} = 9.75/8 = 1.2 minutes".

W. O. Hinkley

Determination of Strontium in the Presence of Calcium

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IN CONNECTION with a phase rule study of the ternary system calcium nitrate–strontium nitrate–water in process in this laboratory an accurate method for the determination of strontium in the presence of calcium was needed. As the system is being studied with the idea that the data gathered may be used as the basis for a fractional crystallization process for preparing pure strontium nitrate from impure or low-grade strontium ores, it was further desired that the analytical procedure be capable of use as a technical method.

TABLE I. SOLUTION OF CALCIUM NITRATE AND STRONTIUM NITRATE IN ACETONE AT ROOM TEMPERATURE

$Ca(NO_3)_2$		$Sr(NO_3)_2$	
Time elapsed, minutes	Salt, gram per ml. of soln.	Time elapsed, minutes	Salt, gram per ml. of soln.
12	0.3118	13	0.0004
25	0.3377	25	0.0007
37	0.3713	57	0.0010
56	0.4189	103	0.0016
78	0.4532		
106	0.4642		

The classical method for the determination of strontium in the presence of calcium is by the extraction of the calcium nitrate from the mixed alkaline earth nitrates with a mixture of absolute alcohol and absolute ether (*1*). These solvents are both expensive and hygroscopic, this latter feature being especially important in many localities. The acetone extraction method of Shreve and co-workers (*2*) seemed to obviate some of these undesirable features. The results claimed for it were substantiated by trial, but only in mixtures containing a calcium to strontium ratio of 3 to 1 or richer in strontium. With increasing proportion of calcium to strontium in the mixture of the salts the method becomes less and less reliable.

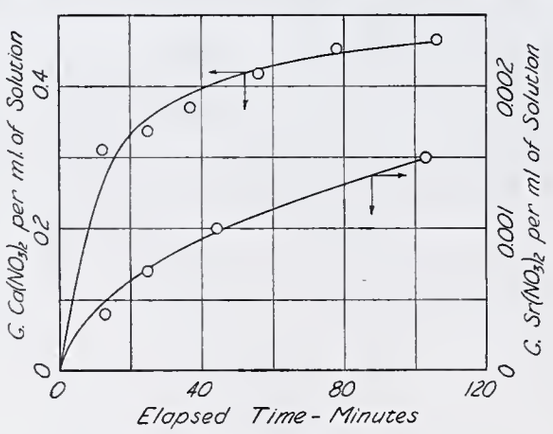

FIGURE 1

As acetone is both cheap and ordinarily readily available, it was decided to attempt to modify the acetone extraction method so that sufficiently accurate results could be obtained. Accordingly, the work of Williams and Briscoe (*3*) was repeated in part, and their finding that calcium nitrate is extremely soluble and strontium nitrate essentially insoluble in acetone at room temperature was confirmed. It was also found that the rate of solution of calcium nitrate in an acetone solution of calcium nitrate was too slow at the end of an hour to make this solution a good solvent. Results of this work are shown in Table I and Figure 1.

Among the variations in procedure tried were varying the length of time with a single extraction, repeated extractions of differing times, ratio of acetone to total salts, and finally a continuous extraction process using Jena sintered-glass filter crucibles in a modified Soxhlet apparatus. None of these gave satisfactory results.

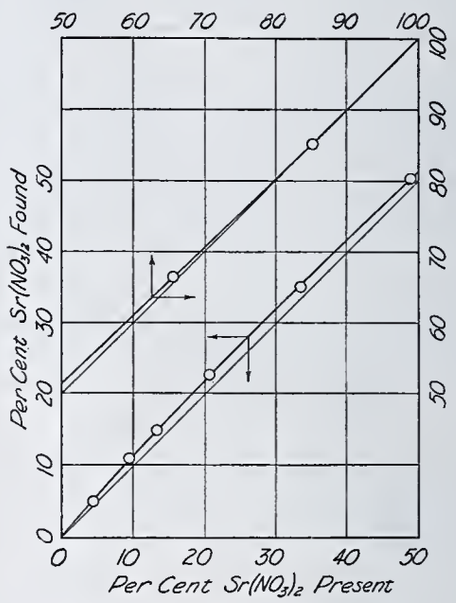

FIGURE 2

It was then decided to standardize a procedure, and to obtain a calibration curve for this method. The variables which it was deemed necessary to control were ratio of solvent to total salts, time of extraction, and manner of extraction. Accordingly, the following procedure was adopted and rigidly followed:

For the analysis of a sample containing other substances than calcium and strontium nitrates it is necessary to isolate these alkaline earth nitrates as in the gravimetric analysis for calcium in limestone. Briefly, this consists of putting the sample in solu-

TABLE II. CALIBRATION OF ANALYTICAL PROCEDURE

	Strontium Nitrate Present							
	% 4.42	% 9.36	% 13.24	% 20.59	% 33.30	% 48.84	% 65.55	% 85.10
Trial	Strontium Nitrate Found							
	%	%	%	%	%	%	%	%
1	5.00	10.90	14.92	22.52	34.95	49.95	66.55	85.10
2	5.45	9.10[a]	14.67	24.40[a]	36.85[a]	50.45	66.73	85.20
3	5.34	10.70	15.22	21.85	34.90	50.70	66.45	85.10
4	4.46	11.20	14.47	23.75	35.50	50.15	66.25	83.10[a]
5	3.89[a]	11.10	15.41	23.05	34.75	50.40	66.65	85.30
6	4.92	11.50	...	....	....	...	...	....
7	5.32	11.60	...	....	....	...	...	....
8	5.45	10.90	...	....	....	...	...	....
9	4.98	10.50	...	....	....	...	...	....
Av.	5.12	11.05	14.94	22.79	35.02	50.35	66.53	85.20
Av. deviation	0.70	1.69	1.70	2.20	2.72	1.51	0.98	0.10

[a] Not used in average.

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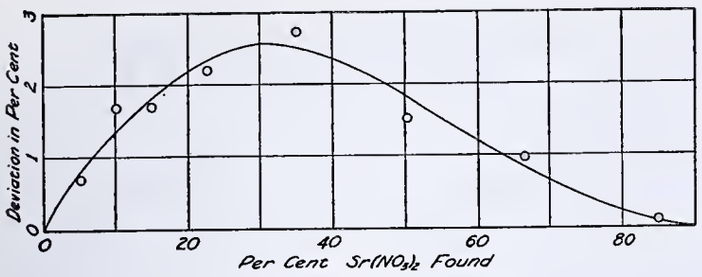

FIGURE 3

tion with hydrochloric acid, after fusing it with sodium carbonate if necessary. The silica is then removed by repeated dehydrations and filtered off, followed by the removal of iron and aluminum as hydroxides by precipitation with ammonium hydroxide. The alkaline earth metals are then precipitated as carbonates with ammonium carbonate and filtered off from the solution. Treatment of the carbonates with nitric acid will then yield the desired solution of the alkaline earth nitrates. This procedure makes no provision for separating barium from strontium and calcium.

The solution, of sufficient size to yield about 1 gram of anhydrous salts, containing the mixed calcium and strontium nitrates, is evaporated on a steam plate for from 12 to 24 hours, and finally dried in an oven at 140° C. for 24 hours. Drying at a lower temperature was found to be too slow, and tests showed no detectable decomposition of the strontium nitrate at this temperature and for this time. Both the evaporation and drying were generally carried out in 40 × 25 mm. weighing bottles with the covers held slightly open for the escape of vapor by a U-shaped wire clip on the top of the bottle. The mixed anhydrous nitrates are then cooled in a desiccator over phosphorus pentoxide (calcium nitrate is an excellent desiccant itself), and weighed. They are then extracted with C. P. acetone, using 4 ml. of acetone per 100 mg. of salts. The salts are transferred to an evaporating dish for the extraction with the extractant acetone, and thoroughly pulverized with a stirring rod, the end of which has been enlarged. During the extraction the contents of the dish are stirred every 8 to 10 minutes, the dish being covered with a watch glass at all other times. At the end of exactly one hour the residue in the dish is filtered off in a No. 3 Jena sintered-glass filter crucible, dried at 140°, and weighed.

Solutions containing known amounts of both alkaline earth nitrates were then prepared, and the foregoing procedure was applied to them. The data in Table II were obtained. These are shown graphically in Figure 2, which shows the values obtained by this analytical procedure compared with the true values. The maximum deviation is in the range of 10 to 60 per cent strontium nitrate. In Figure 3 is plotted the deviation in per cent against the per cent strontium nitrate found in the sample by this analytical method. The deviation is subtracted from the per cent strontium nitrate determined to give the true value.

Although a sample yielding about 1 gram of mixed alkaline earth nitrates was preferred, samples as small as 0.40 gram and up to 3 grams were run by this method. The strontium content of these samples ran from a minimum of 4 to 5 mg. up to practically the entire weight of the sample. In running triplicate samples, almost invariably a pair of values would check to within 5 parts in 1000, based on the original weight of the nitrates. This method has been used on all the analytical work on the phase rule study referred to earlier, and the results have been most satisfactory in that they are thoroughly consistent among themselves.

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Analysis of the Ternary System Ethanol-Methanol-Water

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A rapid and accurate physical method of analysis is presented for the ternary system ethanol-methanol-water, which requires the boiling point, refractive index, and specific gravity of the sample. The composition is then read directly from a chart. No assumptions are involved and no adjustment to a definite water concentration is needed.

THIS ternary system is encountered in some antifreeze solutions and certain denatured alcohol formulas and pharmaceutical preparations, although the analysis was developed for use in a study of liquid-vapor equilibrium which will be published later.

Early methods of analysis applied to this system had as their chief purpose the determination of small amounts of methanol in ethyl alcohol and water. Chemical methods of analysis were to oxidize the methanol or subject it to some other chemical reaction, then determine the compound formed.

Leach and Lythgoe (*3*) developed a purely physical analysis for the ternary system, using the specific gravity and refractive index. The method embodied two approximations: that the concentration of total alcohol is a function of gravity only, and that the refractive index is linear with the ratio of the two alcohols. The method is necessarily inexact, and has the disadvantage that the rather inaccessible and not highly accurate Hehner alcohol tables must be used to avoid serious errors.

Lange and Reif (*2*) obtained better accuracy with a similar method, by adjusting the alcohol concentration to 50 per cent by volume, then determining gravity and refractive index.

Williams (*6*) reviewed Leach and Lythgoe's method, and improved upon it by adjusting the sample concentration to 20 per cent by weight of total alcohol before making final gravity and refractometer determinations.

Mortimer (*5*) developed a ternary chart for alcohol concentrations up to 40 per cent by weight in terms of Zeiss refractometer readings, with which the proportion of the two alcohols may be resolved.

Macoun (*4*) improved Lange and Reif's method somewhat by adjusting the sample to a concentration of 40 per cent alcohol by weight. The weight per cent is independent of temperature whereas the volume per cent (used by Lange and Reif) is not. Macoun also prepared a table of alcohol concentration in terms of refractometer readings for values between 39.5 and 40.5 weight per cents of alcohol, and temperatures from 15.6° to

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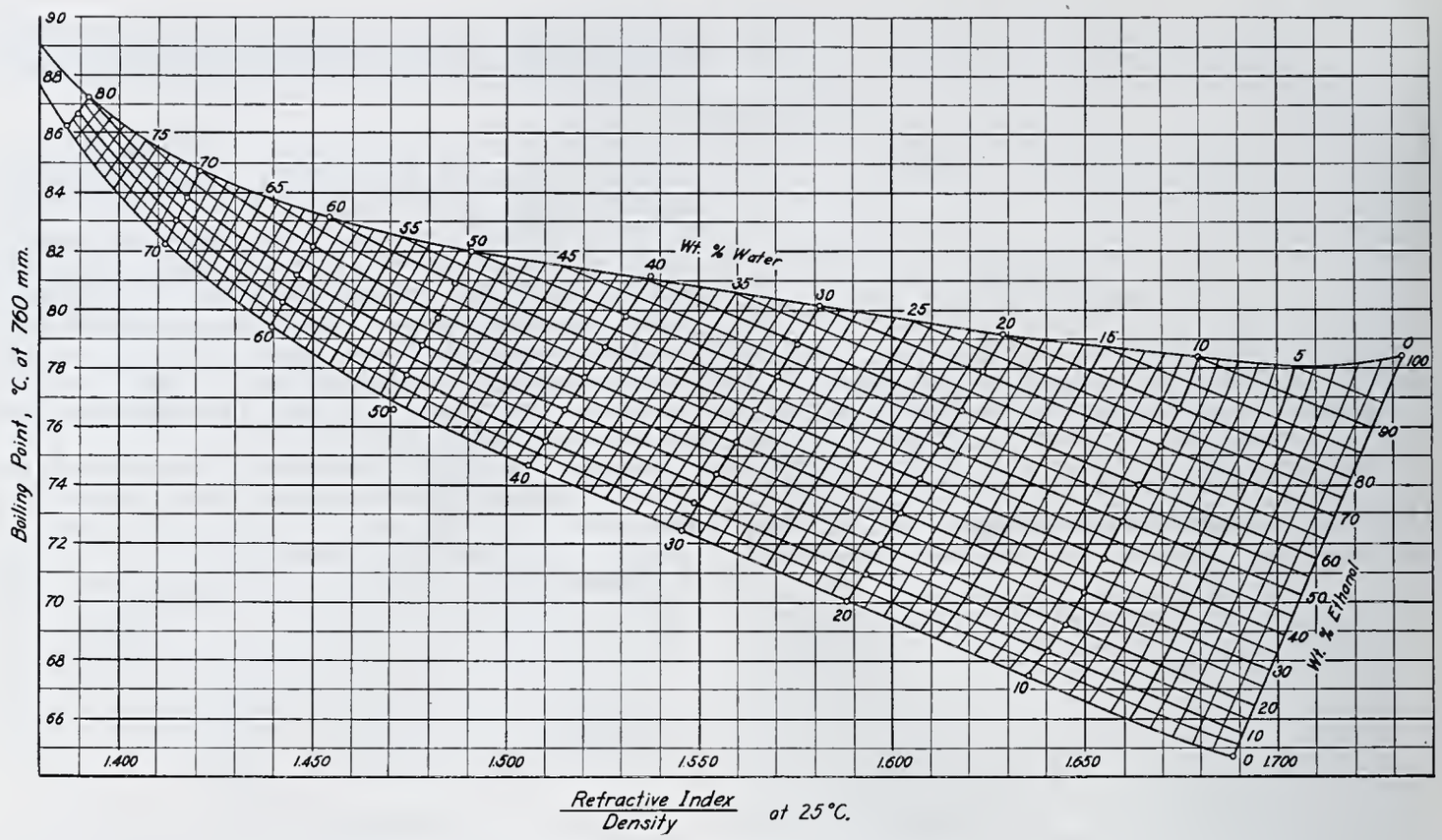

FIGURE 1. ANALYSIS OF ETHANOL-METHANOL-WATER MIXTURES

22° C. The relative amounts of the alcohols were computed by the linearity approximation used by Leach and Lythgoe. A footnote to the article is significant:

At this point the specific gravities of aqueous ethyl alcohol and aqueous methyl alcohol are close together, while their refractive indices lie far apart. Certain errors inherent in this type of determination are therefore greatly reduced.

All the foregoing methods embody the first approximation in that they depend upon gravity alone for the water content, although no brief is held that the gravity is completely independent of the ratio of the two alcohols.

By inclusion of a third physical constant, the boiling point, a method free from approximations was obtained. A series of 56 determinations was made upon prepared samples, systematically varied in composition. The data were then plotted in various ways. The Lorenz-Lorentz relation (which is theoretically inapplicable to mixtures) was tried, but showed no particular promise. The final plot (Figure 1) of boiling point (° C. at 760 mm.) against the refractive index-density ratio at 25° C. (n_d/D_4^{25}) was selected as giving the most uniform spread of and least curvature to the lines of constant composition. Use of the chart is straightforward—the methanol content is obtained by difference. A sample can be analyzed in about 15 minutes' time.

Two unknowns made up by one person and tested by another who had had no previous experience with the method gave the results in Table I. While additional confirmatory determinations would be in order, the method contains no inherent errors and the consistency with which the data on known mixtures fell upon the smooth curves indicate the chart to be accurate to within 0.2 per cent for water and for ethanol. The same degree of accuracy for a determination requires observations of the gravity and refractive index accurate only to the third decimal place with the boiling point accurate to 0.1 ° C.

To test solutions of composition outside the zone of experimental points on the chart: those containing less than 20 per cent of alcohol may be concentrated by distillation or be enriched by the addition of standardized aqueous alcohol; those containing less than 10 per cent of water may be diluted.

Apparatus and Reagents

Specific gravities were taken at 25° C. as D_4^{25} using a Geissler-type gravity bottle. Refractive indices were determined at 25° C. with an Abbe instrument against the sodium D line. Boiling points were obtained with the conventional Cottrell apparatus and a calibrated Anschutz thermometer. This Cottrell boiler has been used on these same mixtures before (*1*), and it can be made in any laboratory equipped with the usual glass-blowing facilities. The observed boiling points were corrected to 760 mm. by the formula

$$\text{Correction ° C.} = \frac{273.1 + t_{obs.}}{6.0}\,(2.8808 - \log p_{mm.})$$

The alcohols were C. P. grade anhydrous methanol and 95 per cent ethanol. They were fractionated through a 3-foot (91.44-cm.) packed column and the heads and tails rejected. Gravity, refractive index, and boiling point determinations on the middle fractions showed no indications of interfering substances. The distilled water for making up the stock solutions was acidified with sulfuric acid, treated with permanganate, and redistilled. Care was taken to keep all solutions in glass-stoppered bottles, and to expose them to the atmosphere as little as possible.

TABLE I. ANALYSES OF UNKNOWNS

	Sample I		Sample II	
	Taken	Found	Taken	Found
	Wt. %		Wt. %	
H_2O	40.7	41.0	10.8	11.8
MeOH	9.6	10.0	49.6	48.5
EtOH	49.7	49.0	39.6	39.7

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Rapid Method for Determining Potassium in Plant Material

Perchloric Acid Digestion and Chloroplatinic Acid Precipitation

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THE need for a more rapid method for determining potassium in plant material was emphasized by the referee work on ash and the recovery of mineral elements in ash, reported by St. John and Midgley (*11*) and St. John (*10*), which showed that higher ashing temperatures caused an apparent loss of potassium. Methods reported in the literature, involving a number of steps and separations, are somewhat time-consuming and provide more opportunity for loss of potassium.

This laboratory has contributed papers on methods for more rapid oxidation of organic matter, or increased recovery of elements by oxidation with combined nitric, sulfuric, and perchloric acid digestion (*2*, *4*, *5*, *6*). Perchloric acid is increasingly used for this general purpose. Smith (*13*, *14*) has studied perchloric acid and its use in the determination of a number of elements and has presented a method for potassium. Gieseking, Snider, and Getz (*7*) determined potassium and other elements by perchloric acid digestion, followed by the cobaltinitrite method described by Schueler and Thomas (*12*).

Method

A modified method for the determination of potassium has been developed which involves the nitric-perchloric acid digestion of the plant material and chloroplatinic acid precipitation of the potassium, and which eliminates a number of time-consuming steps. Plant products of somewhat widely varying character have been analyzed by this method, presented below, and also by the A. O. A. C. (*1*) method where the plant material is ashed after moistening with sulfuric acid. Temperature was controlled at 600° C. in this work.

Weigh 1 or 2 grams of the sample into a 150-ml. beaker. Add 30 ml. of concentrated nitric acid, cover, and digest over a low flame or on a hot plate until all easily oxidizable organic matter is oxidized. It is occasionally necessary to add a second or third portion of nitric acid. Cool slightly, add 10 ml. of 60 per cent perchloric acid, and continue digestion until the solution is colorless and fumes of perchloric acid begin to appear. Continue heating until all perchloric acid is driven off, taking care to avoid spattering. After fumes cease to appear, place the beaker in a muffle furnace regulated to a temperature of 350° C. and bake for 15 to 30 minutes to remove last traces of acids and any ammonium salts. Remove from the muffle, cool, and transfer the precipitate to a 7-cm. No. 4 Whatman or equivalent filter paper, with a minimum quantity of near-boiling water. The use of a policeman is essential in this transfer. Wash thoroughly with boiling water and collect the filtrate in a 100-ml. beaker, keeping the filtrate to a minimum volume. Evaporate on a steam bath or hot plate to 5 or 10 ml., but not to dryness. Acidify with concentrated hydrochloric acid. Add chloroplatinic acid to precipitate all potassium and sodium according to the A. O. A. C. method for potassium in fertilizers, and wash with 80 per cent alcohol, then with ammonium chloride saturated with potassium chloroplatinate, and finally with 80 per cent alcohol. The results may be checked to determine the presence of any insoluble residue by dissolving the weighed potassium chloroplatinate with near-boiling water, followed by alcohol, drying, cooling, and reweighing.

Discussion

Comparative analyses (Table I) were made of two practical feeding mixtures and of samples of alfalfa, corn, potatoes, soybeans, wheat, and apple juice by the method presented in this paper and the A. O. A. C. sulfuric acid ashing method, using the rapid method for potassium only. Results are calculated in terms of the percentage of the element potassium in the original sample of feed rather than in the ash, and are corrected for insoluble residue.

The data in Table I indicate that satisfactorily consistent results are obtained by the potassium method described in this paper. In general, the agreement among replicates is better than when the results are obtained by the present official method. It is easier to obtain satisfactory agreement by use of the perchloric acid digestion.

The method here described gives a somewhat higher value for potassium in some plant materials than the A. O. A. C. method. With corn, potato, and fruit juice, the percentage of potassium is essentially the same by the two methods. Gieseking, Snider, and Getz (*7*) obtained somewhat lower results (with the A. O. A. C. method) than when using a perchloric acid digestion and cobaltinitrite precipitation.

An interesting and unexpected result is evident from a study of the original data on which the results in Table I are based. In determining potassium in fertilizers, difficulty has been experienced with an insoluble residue which accompanies the chloroplatinate precipitate as reported by Ford (*3*). The authors found an insoluble residue in the potassium precipitate from these samples, when using the A. O. A. C. method. In most cases when utilizing the perchloric digestion method there was no insoluble residue. The amount of insoluble residue was determined in each case by dissolving the soluble

TABLE I. POTASSIUM IN PLANT MATERIALS

	Perchloric Acid Digestion	A. O. A. C. on Ash
	%	%
Poultry feed	0.767	0.562
	0.750	0.504
	0.780	0.642
	0.766	0.597
	0.790	0.544
	0.807	
	0.821	
Av.	0.783	0.572
Dairy feed	0.647	0.496
	0.655	0.442
	0.655	0.564
	0.655	
	0.656	
Av.	0.654	0.501
Alfalfa	1.015	0.912
	1.031	0.859
	0.986	0.930
		0.896
		0.820
Av.	1.011	0.883
Corn	0.257	0.258
	0.260	0.253
	0.242	0.264
		0.260
Av.	0.253	0.259
Potato	0.383	0.396
	0.443	0.457
	0.409	0.416
Av.	0.412	0.423
Soybean	2.180	1.866
	2.160	1.798
	2.047	1.843
	2.078	1.678
	2.063	1.862
	2.205	
	2.269	
Av.	2.143	1.809
Wheat	0.305	0.169
	0.310	0.169
	0.300	0.231
	0.310	0.210
		0.246
Av.	0.306	0.205
Apple juice	0.126	...
	0.130	...
	0.130	...
	0.146	...
	0.136	...
Av.	0.134	0.133[a]

[a] The authors extend thanks to A. M. Neubert of this division for this analysis which he is publishing elsewhere.

Table II. Recovery of Potassium

	(1)	(2)	(3)	(4)
	16.5933	16.7007	16.9511	17.0389
	16.5340	16.6395	16.8886	16.9777
	0.0593	0.0612	0.0625	0.0612
Potassium recovered, gram	0.00954	0.00984	0.01005	0.00984

potassium chloroplatinate with hot water and reweighing the crucibles in which the original precipitate had been weighed.

The A. O. A. C. method for plant material does not specify the temperature for ashing and for driving off ammonium salts. However, in using this method, the samples were ashed at 600° C. and the ammonia was driven off at 550° C., since ammonium oxalate is not volatile at 350° C. In the proposed method, ammonium salts were driven off at 350° C., at which temperature both ammonium chloride and nitrate are volatile. Utilization of these controlled temperatures tended to avoid the loss of potassium due to volatilization by localized overheating during ashing or while driving off the ammonia.

The proposed method apparently gives satisfactory results without removal of calcium and other metals. Wiley (*16*) states that a separation of alkali earths, if potassium alone is to be determined, is superfluous since calcium and magnesium chloroplatinate are even more soluble in 90 per cent alcohol than is sodium chloroplatinate. Mellor (*9*) quotes earlier workers to show that calcium chloroplatinate is soluble in alcoholic solution, and a saturated solution contains 53 per cent of the salt. The salt is soluble in ethyl alcohol of specific gravity 0.8035 at 15°, and is decomposed into its component chlorides. Potassium has apparently been satisfactorily determined on soils by the calcium carbonate–ammonium chloride fusion method for many years, without removing calcium.

The method here described is more rapid because of elimination of a number of steps. Nitric-perchloric acid digestion is more rapid than ashing. Digestion is made directly in a beaker, saving a transfer. The time required for evaporations is reduced to a minimum, since there is only one transfer and filtration before precipitation. This filtration requires much less washing than filtering out precipitated iron and aluminum, and evaporation is carried to 10 ml. rather than to dryness. There is no evaporation of a concentrated solution of ammonium salts, since none have been added and thus the difficulty with creeping salts is minimized and considerable time is saved. Any small amount of ammonia present in the original material after digestion is volatilized, and any remaining silica is dehydrated during the baking at 350°. Filtration and washing after baking are much more rapid, and the possibility of potassium loss is reduced in comparison to the method where calcium is separated. With the proposed method, it is easily possible to start and complete the determination of potassium on a set of samples within an 8-hour day.

The somewhat higher results obtained in some cases by the procedure here described than by the A. O. A. C. method are believed to demonstrate the recovery of added potassium from the original sample, since solubility and microscopic examination of the precipitates indicated pure potassium chloroplatinate. Any impurity in the precipitate or any added potassium due to the reagents or procedure should have given higher results in the case of all samples. Further, utilization of the described method with pure salts gives essentially 100 per cent recovery. Data in Table II (1) and (2) are obtained by precipitating the potassium from a solution of potassium chloride containing 0.00996 gram of potassium. To similar aliquots of the same potassium chloride solution there was added a solution of calcium chloride containing about 52 mg. of calcium chloride, equivalent to about the amount present in an average sample of alfalfa. The recovery of potassium is very satisfactory, as shown by (3) and (4), and it is evident that the presence of a comparatively large amount of calcium has not interfered with the complete recovery of potassium; in fact, the amount of potassium recovered is within ±0.1 mg. of the amount in the solution, which is a variation of less than 1 part in 600. A determination of potassium on the calcium chloride solution gave practically negligible results.

The added number of steps in the A. O. A. C. procedure offers greater opportunity for losses. Potassium may be lost during the ashing procedure (*10*, *11*), or by spattering and through the volatilization of potassium salts during ashing (*16*).

It may be advantageous to use a chloroplatinic acid solution made up with hydrochloric acid, as mentioned by Tenery and Anderson (*15*). A large excess of chloroplatinic acid in precipitation is believed essential. The authors have found the chloroplatinic precipitation in hydrochloric acid preferable to precipitation in alcohol as recommended by Smith (*13*).

Caution. Kahane (*8*) and Cook (*2*) have summarized the care that should be taken in digesting with perchloric acid. Thorough digestion with nitric should be completed before the addition of perchloric. No difficulty has been experienced in this laboratory in making hundreds of digestions with perchloric acid.

Summary

A rapid procedure for the determination of potassium in plant material involves a combination of the perchloric acid method of digesting the sample and the familiar chloroplatinic acid precipitation. A number of steps employed in the more commonly used method have been eliminated. In addition to rapidity, ease of manipulation, and ease of obtaining satisfactory agreement of results, the method seems to give better recovery in some instances than the present official method for plant material.

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Correction. In the paper "Rapid Turbidimetric Method for Determination of Sulfates" [Ind. Eng. Chem., Anal. Ed., 14, 119 (1942)] Whatman No. 42 paper should have been mentioned in the procedure, not Whatman No. 12 paper.

J. F. Treon, Jr.

Determining the Fusain Content of Illinois Coals

A Comparison of Chemical and Petrographic Methods

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THE method commonly referred to as microscopic count or petrographic analysis used for quantitatively determining the different ingredients of banded coal has been standard practice for several years in the coal laboratory of the Illinois State Geological Survey. However, this type of analysis is long and tedious, is subject to personal errors, and does not permit concurrent duplicate determinations. Because of these disadvantages a less time-consuming and less laborious method for determining fusain is desirable. This investigation, therefore, was for the purpose of determining whether the chemical method described by Fuchs, Gauger, Hsiao, and Wright (*2*) would provide values for fusain in sufficiently close agreement with those obtained by the petrographic method to justify its exclusive use.

TABLE I. PETROGRAPHIC ESTIMATION OF FUSAIN CONTENT IN A FINE COAL

3/8-inch screenings (raw carbon)[a]

Screen Mesh No.	Particle Sizes Retained on Screens	Screening Analysis	
	Mm.	*Grams*	%
8	+2.36	7.40	3.87
10	−2.36 +1.651	22.10	11.56
14	−1.651+1.17	26.50	13.86
20	−1.17 +8.33	24.40	12.76
28	−8.33 +0.590	21.40	11.19
35	−0.590+0.417	18.30	9.57
48	−0.417+0.300	14.20	7.43
65	−0.300+0.208	10.50	5.49
100	−0.208+0.150	10.10	5.28
150	−0.150+0.104	6.10	3.19
200	−0.104+0.075	6.50	3.40
300	−0.075+0.046	3.30	1.73
Pan	−0.046	20.40	10.67
		191.20	100

[a] Sample from preparation plant of Madison County, Ill., mine.

Petrographic Method

The procedure involves two distinct series of steps after preparation of the sample and riffling a desired amount (approximately 500 grams). In the first step the sample selected by riffling is weighed and separated into size classes by screening through a series of Tyler standard sieves. Twelve sieves generally are used and these are grouped into two nests of six sieves each for convenience in using the Ro-Tap shaker. The sieves range from No. 8 mesh to No. 300 mesh. The size of the openings in the individual sieves decreases in the ratio of the square root of two. The openings of the No. 8 mesh screen are 2.36 mm. square, and of the No. 300 mesh are 0.046 mm. square. The material retained on eleven of the screens, after shaking through the first nest (8- to 35-mesh) for 10 minutes and the second nest (48- to 300-mesh) for 15 minutes, consists of sorted particles, each group having a relatively small size range. In addition to the sized material there are two unsorted batches, the 2.36-mm. material retained on the No. 8 mesh screen and the contents of the pan which passed through the No. 300 mesh screen (Table I).

The essential purpose of the screening is to facilitate the second step of manually separating and counting the component particles. For this work an ore-dressing type of binocular microscope is used.

In the separation and counting of fusain particles use is made of the distinctly different appearance of fusain and of the other constituents. In contrast to particles of the other constituents, fusain particles are flaky and needle-shaped with surface striations and pits often characteristic. These characteristic differences are maintained throughout the entire range of sizes used in the petrographic method of estimation.

In making fusain estimations the number of particles separated and counted in order to arrive at the correct fusain percentage in each size class is dependent upon the frequency of distribution of fusain in the particular class. For the size classes containing less than 10 per cent fusain a count of 2500 particles gave results which could be duplicated within 0.2 per cent by different operators. For size classes which had in excess of 10 per cent fusain good results were obtained by counting as few as 1000 particles in some classes (Table II).

It is obvious that the fusain percentages obtained by separating and counting particles of coal in this manner are not true weight percentages. If all particles in the two separated groups of fusain and nonfusain were spheres of the same size and specific gravity, true weight percentages could be calculated from the count data. It is known, however, that the ingredients of banded coal do not break down into spherical particles and that specific gravity is variable. Notwithstanding this, it was assumed, for practical purposes in obtaining satisfactory results, that every particle of coal separated and counted could be regarded as having the same size and specific gravity. The validity of this assumption was tested by weighing the fusain and nonfusain of ten size classes, cal-

TABLE II. PETROGRAPHIC ESTIMATION OF FUSAIN CONTENT IN A FINE COAL

3/8-inch screenings, microscopic count

Screen Mesh No.	Particle Sizes Retained	Separation and Count			Distribution of Fusain		
		Fusain	Non-fusain	Total	Fusain and non-fusain	Fusain	%
	Mm.				*Grams*	*Grams*	
8	+2.36	104	1700	1804	7.40	0.43	5.76
10	−2.36 +1.651	142	2021	2163	22.10	1.45	6.56
14	−1.651+1.17	151	1950	2101	26.50	1.91	7.19
20	−1.17 +0.833	181	2196	2377	24.40	1.87	7.67
28	−0.833+0.590	210	2348	2558	21.40	1.76	8.21
35	−0.590+0.417	251	2489	2740	18.30	1.68	9.16
48	−0.417+0.300	234	2125	2359	14.20	1.41	9.92
65	−0.300+0.208	333	2584	2917	10.50	1.20	11.42
100	−0.208+0.150	322	2058	2380	10.10	1.30	12.90
150	−0.150+0.104	298	1400	1698	6.10	1.07	17.55
200	−0.104+0.075	264	800	1064	6.50	1.61	24.81
300	−0.075+0.046	408	500	908	3.30	1.48	44.93
Pan	−0.046	560	180	740	20.24	15.44	75.67

Total weight of sample, grams	191.20
Weight of nonfusain, grams	158.59
Weight of fusain, grams	32.61
Content of fusain in sample, %	17.06

TABLE III. COMPARISON OF FUSAIN VALUES BY COUNT AND BY WEIGHT

Microscopic method

Grade Size (Screen Mesh)	Per Cent by Weight	Per Cent by Count
8–10	2.3	2.6
10–14	2.8	2.5
14–20	4.0	3.6
20–28	3.9	3.9
28–35	4.1	3.9
8–10	1.5	2.1
10–14	2.2	1.8
14–20	2.4	2.3
20–28	2.9	2.4
10–14	2.7	2.6

[1] Present address, Battelle Memorial Institute, Columbus, Ohio.

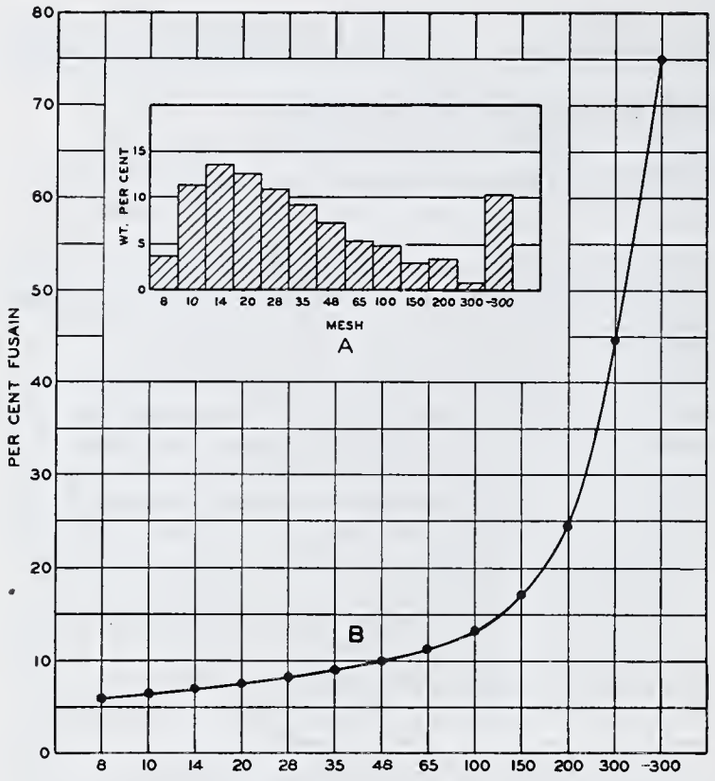


FIGURE 1. DISTRIBUTION OF FUSAIN

A. Histogram showing distribution of coal by weight percentages on screens after Ro-Tap operation of ten minutes for +35-mesh and 15 minutes for −35-mesh screens. Sample same as Table I

B. Direct plot curve showing distribution of fusain by weight percentages in coal retained on screens in *A*

culating true weight percentages, and comparing these with results obtained from count data. The results so obtained are in reasonably close agreement (Table III).

The estimation of fusain by the petrographic method here described is probably subject to unavoidable human errors and is time-consuming. The identification and separation of fusain from nonfusain in the fine sizes are particularly difficult. Eye and muscular fatigue are probably the chief causes of error in identifying, separating, and counting the extremely fine particles. An experienced petrographic observer working carefully can complete an analysis of a coal sample in about one week, and there is no short cut in making a duplicate count. An advantage afforded by petrographic analysis lies in the data obtained with which curves can be drawn showing size frequency distribution of fusain in the sample (Figure 1).

Chemical Method

The chemical method as described by Hsiao and co-authors (*2*) depends upon the relative susceptibility to oxidation of fusain and nonfusain coal material. Briefly, this procedure consists of oxidizing 1-gram portions of −200-mesh coal by boiling in 8 *N* nitric acid for 2-, 3-, and 4-hour periods. After proper washing with sodium hydroxide, hydrochloric acid, and water the residues are dried at 105° C., weighed, and ashed, and the weights of ash are deducted from the weights of oxidation residues. These ash-free residues are then calculated to per cent of moisture- and ash-free coal and plotted against oxidation time. A straight line is drawn through these points (through the last two points if impossible to draw through all points) and extrapolated to cut the zero time axis, and the corresponding residue value is taken as the fusain value. Moisture and ash determinations were made by A. S. T. M. procedures (*1*).

Figure 2 presents curves for three of the samples studied, showing their extrapolation to the zero time axis, as well as a typical oxidation curve (curve *A*) for times from 10 minutes to 4 hours. This curve appears to be made up of two parts, the first or curvilinear portion representing the oxidation of nonfusain coal material and the second flat portion representing the oxidation of the fusain. The curved portion appears to represent a first-order reaction while the flat portion appears to represent a zero-order reaction where extrapolation is easily possible.

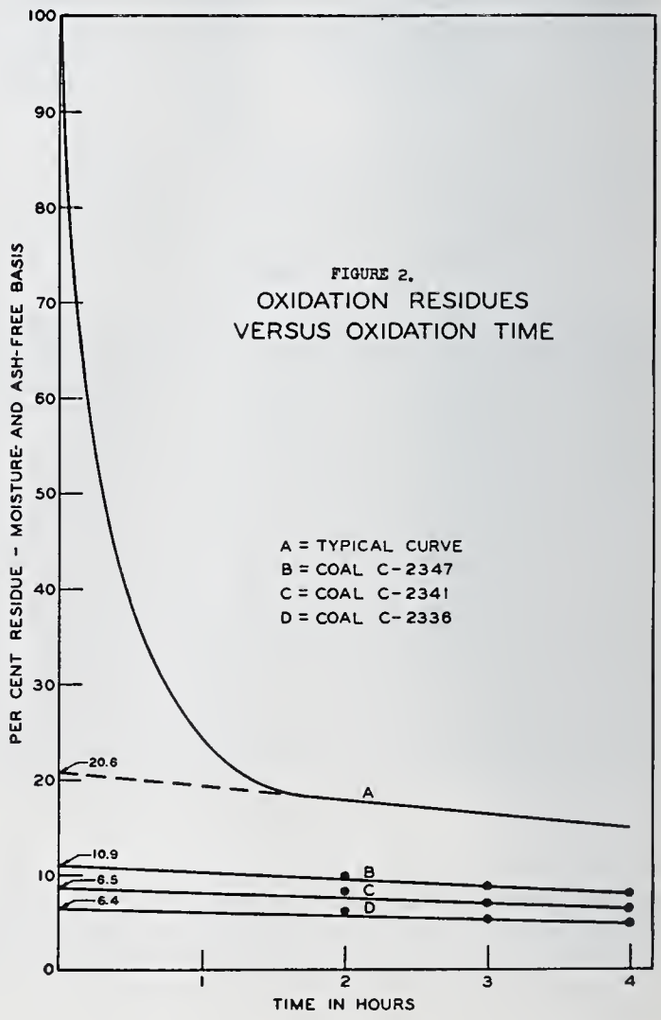
Results

Thirteen coal samples were analyzed for fusain content independently by both methods. A comparison was made of the results and certain incidental factors were also observed, such as length of time required for the analyses and the probability of errors. The fusain content of the thirteen samples as determined chemically and microscopically is given in Table IV. The values check reasonably well. In only four of the samples was the difference in the percentage figures more than 2 per cent. In the other nine samples the difference between chemical and microscopic results varied from 0.0 to 1.8 per cent.

TABLE IV. RESULTS OF TESTS

Lab. No.	Moisture in Coal	Ash in Coal	Fusain: Chemical method[a]	Fusain: Petrographic method	Difference
	%	%	%	%	%
C-2336 (1)	5.1	32.2	6.4	6.4	0.0
C-2337 (2)	6.5	24.2	5.4	7.2	+1.8
C-2338 (3)	5.5	41.5	14.2	9.4	−4.8
C-2339 (4)	7.1	25.6	18.0	15.2	−2.8
C-2340 (5)	2.7	18.8	19.0	19.5	+0.5
C-2341 (6)	5.1	24.2	8.5	7.5	−1.0
C-2342 (7)	6.1	19.3	10.1	7.4	−2.7
C-2343 (8)	5.2	11.0	11.5	12.6	+1.2
C-2344 (9)	5.9	23.2	12.8	10.4	−2.4
C-2345 (10)	6.2	19.2	15.9	14.4	−1.5
C-2346 (11)	5.1	10.9	4.2	4.0	−0.2
C-2347 (12)	7.6	13.4	10.9	10.8	−0.1
C-2348 (13)	5.5	9.8	13.8	12.1	−1.7

[a] Moisture- and ash-free basis.



The time necessary for conducting the chemical determinations, including duplicate tests of each sample, was 2 days, as compared to 5 to 10 days required for the petrographic estimations.

Certain of the residues from the chemical analyses were saved and examined carefully under the microscope. No organic material other than fusain could be found. The fusain in these residues was unusually easy to identify.

Conclusions

These tests indicate that in the majority of cases practically the same results are obtained by each method. Where different values are obtained, the greater complexity of the petrographic method and its greater dependence on personal judgment seem to favor the results obtained by the oxidation method.

The advantages of using the chemical method as indicated by these tests are summarized as follows: A considerable saving of time is effected. Duplicate analyses can be made simultaneously. There is less liability of human error. The values obtained represent fusain on an ash- and moisture-free weight basis.

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Expected Service Effectiveness of Preservative Treatments Applied to Millwork

A Laboratory Method of Comparison

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A practical laboratory method for evaluating and comparing the effectiveness of various millwork preservatives is described. This method provides moisture for the specimen in the same manner in which most of the moisture is supplied to millwork in service—namely, by condensation.

The specimens are compared either by visual examination of the split specimen or by microscopic examination of sections of the specimens.

CONCENTRATION of effort, during the past five years, on the development of methods for comparing the relative toxicities of various millwork preservative treatments has suggested the need for a technique to evaluate the expected field effectiveness of preservative treatments, particularly with regard to the control of blue stain fungi.

The first step in this direction was made by Hubert (*1*), who in 1938 reported a study on optimum time of immersion of millwork in preservative solutions required to effect a satisfactory treatment. In order to correlate depth of penetration of preservative with the effectiveness for control of fungi, he took 0.125-inch thick sections at various points in the bottom rail of a treated window sash and exposed them to fungi in Kolle flasks. Following this test procedure, the authors' results indicate that most millwork treatments in commercial use have shown adequate control of the important blue strain organisms, particularly when the 0.125-inch sections were taken within 2 inches of the "end" of the rail (where it meets the stile), but sections taken at a greater distance from the end showed less fungus control.

The authors have also noted from observations on window sash in service that where stain occurs, it often appears under the paint coat, suggesting that the stain fungi grew from within the wood rather than from germinating spores on the paint coat. Since present preservative treatments of sash and other millwork are applied as dips rather than by pressure, the wood is not completely impregnated. This fact, coupled with observations on stain development, suggests the desirability of making tests to determine the relative value of commercial preservatives for preventing fungi from growing from the inner untreated portion of the wood through the treated area and on through the paint coats.

Various methods were tested, and as a result of this research the following method is suggested as reliable and practical for testing the relative effectiveness of millwork preservatives.

The Method

In setting up a method in the Curtis Companies Incorporated research laboratory, it seemed desirable to use sections of bottom rails of window sash, treated in accordance with industrial practice, in order to eliminate the artificial character present when used in blocks either completely impregnated or of such shape and so treated that results comparable with those observed in actual field service could not be expected.

In order to carry out further the correlation between laboratory tests and field conditions, all culture media were eliminated. The test specimens themselves provided the medium in the untreated interior portion. Inoculation of the untreated sapwood was accomplished by spraying onto it a spore suspension of the test organism with a small spray atomizer.

A method for providing enclosure, support, and moisture was developed for this work. Adhering to the desirability of a close correlation between the laboratory method and actual service conditions, condensation was employed to provide the specimens with moisture. This was accomplished by chilling the specimen to approximately 45° F., then rapidly raising the relative humidity of the air surrounding the specimen until a heavy condensation was produced on the specimen. (This principle was originally suggested by C. Audrey Richards. Assistance in planning this procedure was also given by Theo. C. Scheffer.)

By performing this operation under identical conditions on all specimens, good control of moisture deposit was obtained. The frequency at which this operation was performed governed the average moisture condition of the specimens.

It is believed that this method has none of the disadvantages, such as overwetting, which is sometimes experienced

FIGURE 1. METHOD OF SUPPORTING SPECIMEN IN JAR

in the Kolle flask method (*2*) where the specimen receives its moisture by lying near, or in actual contact with, the moisture-bearing substrate. There is also no possibility of conduction of malt or other substances from the culture medium by the fungus mycelia.

Apparatus

No complex or expensive equipment was required. Ordinary wide-mouthed 2-quart glass fruit jars were used, and were modified with a minimum of labor (Figure 1).

The porcelain liners were removed from the lids and to each lid was soldered a support wire shaped at the lower end to fit into a weather-strip groove already in the section of the bottom rail. The wire was of sufficient length to support the specimen at least 1.5 inches above the bottom of the jar. About 0.75 inch of water was placed in each jar.

Three wire trays were constructed to hold 10 jars each. The jars were set in these trays permanently and removed only when the specimen was to be examined.

A water pan sufficiently large to hold one tray and about 6 inches deep was constructed. The tray was supported on brackets about 8 inches from the laboratory bench top and was filled with water to a depth of about 2 inches. Bunsen burners were used to heat this pan and its contents.

The usual incubation facilities were used. Wet incubation was not required. A standard household type of electric refrigerator was used to chill the trays of jars.

Preparation of Test Blocks

The test pieces were obtained from a piece of straight-grained Ponderosa pine sapwood, 1.375 inches × 4 inches × 16 feet, selected from stock according to specifications outlined in the N. D. M. A. standard wood block method (*2*). Liberal use of the benzidine-nitrite indicator assured that no heartwood was present.

After the piece had been machined to the detail of the bottom rail of the sash, thirty-six samples 5 inches long were cut from this piece and a 2.25-inch piece of bottom rail weather strip was stamped into each specimen. For each solution to be evaluated six pieces were prepared: three pieces to be inoculated on unpainted end grain and three pieces to be inoculated on painted end grain. Likewise, two sets of three were run (untreated) for controls.

Treatment of Test Blocks

All solutions were prepared according to manufacturer's directions or the usual industrial procedure.

The eighteen sections 5 inches long to be inoculated on unpainted surfaces were painted with two coats of interior undercoater, on the ends only, before dipping. The eighteen sections to be paint-infected were cut to 2.25-inch lengths and were likewise painted on the ends with two coats of interior undercoater before dipping. After the paint had dried thoroughly, the pieces were given the standard 3-minute immersion in five different preservative solutions. In each case three of the 5-inch pieces and three of the 2.5-inch pieces were treated.

Shortly after treating, 2.25 × 3 inch pieces of glass were glazed in all sections. In the case of the 5-inch pieces the glass was glazed in the center of each piece and the ends were cut off at the edges of the glass, leaving a 2.25-inch piece with unpainted ends to be used for wood infection.

After one week's standing all specimens were painted on the interior side with an interior undercoater diluted 8 parts of paint to 1 part of linseed oil and 1 part of turpentine. The second coat on the interior side was composed of half undercoater and half gloss enamel diluted 8 to 1 with turpentine. The third coat on the interior was straight gloss enamel. The surfaces which would normally be on the outside of the house were painted two coats of an exterior undercoater followed by one coat of gloss white exterior paint. The composition of the paints used in this test is given in Tables I and II.

After standing 2 months more the specimens were sterilized in a sterilizing chamber, using a General Electric germicidal lamp which emits an ultraviolet radiation of 253 Å. (Figure 2). The lamp used was a Type T-8, 15-watt, 110-volt, 60-cycle, which emits 15 to 20 microwatts per square centimeter of 2537 Å. units at 1 meter. The specimens were supported from the fruit jar lids as shown in Figure 1.

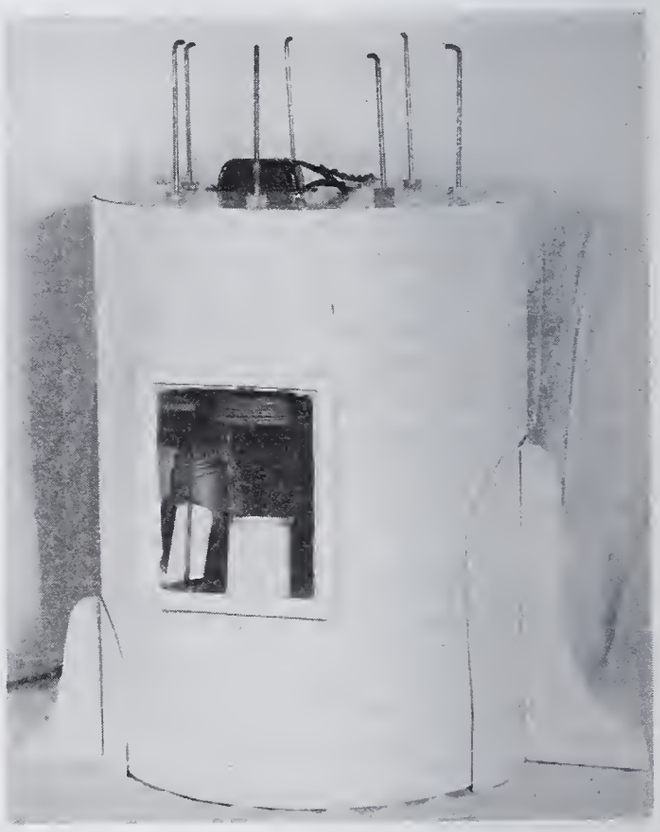

FIGURE 2. ULTRAVIOLET RADIATION STERILIZATION CHAMBER

While the specimens were suspended on the wire, the jar lids and specimens were suspended on the hooks at the lower ends of the rods, seen projecting through the top of the sterilization chamber. During the process of radiation the specimens were raised out of the jars and the rods were turned so each face of each block was exposed to the rays for 20 minutes.

The fruit jars, previously sterilized in an autoclave for 15 minutes at 15 pounds' pressure, with lids loose, were also in the sterilizing chamber during sterilization of the specimens, and at the end of the sterilizing period the specimens were lowered into the jars by means of the rods, which eliminated contamination of the specimens which might have occurred had the chamber been opened and the specimens lowered into the jars by hand.

Inoculation of Test Specimens

Throughout the entire series, *Hormiscium gelatinosum* (F. P. L. 595) was used as the test organism.

Spore suspensions of *Hormiscium gelatinosum* were prepared by shaking small volumes of inoculum in sterile water in a small sterile DeVilbiss throat atomizer. This suspension was then sprayed directly on the surfaces to be inoculated. All specimens were inoculated, on both ends only, in a transfer chamber which was equipped with the germicidal lamp described above. This lamp was not left on during inoculation but was lighted for several minutes before inoculation in order thoroughly to sterilize the air in the chamber.

Incubation and Condensation Cycles

The completely assembled jars were placed in their trays, run immediately through the first condensation cycle, then placed in the incubator overnight. These cycles were performed daily throughout the entire period of the test, except Sundays and holidays.

It was found convenient to place the trays in the refrigerator the first thing each morning, for a period of 2 hours. This was found to be sufficient to give the degree of chilling necessary for a heavy deposit of condensation. Upon removal from the refrigerator, the trays were set in the water pan, where heat was immediately applied. The temperature of the water was raised uniformly, and as rapidly as possible, until actual water drops could be seen standing on the specimens. The tray was then placed in the incubator until the following morning. All specimens were incubated at 79° F.

The condensation cycles were repeated daily until the blue stain had started to grow vigorously and then the cycle was repeated often enough to maintain a standing film of water on the specimens. It was found that a repetition of the cycle every other day provided enough moisture for rapid growth of the fungi after it had started to grow. After 12 weeks of incubation a heavy growth of blue stain was very evident on the untreated controls, so the test was terminated and the specimens were examined.

In this test there was no evidence of any common mold growth, which is common with other means of sterilization, thus testifying to the efficiency of the sterilizing lamp.

Expression of Results

As with all other methods of evaluating resistance to blue staining organisms, the results are expressed qualitatively rather than quantitatively. In this work, however, it was possible to express three classifications of resistance: low, medium, and high. This was possible because the examination was made microscopically, and the criterion was the concentration of mycelia in the cell structure near the paint film relative to the untreated controls. There was so marked a difference between those showing the highest degree of control

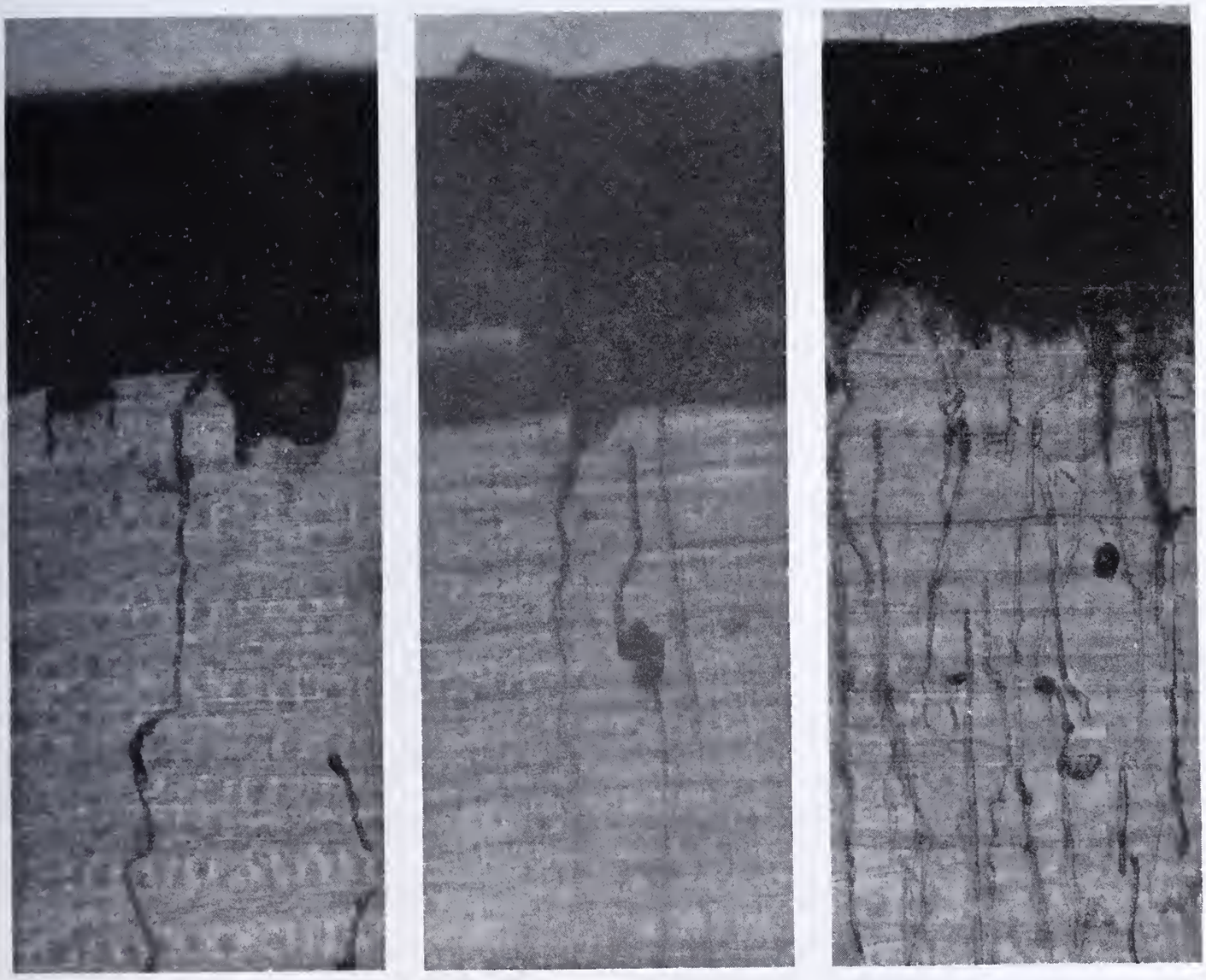

FIGURE 3. EFFICIENCY OF TREATING SOLUTION

× 120 photomicrograph of paint film and treated wood section
Left. High efficiency, two blue stain hyphae
Center. Medium efficiency
Right. Low efficiency, indicated by abundance of blue stain hyphae

Table I. Composition of Exterior Paints

Composition of Pigment	% by weight		Composition of Vehicle	% by weight
First and Second Coats—Pigment 64%, Vehicle 36%				
Lead-zinc pigment		30	Linseed oil	64
White lead sulfate	10		Japan drier	15
Zinc oxide	20		Mineral spirits	6
White lead carbonate		36	Turpentine	15
Titanium-magnesium pigment		34		100
		100		
Third Coat—Pigment 65%, Vehicle 35%				
Zinc-lead pigment		45	Linseed oil	89
Basic sulfate white lead	16		Japan drier	11
Zinc oxide	29			100
White lead carbonate		18		
Titanium-magnesium pigment		32		
Titanium dioxide		5		
		100		

Table II. Composition of Interior Paints

Composition of Pigment	% by weight	Composition of Vehicle		% by weight
First Coat—Pigment 65%, Vehicle 35%				
Lithopone	82.0	Linseed oil		10
Zinc oxide	9.0	Varnish		50
Calcium carbonate	4.5	Vegetable oils	22	
Magnesium silicate	4.5	Resins	41	
		Mineral spirits	37	
	100.0	Mineral spirits		40
				100
Second Coat—50% by Volume of First Coat Material, 50% by Volume of Third Coat Material				
Third Coat—Pigment 40%, Vehicle 60%				
Titanium dioxide	33	Varnish		100
Lithopone	67	Resins	20	
		Vegetable oils	31	
	100	Mineral spirits	49	

and the untreated specimens that there was no question about the certainty of the intermediate or medium state.

Each specimen was split radially into pieces about 0.25 to 0.375 inch thick by means of a chisel. Each piece was examined for uniformity of staining. Several small cubes were cut from each specimen selected, so that the paint film would be included. The cubes were placed in boiling water to displace air, and sections were cut with a microtome and examined with a 16-mm. objective × 12 eyepiece.

The degree of staining of each specimen was designated as follows:

+. Very difficult to find any mycelium in the treated layer of wood immediately beneath the paint film, as shown in Figure 3 (left).

++. Moderate growth of mycelium in the treated wood but not nearly so badly infested as the untreated controls (Figure 3, center).

+++. Very heavy growth of mycelium in the treated wood, evidencing no appreciable amount of control as compared to untreated wood (Figure 3, right).

Each individual specimen was rated as above, and all six blocks (for each solution) were evaluated by averaging all plus marks, assigning a value of 1 for each plus mark. The average value for all plus marks for each solution was then calculated—for instance, all six control blocks were rated +++, which gave an average rating of 3.0 and all six blocks treated with 2-chloro-*o* phenylphenol plus water-repellent were rated +, which gave an average rating of 1.0.

It was decided to classify all solutions having an average rating between 2.0 and 3.0 as being low in resistance, all between 1.0 and 2.0 as being medium in resistance, and all from 0 to (and including) 1.0 as being high in resistance.

Since no treatment absolutely prevented the growth of an occasional hypha, no solution had a higher rating than 1.0. On this basis of rating, it was easy to classify each individual method of treatment in one of the three groups.

It is also possible to evaluate the different solutions for the extent of control of the growth of blue stain by splitting the entire specimen in several pieces and observing the penetration of the mycelia into the treated wood. By comparing specimens in this manner, they can be classified into the same general groups as mentioned above.

Results

The treatments tested and the results of the microscopic examination are shown in Table III. These results are based on examination of "wood-inoculated" specimens only. None of the "paint-inoculated" specimens showed enough fungus growth to be evaluated on this basis.

The test proves that combinations of toxics seem to be more effective in preventing the growth of blue stain than either pentachlorophenol or 2-chloro-*o*-phenylphenol used alone.

Probably the most interesting result is that the addition of a water-repellent raises the resistance of a 2-chloro-*o*-phenylphenol solution from low to high. This indicates that the addition of a water-repellent actually increases the value of a toxic solution in preventing the growth of blue stain.

While the method described in this paper may be simplified and made more quantitative, it is believed that it offers a distinct step towards the attainment always worked for in the laboratory: close correlation with actual service experience.

The following features suggest the usefulness of the method:

1. The test specimens are taken from actual window bottom rails.
2. The treatments are applied in the normal way, just as they are used in commercial operations.
3. Treatments, not just treating solutions, are evaluated.
4. The organism most difficult to control by preservative treatment was used for the evaluation.
5. All culture media were eliminated. Infection was produced directly on untreated wood, which appears to be the most likely to occur in actual service, since access to the untreated sapwood core is most frequently gained through checking of the treated exterior portion of the wood and occasionally by damage of the treated exterior portion.
6. Moisture was provided by condensation, which is the manner in which most moisture reaches products in actual service inside a building.
7. This method not only compares one treatment against another, but it furnishes information which aids in determining which treatment will be more completely effective in actual service.

Table III. Results of Microscopic Examination

Degree of Control	Formula of Solution Tested	% by weight	Average Microscopic Rating
Low	2-Chloro-*o*-phenylphenol	5.0	2.67
	Mineral spirits	95.0	
Medium	Pentachlorophenol	5.0	1.5
	Diacetone alcohol	5.0	
	Mineral spirits	90.0	
Medium	2-Chloro-*o*-phenylphenol	2.0	1.5
	Pentachlorophenol	1.5	
	Tetrachlorophenol	1.5	
	Diacetone alcohol	5.0	
	Mineral spirits	90.0	
High	2-Chloro-*o*-phenylphenol	1.5	1.0
	Pentachlorophenol	2.0	
	Tetrachlorophenol	1.0	
	sym-Trichlorophenol	0.5	
	Diacetone alcohol	5.0	
	Mineral spirits	90.0	
High	2-Chloro-*o*-phenylphenol	5.15	1.0
	Water repellent	13.75	
	Pine oil	2.00	
	Mineral spirits	79.10	

Acknowledgment

The authors wish to thank C. Audrey Richards for her helpful advice and suggestions, and Theo. C. Scheffer, whose suggestions have been of much value. The authors are indebted to R. W. Lehman and E. A. Patton for assistance in developing the various features of this method and for helping carry it through to completion.

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Presented before the N. D. M. A. Preservative Standards Advisory Committee meeting at the Forest Products Laboratory, Madison, Wis.

Colorimetric Determination of Diethylstilbestrol

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AND
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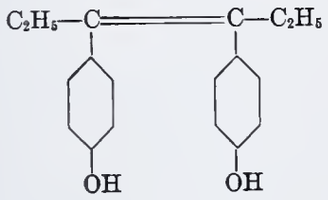
THE universal interest in diethylstilbestrol as a substitute for the naturally occurring estrogens has resulted in the proposal of several methods for its quantitative estimation. Most of the methods (*3*, *4*, *5*, *8*) are not applicable in the presence of the usual tablet compositions. Dechene (*2*) recently applied the "xanthoproteic" reaction to the compound and used this as a basis for a colorimetric method. According to this author, this reaction is not applicable in the presence of any other "phenyl compounds".

In undertaking this investigation, the writers sought a reaction which was specific for one of the functional groups present. As seen from the structural formula, diethylstilbestrol contains two phenolic hydroxyl groups:



Since colorimetric methods for the determination of phenols are well established, the writers applied several of these reactions to the compound and found that the reaction of Folin and Ciocalteu (*6*, *7*) gave the best results. In this reaction the labile complex phosphomolybdic phosphotungstic acids are reduced by the phenolic hydroxyls to give a blue solution of tungstic oxides. It was found that the color produced was stable and readily adaptable to colorimetric or photometric determination and gave a linear relationship over the range of concentrations required. The presence of alcohol, sugars, talc, and fatty acids and their salts did not interfere. Water-soluble colors were eliminated by the technique described below. Figure 1 shows the straight-line graph obtained by plotting the concentrations against the photometer readings. Table I gives data on the extraction of diethylstilbestrol from an artificial mixture. The method was applied to the analysis of commercial tablets and the results are given in Table II.

Experimental

EQUIPMENT. A neutral wedge photometer with a B. & L. Smoke C. glass wedge and filter No. 65 (*1*) was used with a 2.5-cm. (1-inch) cell. A Hellige Duboscq colorimeter was also used.

COLOR REACTION. Diethylstilbestrol (0.2 to 0.8 mg.) in 10 ml. of 30 per cent alcohol contained in a 100-ml. volumetric flask is diluted with 50 ml. of distilled water and 5 ml. of reagent (*7*) are added. After mixing, 15 ml. of 20 per cent sodium carbonate (anhydrous) solution are added and the solution is diluted to the mark with distilled water, mixed, and allowed to stand at room temperature for 1 hour, at which time the color is fully developed. The concentrations are plotted against photometer readings to give a graph, Figure 1, from which the concentration of an unknown solution may be read.

ANALYSIS OF COMPRESSED COLORLESS TABLETS. A number of tablets equivalent to 10 mg. of diethylstilbestrol are weighed and ground. An accurately weighed sample equivalent to approximately 5 mg. of diethylstilbestrol is transferred to a 100-ml. volumetric flask, 30 ml. of alcohol are added, and refluxed on a water bath for 15 minutes. The solution is then cooled and made to volume with distilled water, mixed, and filtered. A 10-ml. aliquot is treated as described above and, at the same time, a standard containing 0.5 mg. of diethylstilbestrol per 10 ml. in 30 per cent alcohol is treated similarly. After 1 hour the colors are compared in a colorimeter. This unknown solution may also be examined photometrically and the concentration derived from the graph.

COLORED TABLETS. The weighed powdered sample, prepared as above, is transferred to a 100-ml. separatory funnel containing 50 ml. of water and extracted with one 20-ml. and three 10-ml. portions of ether. The ether extracts are combined and evaporated. The residue is dissolved in 30 ml. of alcohol, transferred to a 100-ml. volumetric flask, made to the mark with distilled water, and filtered. A 10-ml. aliquot is used for the analysis.

TABLE I. EXTRACTION OF DIETHYLSTILBESTROL FROM A TABLET MIXTURE

Sample[a]	Ether Used	Diethylstilbestrol Present	Diethylstilbestrol Recovered
	Ml.	*Mg.*	*Mg.*
1	20	5	3.4
2	20, 10	5	4.6
3	20, 10, 10	5	4.8
4	20, 10, 10, 10	5	4.6

[a] 1 gram of mixture of starch, lactose, and stearic acid containing diethylstilbestrol.

TABLE II. ANALYSIS OF COMMERCIAL TABLETS OF DIETHYLSTILBESTROL

Tablet	Quantity Recovered: Colorimeter[a]	Quantity Recovered: Photometer[b]	Quantity Recovered: Av.	Labeled Quantity	Difference Found
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*
A	0.48	0.49	0.49	0.5	−0.01
B	0.53	0.56	0.55	0.5	0.05
	1.08	1.16	1.12	1.0	0.12
C	0.98	0.99	0.99	1.0	−0.01
D	0.51	0.57	0.54	0.5	0.04

[a] Standard set at 20, average of 5 readings.
[b] Average of 5 readings.

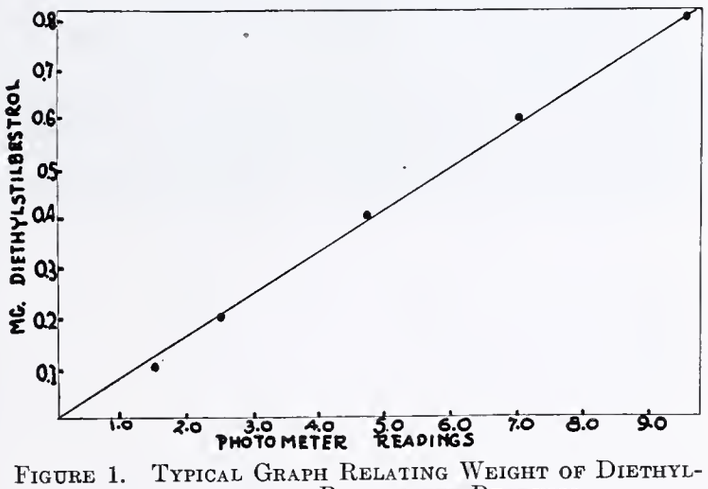


FIGURE 1. TYPICAL GRAPH RELATING WEIGHT OF DIETHYLSTILBESTROL TO PHOTOMETER READINGS

Acknowledgment

The authors gratefully acknowledge receipt of the materials used in this investigation from the following: Abbott Laboratories, North Chicago, Ill.; Eli Lilly and Company, Indianapolis, Ind.; Merck & Co., Inc., Rahway, N. J.; and Carroll Dunham Smith Pharmacal Company, Orange, N. J.

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Spectrophotometric Estimation of Pentachlorophenol in Tissues and Water

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PENTACHLOROPHENOL is used in industry as an agent for the preservation of wood and wood products. Attention was first called to its toxicity in studies on mice by Bechhold and Ehrlich, in 1906 (*1*). Extensive toxicity studies were carried out by Kehoe, Deichmann, and Kitzmiller in 1939 (*3*), and by Boyd, McGavack, Terranova, and Piccione in 1940 (*2*) and 1941 (*4*).

Extension of the investigation necessitated the development of an analytical method. The procedure reported here utilizes the formation and the spectrophotometric determination of a reddish-yellow pigment formed by the action of fuming nitric acid on pentachlorophenol. The method was found dependable, and of sufficient accuracy to enable the authors to follow this compound through the body and to detect amounts below the harmful levels. Further toxicological studies of this compound will be reported elsewhere.

Method

The following method is suggested for the determination of from 0 to 9 mg. of pentachlorophenol in 10 grams of biological material (except pure fat) and of from 0 to 50 mg. of pentachlorophenol in about 5 ml. of water.

REAGENTS REQUIRED. Concentrated hydrochloric acid, specific gravity 1.19, 4 per cent sodium hydroxide, fuming nitric acid, and chloroform.

ORGANS AND TISSUES. The sample is macerated in a meat grinder or mortar and, if necessary, mixed in a "Powermaster kitchen mixer". Ten grams are then weighed into the Pyrex distilling flask (Figure 1) and 20 ml. of water and 2 ml. of concentrated hydrochloric acid are added. The mixture is distilled until 1000 ml. of distillate have been collected in a flask containing 10 ml. of 4 per cent sodium hydroxide. The distillate is heated over a flame to about 75° C., and then half of this volume is measured into a 1-liter beaker, slowly concentrated on a hot plate to 50 ml., then transferred to a 250-ml. Erlenmeyer and evaporated to about 2 ml. [The other 500 ml. are set aside as a reserve to supply a suitable aliquot, in case the results of the first determination fall beyond the curve (Figure 2).]

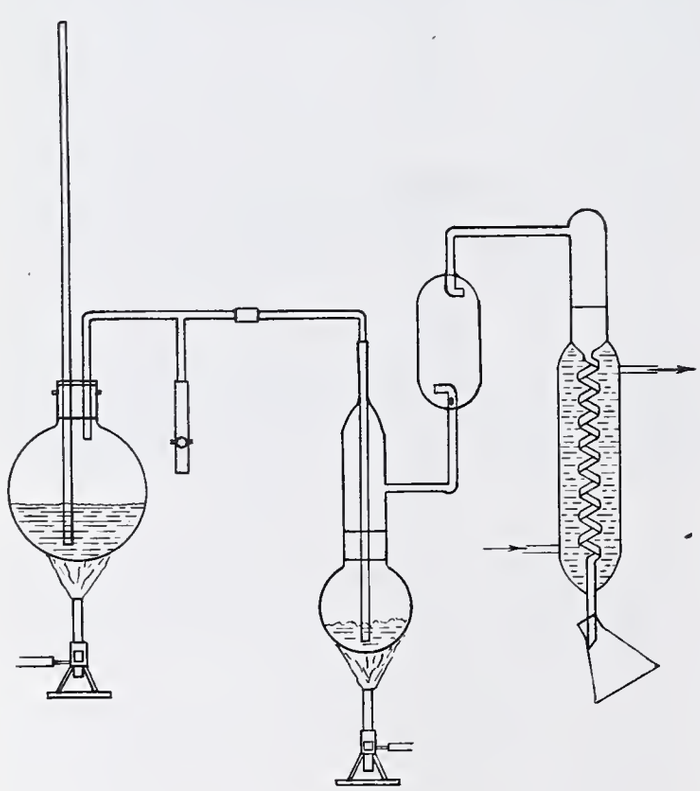

FIGURE 1. DISTILLING APPARATUS

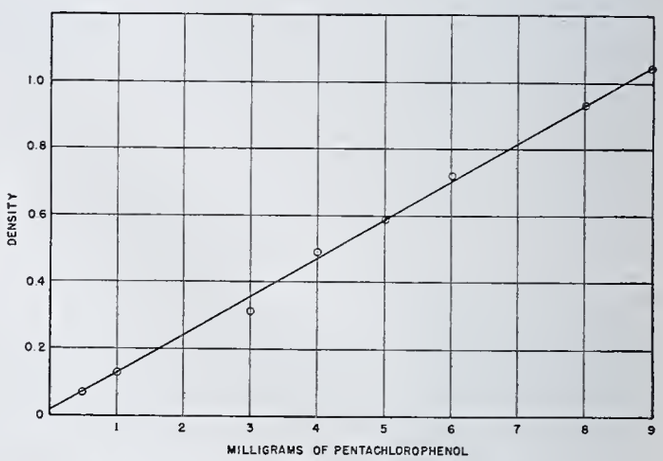

FIGURE 2. RELATIONSHIP BETWEEN DENSITY AND CONCENTRATION, EMPLOYING 10-MM. CELLS

Four milliliters of fuming nitric acid are then slowly added and the precipitate clinging to the bottom of the Erlenmeyer is freed completely with a stirring rod, which is then rinsed with 1 ml. of fuming nitric acid. The solution is placed in ice for 20 minutes and is then washed with about 100 ml. of water into a 250-ml. separatory funnel. The reddish-yellow pigment is extracted with three 8-ml. portions of chloroform, permitting 5 minutes for each separation. The combined chloroform extracts are washed with a single 100-ml. sample of water (to remove water-soluble nitrates) permitting 10 minutes for the separation, then filtered through 2 layers of Whatman No. 1, 9-cm. filter paper wetted with chloroform into a 25-ml. glass-stoppered cylinder. After diluting with chloroform to 25 ml., the concentration is determined by means of standard curves (Figures 2 and 3) using 10-mm. or 4-inch matched Aminco cells and the spectrophotometer at 460 mμ.

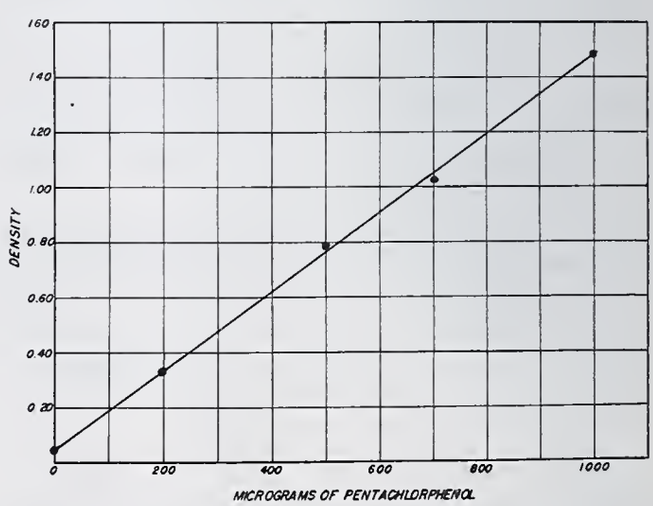

FIGURE 3. RELATIONSHIP BETWEEN DENSITY AND CONCENTRATION EMPLOYING 4-INCH CELLS

TABLE I. ANALYSES OF CONTROL BLOOD, URINE, ORGANS, AND TISSUES ASSUMED TO BE FREE OF PENTACHLOROPHENOL

Sample	Pentachlorophenol Found Mg.	Sample	Pentachlorophenol Found Mg.
Rabbit blood, 10 ml.	0	Ground rat, 10 grams	0
	0		0
	0		0.03
	0.02	Beef brain, 10 grams	0
	0		0
	0	Rabbit muscle, 10 grams	0
75 ml.	0	Rabbit liver, 10 grams	0
	0	Rabbit heart, 10 grams	0
100 ml.	0.01	Rabbit kidneys, 10 grams	0.03
	0.01	Human urine, 100 ml.	0.01
Rabbit urine, 10 ml.	0		0.01
	0		0.02
	0		0
	0.01		0
	0		

Figure 4 gives the transmission curve of the pigment formed under these conditions; Figures 2 and 3 give the relationship between density and concentration of pentachlorophenol.

BLOOD AND URINE. Whole blood or urine (10-ml. samples) is distilled directly, after treatment with 20 ml. of water and 2 ml. of hydrochloric acid, but only 300 ml. of distillate are collected in an Erlenmeyer containing 3 ml. of 4 per cent sodium hydroxide. This is evaporated on a hot plate to 20 ml., transferred to a 250-ml. Erlenmeyer, and evaporated to 2 ml. The analysis is then continued as under "Organs and Tissues". In the case of blood, paraffin is added to reduce foaming.

TABLE II. RECOVERY OF PENTACHLOROPHENOL

(Added to 10-ml. samples of blood and urine and to 10-gram samples of organs and tissues)

Added Mg.	Recovered Mg.	Added Mg.	Recovered Mg.
Rabbit Blood			
0.03	0.03	0.14	0.12
0.03	0.04	0.14	0.12
0.03	0.025	0.145	0.125
0.06	0.055	2.45	2.50
0.06	0.075	4.54	4.40
0.06	0.055	4.54	4.31
0.11	0.095	6.8	7.0
0.11	0.093	6.8	7.4
0.11	0.10	6.8	6.7
Rabbit Urine (25-ml. samples)		Human Urine	
		4.54	4.54
0.08	0.09	4.54	4.90
0.09	0.09	5.5	5.0
0.09	0.10	5.5	5.1
0.10	0.08	6.0	6.0
0.18	0.19	6.8	5.4
0.18	0.19	6.8	6.9
		9.95	9.6
Ground Rat		Rabbit Muscle	
0.24	0.22		
0.24	0.21	2.5	2.4
0.24	0.22	5.0	4.9
0.24	0.23		
0.43	0.44	Rabbit Liver	
0.43	0.43		
1.5	1.4	5.0	4.4
1.5	1.4	8.0	8.0
2.5	2.5	8.0	7.6
2.5	2.3		
4.8	4.5	Rabbit Heart	
Rabbit Lung		2.5	2.55
		4.5	4.0
2.5	2.3		
4.8	5.3	Rabbit Kidneys	
4.8	5.1		
		2.5	2.4
Rabbit Fat		4.5	4.6
		Beef Brain	
4.0	2.1		
4.0	1.9	1.5	1.4
8.0	4.0	2.5	2.42
8.0	3.6	2.5	2.38
		5.0	4.5
		5.0	4.4

Table I gives the values obtained by analyzing samples of normal blood and tissues for pentachlorophenol. Table II indicates the recoveries of pentachlorophenol added to blood, urine, and tissues, respectively.

WATER. The steam-distillation is omitted in the absence of other material reacting with nitric acid. A 2–5 ml. sample is pipetted into a 50-ml. Pyrex test tube, and from 3 to 15 ml. of fuming nitric acid are added (depending on the amount of pentachlorophenol in the sample); the whole is then allowed to stand in an ice bath for 20 minutes. From this point the analysis is continued as described above, 10-mm. cells being employed for the spectrophotometric analysis.

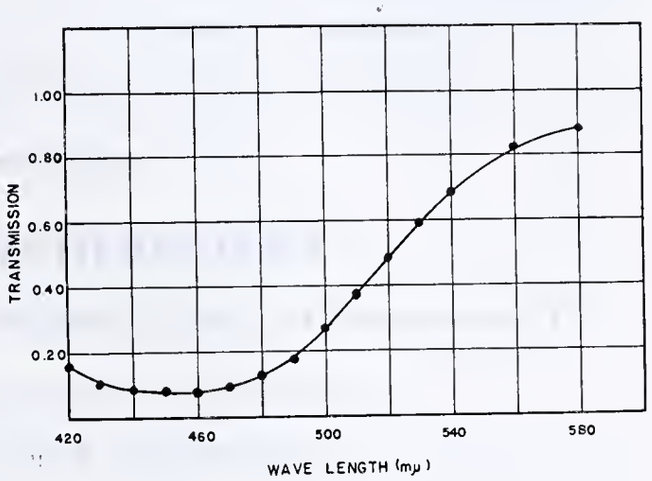

FIGURE 4. TRANSMISSION CURVE FOR PIGMENT FORMED

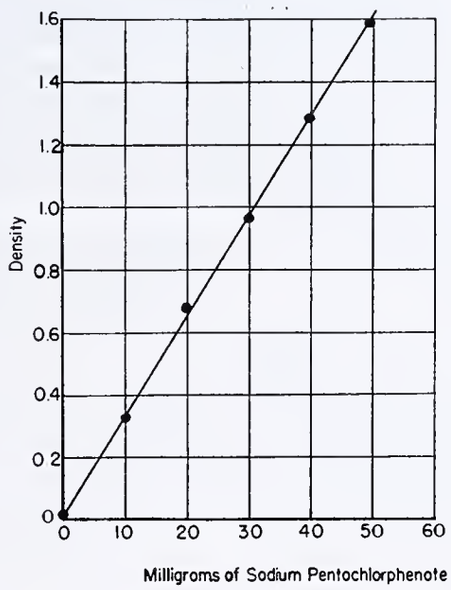

FIGURE 5. RELATIONSHIP BETWEEN DENSITY AND CONCENTRATION

10-mm. cells employed. Concentration of sodium pentachlorophenate in sample of water is read directly from this graph.

Figure 5 gives the relationship between density and the quantity of pentachlorophenol (in milligrams) present in the original sample. The final estimation can be carried out with a colorimeter by using standard solutions. When 28 mg. of pentachlorophenol as the sodium salt were added to 2-ml. samples of water, the following quantities were recovered: 27.2, 27.4, 28.0, 27.7, 28.0, 28.6, 26.9, 27.7, 28.6, 28.6, and 29.1 mg.

PRECAUTIONS. It is advisable to read the solutions in the spectrophotometer within 10 minutes; the color is stable, but droplets of water may settle out if the solutions are allowed to stand for longer periods.

Figures 2, 3, and 5 were obtained with the author's photoelectric spectrophotometer and with their equipment; therefore, if accuracy is desired, it is not advisable to use them with other spectrophotometers and cells. It is suggested that other investigators prepare their own figures. The cells, even though they are matched, should not be interchanged and should always be placed in the same position.

It is imperative that any precipitate which may form be loosened completely from the bottom of the Erlenmeyer after the 4 ml. of nitric acid have been added.

Errors will not be introduced if samples go to dryness on the hot plate; the precipitate is brought in solution with about 2 ml. of water.

Discussion and Summary

A method for the determination of pentachlorophenol in biological material and in water is based on the formation and spectrophotometric determination of a reddish-yellow pigment formed by the action of fuming nitric acid on pentachlorophenol.

Substances other than pentachlorophenol may react with nitric acid to form colors which may occasionally give false positive results. In no instance, however, did these errors exceed 0.03 mg. per sample (Table I).

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Determination of Nitrites

Discussion of the Shinn Method As Applied to Examination of Water

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AND
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THE diazotization of sulfanilic acid by the available nitrite in water, using α-naphthylamine as a coupling agent, has continued throughout the years as a colorimetric method for determining nitrites in water (*1*). The diazo reaction has been used for the determination of minute quantities of certain organic substances, many of which in turn could have been used for determining nitrites. One of the compounds diazotized and determined colorimetrically by available nitrite has been sulfanilamide.

The diazotization of sulfanilamide by available nitrite, using α-naphthylamine as a coupling agent, was eliminated some time ago. Dimethyl-α-naphthylamine was next used, but found unsatisfactory because of the necessity of using a catalyst for rapid development of color in dilute solutions.

Bratton and Marshall (*3*) investigated many new coupling agents for the diazotization of sulfanilamide and found *N*-(1-naphthyl)-ethylenediamine dihydrochloride the most satisfactory. They state that this new coupling agent is far better than dimethyl-α-naphthylamine, because of its producibility and purity, greater rapidity of coupling, increased sensitivity, and increased acid solubility of the azo dye formed.

Shinn (*5*) was the first to make use of sulfanilamide and the coupling agent, *N*-(1-naphthyl)-ethylenediamine dihydrochloride, as a means of determining nitrites; in fact, her article was brought to the authors' attention because she had found these reagents far superior to sulfanilic acid and α-naphthylamine. Shinn points out that sulfanilamide is more stable than sulfanilic acid, both in the dry state and in solution; furthermore, it reacts more rapidly in the coupling process. It has been recognized that the coupling of diazotized sulfanilic acid with α-naphthylamine is relatively slow (*2*) and that the color developed must be read within 30 minutes (*1*) because it is so unstable. The authors' experience has shown that, with the standard procedure (*1*), full color development is not reached until 30 minutes' standing with subsequent fading after 30 minutes.

Because of the qualification of the new coupling agent, the use of sulfanilamide, and the resulting improved diazo reaction, it appeared that the use of these two compounds for determining nitrites in water would be worthy of consideration. The Shinn method (*5*) for nitrites was investigated in order to determine its applicability to water because of the need for a more satisfactory method than the standard procedure (*1*).

The Shinn method, as published, prescribes an unknown of no greater volume than 35 ml. which, after the addition of reagents, is diluted to 50 ml. This procedure would be awkward and contrary to best waterworks analytical procedure. Good analytical technique would necessitate the use of 50-ml. or 100-ml. water samples of unknown nitrite content since comparatively few waterworks laboratories have photoelectric colorimeters, but will continue to use for some time to come the Nessler tubes to which they are accustomed. The method emphasizes the increase in sensitivity with decreasing final volumes of the unknown nitrite solution.

As published, the article points out that samples of unknown nitrite content, containing less than 0.0025 mg. of nitrite (NO_2) or 0.000761 mg. of nitrite nitrogen (N) per 50 ml., or 0.050 p. p. m. of nitrite or 0.015 p. p. m. of nitrite nitrogen, gave colors too faint to be read with any reasonable degree of accuracy with a photoelectric colorimeter. If this were the case it would be impossible to apply the method to waterworks practice, since a large portion of the water supplies contain less than 0.015 p. p. m. of nitrite nitrogen. Any method to be given serious consideration must be sensitive to 0.001 p. p. m. of nitrite nitrogen.

Although Shinn recommended that the unknown be limited to 35-ml. volume, it was found by preliminary investigation that like amounts of nitrite nitrogen, treated before dilution, developed less color than when they were diluted to a final volume of 50 ml. before addition of the reagents. The sensitivity of the reagents increased with increasing dilution, and maximum color was not developed until the volume before the addition of the reagents became 40 ml. or more. This fact overcame the first objection to the method.

This led to an intensive study to determine how equivalent amounts of nitrite nitrogen, developed in dilute or concentrated volumes, would give equivalent readings for all amounts

of nitrite nitrogen, including those between 0.015 and 0.001 p. p. m. Such a finding would eliminate once and for all the effect of water dilution even in those cases where small amounts of nitrite nitrogen were to be found.

One of the very early developments of the investigation disclosed the necessity of thorough mixing after the addition of each chemical to obtain reliable and consistent results. The ammonium sulfamate was next eliminated, since it had no practical value other than destruction of the excess nitrite in the diazotization reaction, an excess which does not exist in the detection of nitrites in water.

The problem of obtaining equivalent color intensity for equivalent amounts of nitrite nitrogen, by adding reagents to the nitrite in the more concentrated condition as prescribed by Shinn, or in the more dilute condition for use with water, appeared to have a bearing on the pH of the unknown nitrite volume. The acid concentrations, pH, and time for color development were investigated. It was found that equivalent color intensities for equivalent amounts of nitrite nitrogen could be developed by using 2 ml. of 1 to 1 hydrochloric acid in the reaction instead of the prescribed 1 ml.

Because of the fairly large total volume of reagents required some consolidation of the reagents and their volumes was deemed advisable. This was accomplished by using 2 ml. of a 0.5 per cent sulfanilamide solution in 1 to 1 hydrochloric acid, instead of 5 ml. of a 0.2 per cent water solution of sulfanilamide and 1 ml. of 1 to 1 hydrochloric acid. The acid solution had the additional advantage of aiding in the dispersion of the sulfanilamide. When stored in a refrigerator, there is little danger that the sulfanilamide will crystallize out.

By using this modified reagent, the time required for full development of color was found to be 15 minutes, and the color intensity was not only equivalent as developed in both dilute and concentrated volumes for like quantities of nitrite nitrogen, but was of sufficient magnitude in the range of even 0.015 to 0.001 p. p. m. of nitrite nitrogen to give differentiations which were as easily detected as those in the standard method (*1*). An advantage of the Shinn method over the standard method is that the maximum color developed in 15 minutes is more rapid and more stable.

In the Shinn method the nitrite value of the sulfanilamide standard is determined from a nitrite standard in which assayed sodium nitrite is used instead of silver nitrite.

Throughout all the investigational work on the use of the two new reagents, sulfanilamide and *N*-(1-naphthyl)-ethylenediamine dihydrochloride, comparisons were made with Nessler tubes containing nitrite standards prepared from assayed sodium nitrite rather than a sulfanilamide standard which is used in a photoelectric colorimeter and whose nitrite value is determined from a nitrite standard prepared from assayed sodium nitrite. Waterworks people are familiar with the old nitrite standard and from a waterworks practice standpoint it would be out of place to discontinue its use at this time.

The simplicity of the preparation of the nitrite standard from the desiccated reagent grade of sodium nitrite should not be overlooked. This has been thoroughly investigated in the laboratory of the Indianapolis Water Company, and found to be reliable and accurate, eliminating the long and laborious procedure using silver nitrite (*4*).

Table I shows the comparison with the present standard method and gives the quantities required by the Shinn method to obtain good color differentiation. Readings as high as 0.0002 mg. of nitrite nitrogen in 50-ml. Nessler tubes can easily be differentiated in increments of 0.00004 mg. of nitrite nitrogen in 50 ml. and readings as high as 0.0005 mg. of nitrite nitrogen in 50 ml. can easily be differentiated in increments of 0.00005 mg. of nitrite-nitrogen in 50 ml. It is suggested that sodium nitrite solutions of two concentrations be used in making up the standards.

Table I. Preparation of Standard Nitrite Solutions

(Quantities for 50-ml. Nessler tubes)

A. P. H. A. Standard Method Nitrite[a] Solution of 1 Ml. = 0.0005 Mg. of NO_2-N		Nitrite Solution of Shinn Method, 1 to 1000, 0.01 per cent 1 Ml. = Approximately 0.00002 Mg. of NO_2-N	
Standard nitrite soln.	NO_2-N	Standard nitrite soln.	NO_2-N
Ml.	*Mg.*	*Ml.*	*Mg.*
0.1	0.00005	2.0	0.00004
		4.0	0.00008
0.2	0.00010	6.0	0.00012
		8.0	0.00016
0.4	0.00020	10.0	0.00020
		1 to 1000, 0.1 per cent 1 Ml. = Approximately 0.0002 Mg. of NO_2-N	
0.7	0.00035	1.25	0.00025
		1.5	0.00030
		1.75	0.00035
		2.0	0.0004
1.0	0.0005	2.5	0.0005
1.4	0.0007	3.0	0.0006
1.7	0.00085	3.5	0.0007
2.0	0.001	4.0	0.0008
2.5	0.00125	4.5	0.0009
		5.0	0.0010

[a] Listed as prescribed in (*1*), although closer increments are probably possible.

Modifications

The 50- or 100-ml. unknowns are compared with a series of standards in matched Nessler tubes instead of against a single standard in a photoelectric or Duboscq colorimeter.

Nitrite standards are prepared from assayed sodium nitrite instead of a sulfanilamide standard whose value has been determined from assayed sodium nitrite.

Ammonium sulfamate is eliminated as reagent.

The solution is thoroughly mixed after the addition of each chemical, whether unknown or standard.

Two milliliters of 0.5 per cent sulfanilamide in 1 to 1 hydrochloric acid are used, instead of 5 ml. of 0.2 per cent sulfanilamide and 1 ml of 1 to 1 hydrochloric acid to increase the sensitivity from 0.015 to 0.001 p. p. m. of nitrite nitrogen, thereby making the sensitivity range comparable to the present standard method range.

Conclusions

The method as published is not applicable to waterworks practice, because of lack of sensitivity in the range from 0.015 to 0.001 p. p. m. of nitrite nitrogen. The present limitation of the water sample to 35 ml. or less is awkward and contrary to waterworks practice. The method can be made applicable by certain modifications.

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Correction. In the article on "Assay of Benzaldehyde" [Ind. Eng. Chem., Anal. Ed., **14**, 154 (1942)] the reference cited in the 35th line, second column, following the words "bromophenol blue", should be (*6*).

Janet G. Dinkelspiel

Determination of Small Amounts of Iodide in Photographic Developers

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The method for the determination of iodide in photographic developers which is described involves the precipitation of halides with silver nitrate, followed by the oxidation of the iodide to iodate while it is in the form of silver iodide. The concentration of iodate is then determined polarographically.

This method can be used for the determination of concentration of iodide as small as 1 mg. per liter of potassium iodide. Amounts around 5 mg. per liter can be determined with an accuracy of about 4 per cent.

A STUDY of analytical methods for photographic developers, which has been under way in these laboratories for some time, has given rise to a new method of analysis for iodide in concentrations as small as $6 \times 10^{-6} M$ in the presence of a thousand times the amount of bromide and large amounts of reducing agents. This method involves the chlorine oxidation of the iodide in the form of silver iodide, followed by the polarographic determination of the iodate formed.

Rylich (*2*) first reported the use of the polarograph for the determination of very small amounts of iodate and bromate. He found that concentrations of iodate as small as $1 \times 10^{-6} M$ could be readily determined polarographically. The potential at which iodate is reduced at the dropping mercury electrode depends on the supporting electrolyte and on the pH of the solution. Below pH 7, the potential varies with pH, but above pH 7, the potential changes only slightly with increasing pH, remaining at a value around 1.1 volts against the saturated calomel electrode. The polarographic analysis of iodate is particularly sensitive because of the six-electron change which occurs in its reduction. This results in six times the current and, therefore, six times the sensitivity of a reduction involving a one-electron change.

Method of Analysis

The complete analytical procedure finally developed was as follows:

Take a 100-cc. sample of the developer and add to it 10 cc. of 0.5 *N* potassium bromide and 60 cc. of concentrated sulfuric acid. Pass steam through the solution until the small bubbles which indicate gas evolution disappear. To the hot solution add 100 cc. of water and 25 cc. of 0.5 *N* silver nitrate and pass steam through the solution or boil it for a few seconds to aid in coagulation of the precipitate. Allow the precipitate to settle and pour off the supernatant liquid, leaving the precipitate in the flask. To the precipitate add 50 cc. of 1 to 1 nitric acid and 250 cc. of water, shake, and allow to settle. Pour off the supernatant liquid and repeat this process with two 250-cc. portions of water. To the precipitate, add 50 cc. of fresh chlorine water, heat until the bromine color disappears, filter, and wash the precipitate with two 5-cc. portions of water. Cool the filtrate under the tap. To the filtrate add 1.0 cc. of 5 per cent phenol and 5.0 cc. of 2.0 *N* potassium hydroxide, and dilute to 100 cc. with water. Bubble nitrogen through the solution and electrolyze on the polarograph.

The wave obtained can be measured by any of the standard methods (*1*). The method used to obtain the values given here is illustrated in Figure 1. The ΔI method could also be used. By this method the current readings at -1.0 and -1.4 volts are recorded and the current difference is plotted against potassium iodide concentration.

Experimental

Before the chlorine water is added to oxidize the iodide to iodate, other reducing agents must be removed. In the analysis of photographic developers, this meant that sulfite and developing agents had to be removed. Sulfite was easily removed by acidification, followed by passing steam through the solution, but in order to separate the iodide from the developing agents, it was necessary to precipitate the halide as the silver salt and wash the precipitate thoroughly.

Since some developers contain little or no bromide, it was found necessary to add some bromide in order to get a sufficiently large amount of precipitate to ensure coagulation of the small amount of iodide present. The silver bromide precipitate acts as a collector for the silver iodide.

The precipitate was washed by decantation in order to save time. In this process, it is inevitable that a few particles of precipitate are lost, but since the total amount of precipitate is relatively large, the loss is not detectable in the final result. When the precipitation is carried out as described, the supernatant solution after the first coagulation is turbid, but this solution was always discarded without any attempt to collect the material in suspension. The error was found to be negligible.

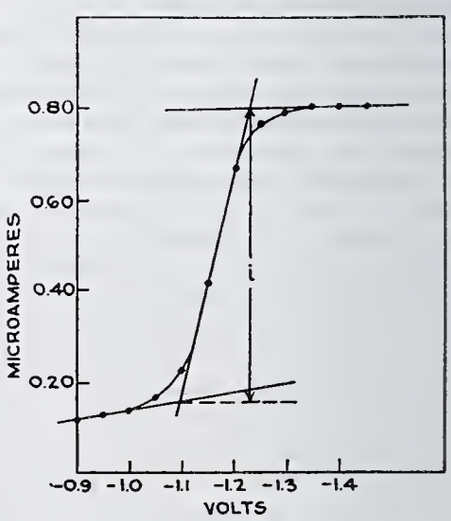

FIGURE 1. METHOD FOR MEASURING WAVE HEIGHT

The precipitate was first washed with nitric acid to aid in the removal of any reducing agent which might be adsorbed to the silver halide. When the nitric acid was omitted, a considerable amount of oxidized material showed up on the polarographic curves as a current at potentials below that of iodate.

In the early experiments on an iodide analysis, it was considered necessary to dissolve the silver halide precipitate before oxidation of the iodide. This caused a great deal of difficulty because the silver thus put into solution had to be re-

TABLE I. OXIDATION OF IODIDE

Potassium Iodide Added	Potassium Iodate Calculated	Potassium Iodate Formed	Conversion of Iodide to Iodate
Mg./l.	*Mg./l.*	*Mg./l.*	%
	With Bromine Water		
5	4.3[a]	3.8	88.4
10	8.6	7.6	88.4
15	12.9	11.4	88.4
	With Chlorine Water		
5	4.3	4.3	100
10	8.6	8.5	99
15	12.9	12.9	100

[a] Final volume of solution 150 cc.

moved before a polarographic wave for iodate could be obtained. A very involved method was worked out but the analysis was not practical until it was found that the iodide could be oxidized just as well while it was still in the form of silver iodide.

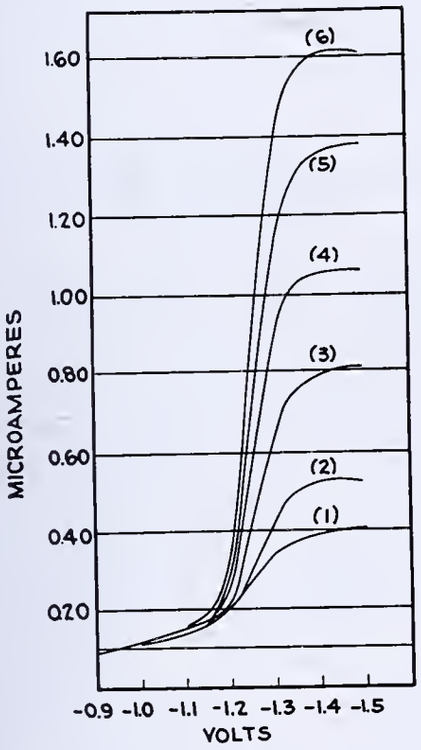

FIGURE 2. IODATE WAVES IN 0.1 *N* KOH AT 25° C. FOR VARIOUS CONCENTRATIONS OF IODIDE IN A PHOTOGRAPHIC DEVELOPER

Concentrations of potassium iodide in mg. per liter: (1) 1.0, (2) 2.0, (3) 4.0, (4) 6.0, (5) 8.0, (6) 10.0. Capillary constants in 0.1 *N* KOH at $E = -1.40$ volts *vs.* saturated calomel electrode: $h = 63.0$ cm., $t = 3.18$, $m^{2/3}t^{1/6} = 1.45$ mg.$^{2/3}$ sec.$^{-1/2}$

In the first experiments on oxidation of iodide in silver iodide to iodate, bromine water was used as the oxidizing agent. Fairly reproducible results were obtained, but on comparing the polarographic wave heights in the analysis with the wave heights obtained with solutions of iodate in the same salt environment it was found that the amount of iodate obtained from a given amount of iodide was less than the stoichiometric value (Table I). It was presumed that this discrepancy was due to the occlusion of some of the iodide in the mass of silver bromide with which the bromine could not react. Chlorine water should eliminate this difficulty by oxidizing both bromide and iodide and thus penetrate the whole particle. The results obtained with chlorine water confirmed this assumption and Table I shows that the results are quantitative.

In order to ensure complete reaction, the mixture of precipitate and chlorine water is heated until the bromine color disappears. This serves the double purpose of completing the reaction and removing the excess bromine. The fine dispersion of silver chloride which remains is removed by filtration. The use of a kieselguhr filter (made by pouring a suspension of kieselguhr in water over the filter) aids in the clean separation of precipitate and filtrate.

Any excess of bromine or chlorine which may be left is then destroyed by the addition of phenol. The amount of phenol is unimportant as long as it is present in excess. The solution is made approximately 0.1 *N* with potassium hydroxide in order to have the iodate wave come at a potential of about 1.1 volts.

As in all polarographic work involving reduction, the oxygen in the solution must be removed by bubbling nitrogen through the solution for 5 to 10 minutes. The removal of oxygen is sufficiently complete when the current is small at a potential around −0.7 volt. Before the solution is electrolyzed the temperature should be checked. A 1° C. change in temperature will make about a 2 per cent change in the wave height. For practical purposes, the temperature should be regulated within 0.5° to 1.0° C.

The polarograph used in this work was a Fisher Elecdropode in which a saturated calomel electrode, connected to the electrolysis cell by means of an agar bridge saturated with potassium nitrate, was used rather than the mercury pool. The stream of nitrogen used to drive out the oxygen was passed through a bottle of water to minimize loss of the sample through evaporation.

A calibration curve was made by analyzing samples of developers containing known amounts of iodide. The wave heights were measured, and these values plotted against the concentration of potassium iodide in the developer. Figure 2 shows a set of curves obtained in a calibration and Figure 3 is the calibration curve. Table II gives the results obtained in some typical analyses. Concentrations of around 5 mg. per liter can be determined with an error of approximately 4 per

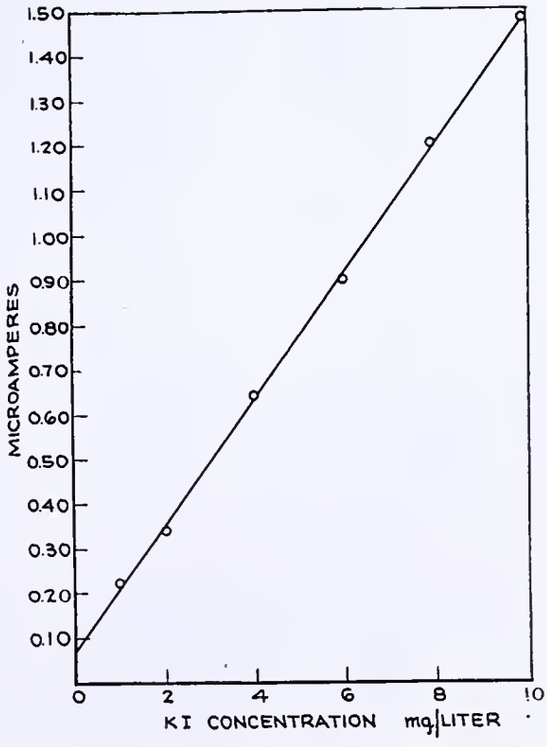

FIGURE 3. CALIBRATION CURVE FROM WAVES IN FIGURE 2

Table II. Oxidation of Iodide

Potassium Iodide Added *Mg./l.*	Potassium Iodide Found *Mg./l.*
2.5	2.6
5.0	5.0
7.5	7.5
10.0	9.9
5.0	4.9
5.0	4.9
5.0	4.8

cent. However, the percentage error increases with decreasing amount of potassium iodide and 1 mg. per liter can be determined with an accuracy of 10 to 12 per cent. Polarographic curves can be obtained with quantities as small as 0.2 mg. per liter. Determinations have been made on various types of developers and in each case the same calibration curve could be used, indicating that variations in the quantities of sulfite, carbonate, bromide, and developing agent were without effect on the test. However, an additional precaution was necessary if thiocyanate was present. Using the above procedure some silver thiocyanate precipitated with the halides, and when the chlorine was added the thiocyanate was oxidized instead of the iodide. The thiocyanate could be removed, however, by steaming the strongly acid solution, letting the hot solution stand for a few minutes, and then steaming the solution again.

Conclusion

Although the method of analysis outlined above has been used only on photographic developers, it should prove useful in any case where it is desired to analyze for iodide in the presence of a thousandfold quantity of bromide and/or such substances as interfere with ordinary iodometric determinations. The final determination of the iodate may be made by one of the usual titration methods if a polarograph is not available.

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Communication No. 834 from the Kodak Research Laboratories.

Detecting Basicity in Slightly Soluble Materials

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IT IS often necessary or advantageous to have available a rapid and sure qualitative test for acid-consuming or basic compounds, such as hydroxides, oxides, carbonates, basic salts, or organic bases. When water-soluble compounds are being examined acid-base indicators can be used, and the presence of hydroxyl ions is quickly manifested by characteristic color changes and by the establishing of certain pH values.

The direct use of such indicators is out of the question with basic substances that are so slightly soluble in water that the equilibrium concentration of hydroxyl ions in the solution is extremely low. Many minerals, rocks, organic bases, industrial products, etc., are in this category. In some cases it is feasible to employ a dilute acid solution to which an indicator has been added: on the addition of basic materials acid is consumed and the consequent rise in the pH value is revealed by a characteristic color change. This procedure is seldom satisfactory for the present instances, since even finely pulverized mineral oxides are often attacked by acids only with extreme slowness. In addition, if the quantity of basic materials is not known, it is tedious to decide the minute quantities of acid to be exposed to this test. Furthermore, this procedure is not applicable with most weak organic bases because of the extensive hydrolysis of the salts formed.

Tichborne (*5*) has recommended a test based on the reaction of metal oxides and hydroxides with sodium bicarbonate:

$$MeO + 2NaHCO_3 \longrightarrow Na_2CO_3 + MeCO_3 + H_2O$$

Any alkali carbonate formed will turn phenolphthalein deep purple. The method is not suited to all cases, since only a limited number of hydroxides and oxides react promptly, and in particular, only when freshly precipitated. Aged or ignited products do not give a clear-cut test. Furthermore, the procedure entails a filtration and the consumption of considerable quantities of test material.

[1] Translated by Ralph E. Oesper, University of Cincinnati.

The method described here for the detection of acid-soluble or acid-binding basic compounds may be carried out well as a spot test. It has been tried on about one hundred materials and has proved excellent for both macro- and microchemical purposes.

The fundamental step of this new method is to bring a test portion of the solid into contact with a reagent solution which contains all the ingredients necessary to the formation of a colored, slightly soluble inner-complex salt, whose precipitation, however, has just been prevented by an adequate hydrogen-ion concentration. In such "equilibrium solutions" the introduction of hydroxyl ions or solid basic materials, which react even partially with dilute acids, is followed immediately by the formation of a colored precipitate. Hydroxides, oxides, carbonates, basic salts, and organic bases respond to this test.

All the organic reagents now in use for the detection or determination of metal ions, by virtue of the formation of slightly soluble inner-complex salts, are acid in character. In many cases the reaction with neutral solutions of the particular metal salts is incomplete, because free acid is produced and this limits the precipitation of the salt. Quantitative precipitation requires buffering, complete neutralization, or often even alkalization, by the addition of ammonia. Alkalization is especially necessary if a reagent must be transformed to an active aci-form. Examples are rubeanic acid, α-nitroso-β-naphthol, etc. Reagents of this type are of no value for the present purpose. Theoretically, detection of acid-soluble basic compounds by the new procedure can be accomplished by means of equilibrium solutions prepared from any of the organic precipitants which give incomplete precipitations because of reversible reactions.

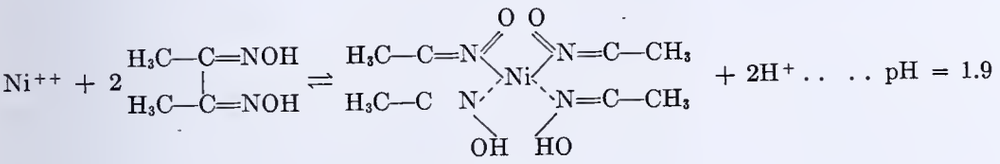
A purely inorganic equilibrium solution is exemplified by the filtrate obtained after the partial precipitation of nickel sulfide by hydrogen sulfide from solutions containing strong mineral acids. Black nickel sulfide appears immediately if these solutions are brought into contact with acid-consuming materials.

However, nickel sulfide equilibrium solutions are not recommended because they are unstable and because hydrogen sulfide reacts with many metal compounds.

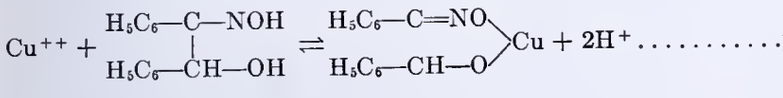
For practical purposes, however, the only suitable organic precipitants are those whose solutions are colorless and which produce colored precipitates. These requirements are well met by dimethylglyoxime, benzoinoxime (cupron), and thioglycolic acid-β-aminonaphthalide (thionalide).

The precipitation of red nickel dimethylglyoxime (*6*), green copper-benzoinoxime (*3*), and brown-black nickel thionalide (*1*) can be represented by the following equations. These reactions are reversible and come to equilibrium, and the precipitation stops as soon as the respective pH is reached.

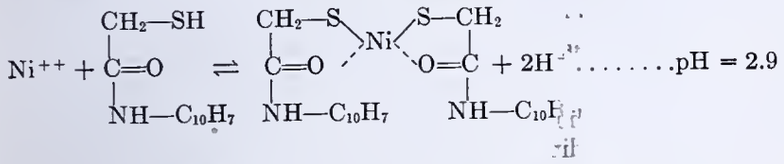
Nickel Dimethylglyoxime



Copper Benzoinoxime



Nickel Thionalide



In the first two instances these pH values were determined electrometrically. The pH of the thionalide equilibrium solution was measured colorimetrically.

Accordingly, if aqueous solutions of nickel salts are treated with alcoholic solutions of dimethylglyoxime or thionalide, or if aqueous cupric solutions react with alcoholic solutions of thionalide (an excess of precipitant in all cases), the clear bright green or bright blue filtrates contain metal ion, reagent, and hydrogen ion in equilibrium corresponding to the foregoing equations. These equilibrium solutions are saturated with respect to the precipitate, a condition that is an advantage from the analytical standpoint. The addition of solids that consume hydrogen ions disturbs the equilibrium, and inner-complex salt, equivalent to the hydrogen ions removed, immediately precipitates. Since this precipitate is highly colored, even traces are easily perceived.

The behavior of slightly soluble basic compounds only is considered here. Patently, solutions of bases (ammonia, etc.) and also salt solutions whose pH is higher than that of the equilibrium solutions will also react. The same will be true of materials that inactivate hydrogen ions by adsorption. Furthermore, metals and alloys that dissolve in dilute acids will also react with equilibrium solutions and cause precipitation of inner-complex salt. The behavior of potassium dichromate and chromate toward nickel dimethylglyoxime equilibrium solution is noteworthy. The former shows no reaction, the latter causes an immediate precipitate. This simple method of detecting the presence of potassium chromate in samples of potassium dichromate will be discussed in a later paper.

The precipitate (red, green, dark brown) is formed on the surface of the basic material and most of it sticks there and so is easy to see. If the sample is colorless, it is possible to detect even traces of basic substances. Since the precipitates are stable, permanent preparations can be made, and basic components, even in mixtures with indifferent materials, can be colored by this process. This method can be used to reveal the presence, and occasionally even the localization, of the basic components of numerous rocks and industrial products, such as cements, pigments, etc.

Organic bases that are only slightly soluble or even insoluble in water, and which consequently do not affect indicators, react nicely toward these equilibrium solutions. It is not even necessary to prepare a solution in alcohol or some other indifferent organic solvent before testing organic preparations as to their basic character or possible content of free bases. The sample is merely placed in one of the equilibrium solutions that contains alcohol, and a color appears if salt-forming basic groups are present. Amphoteric substances whose pH is greater than that of the equilibrium solutions give a positive response to this test. Examples are the aminobenzoic acids, glycine, protein compounds, etc.

Experimental

Reagents (equilibrium solutions). To 50 ml. of a 4 per cent solution of nickel nitrate or copper nitrate add 100 ml. of 1 per cent alcohol solution of dimethylglyoxime, benzoinoxime, or thionalide, warm briefly on the water bath, and filter. If the filtrates are stored in stoppered bottles (neutral glass) they can be preserved for 1 to 2 weeks. The efficacy of the reagent is not impaired if solid reagent separates on standing. Solutions of nickel dimethylglyoxime that remain clear can be prepared by treating a solution of 2.3 grams of hydrated nickel sulfate in 300 ml. of water with 2.8 grams of dimethylglyoxime in 300 ml. of alcohol, and filtering.

Procedure. To a few particles (*circa* 0.5 to 1 mg.) of the finely powdered sample on a white porcelain spot plate, add 1 or 2 drops of equilibrium solution. Mix by means of a fine glass rod or by blowing through a narrow tube. The vulnerability of the sample to the acid in the equilibrium solution determines whether no change is seen, or whether a red (nickel dimethylglyoxime), green (copper benzoinoxime), or dark brown (nickel thionalide) coloration appears at once or after a few minutes. Minute quantities can be tested on a slide, and the development of the color observed under the microscope. Rock and mineral samples can be scraped with a penknife and the resulting powder spot-tested on the main body of the specimen.

The formation of the colored reaction product can be seen well only on colorless substrates. If brown or bright-colored samples are under examination, it is better to make comparative blank tests by substituting a few drops of water for the reagent solution. The formation of the characteristic microcrystals of nickel dimethylglyoxime is easily established if observed under the microscope.

Applications

A positive reaction with each of the three equilibrium solutions was observed with: aluminum hydroxide, aluminum oxide, aluminum phosphate, barium oxide, barium carbonate, beryllium oxide, beryllium carbonate, calcium oxide, calcium carbonate, calcium phosphate, calcium acid phosphate, cadmium oxide, cadmium carbonate, ceric hydroxide, ceric oxide, ferric hydroxide, ferric phosphate, magnesium oxide, magnesium carbonate, magnesium ammonium phosphate, magnesium phosphate, magnesium arsenate, magnesium ammonium arsenate, lead hydroxide, lead oxide, lead carbonate, uranium oxide, ammonium diuranate, zinc carbonate, zinc ammonium phosphate, and zinc phosphate.

A weak reaction was shown by: lead oxide, Pb_3O_4; and mercuric oxide (yellow) stronger than mercuric oxide (red).

No reaction was shown by: bismuth trioxide, ferric oxide (ignited), ferric oxide (Brandt), cobalt oxide, cupric oxide, chromium trioxide, titanium dioxide, magnesium pyro-

phosphate, uranyl hydrogen phosphate, uranyl pyrophosphate, nor by the acidic oxides: stannic oxide, arsenic trioxide, arsenic pentoxide, molybdenum trioxide tungsten trioxide, vanadium pentoxide, and silica (quartz sand).

The following minerals (mostly from Brazilian sources) gave a positive reaction: malachite, serpentine (magnesite), cerussite, smithsonite, bentonite, fuller's earth (iron-free), pumice, montmorillonite, diabase, and bauxite. (The possibility that alkaline earth oxides might be responsible for the positive reaction of bauxite was eliminated. The reaction was just as strong after the finely powdered specimen was treated with both cold and boiling dilute hydrochloric acid, and then thoroughly washed.) Kieselguhr (84 per cent silica, 13 per cent aluminum oxide, 0.25 per cent ferric oxide) gave a positive reaction, but this failed after the sample was ignited (loss 1.27 per cent). Negative reactions were observed with kaolin and bismuthinite.

Finely pulverized window glass and ordinary bottle glass reacted positively; a good grade of chemical glass gave a very weak test. An excellent ampoule glass reacted negatively.

Ampoule Glass. Nickel thionalide equilibrium solution is a more sensitive test for alkalinity than is phenolphthalein. Although nickel dimethylglyoxime equilibrium solution is somewhat less sensitive than the indicator, this may be a real advantage for practical purposes, because the phenolphthalein test is often too delicate when evaluating the quality of a glass with reference to its alkali delivery to water. Ampoule glass of inferior grade can be detected quickly because a little of the powder moistened on a spot plate with 1 to 2 drops of nickel dimethylglyoxime solution shows red at once.

Neutral glass can be tested as to its suitability for ampoule manufacture by comparing the action of a finely powdered sample with that of a pulverized standard glass. Glasses of equal quality develop color of the same intensity, if the grain size of the powders is about the same.

The writers have found that deceptive results are often obtained if the resistance to attack of ampoule glass is tested by the common method of soaking the glass powder for 2 hours in water at 98° C., and then measuring the pH. Even good grades of glass subjected to this treatment sometimes give pH values that may be as high as 8.6. In contrast, large fragments of tubes or ampoules treated with hot water in this manner give pH values that are in accord with the quality of the sample and hence of real value in comparative tests (*2*).

Aluminum Oxide. It is noteworthy that a positive reaction is obtained when any of these equilibrium solutions is brought into contact with aluminum oxide, no matter whether it has been freshly precipitated, aged, or even strongly ignited. The nickel thionalide solution is the most sensitive; a brown color appears almost at once and deepens quickly. The sensitivity of the nickel dimethylglyoxime solution is less, and the copper benzoinoxime solution comes third. This order is observed toward all weakly basic compounds, either inorganic or organic; it is in agreement with the increasing pH values of the three equilibrium solutions.

Analytical application can be made of this behavior of aluminum oxide. The systematic scheme of qualitative analysis always leads to a solution that may contain aluminate. Treatment of this solution with ammonium chloride, or acidification followed by an excess of ammonium hydroxide, will often produce a gelatinous precipitate, a result that seems to establish the presence of aluminum in the original sample. However, this test is far from infallible, because even in the absence of aluminum a gelatinous precipitate may appear. This misleading material is silica, extracted from the glass by strong alkali. Consequently, if only a slight precipitate appears, a confirmatory test for aluminum is imperative. This is no simple matter, as evidenced by the well-known fact that erroneous reporting of the presence of aluminum is one of the most common errors in qualitative analysis. Equilibrium solutions furnish a rapid means of identifying the precipitate as hydrous alumina. The precipitate is washed until the filtrate no longer gives a test for free alkali with equilibrium solution; then it is spot-tested either on the filter or on a porcelain plate with nickel thionalide solution. If aluminum oxide is present, a brown color appears almost immediately and this color deepens on standing.

The weakly acid nickel thionalide solution reacts not only with freshly precipitated alumina, but also with aged or ignited specimens. At first sight, this fact seems incompatible with the known resistance towards acids of aged and particularly of ignited aluminum oxide. In the cold the latter is stable against even concentrated acids. If, in addition, it is recalled that mercuric oxide hardly reacts with the equilibrium solutions, even though it is far more easily dissolved by acids than alumina, it seems clear that the abnormal behavior of the latter toward equilibrium solutions must be ascribed to some cause other than the normal salt formation. In all likelihood, hydrogen ions are adsorbed by alumina and thus rendered inactive. Consequently, alumina should affect equilibrium solutions exactly as does neutralization or buffering. In fact, if alkali-free, ignited alumina is shaken with 0.1 *N* hydrochloric acid and immediately filtered, the filtrate is slightly less acid.

If the assumption is valid, other compounds capable of adsorbing hydrogen ions should behave like alumina. This conclusion has been substantiated in the cases of zirconium oxide and thorium oxide, prepared by igniting the nitrates or hydroxides. These products, whose solubility in acid is extremely low, react positively with nickel-thionalide solution, but much less decidedly than alumina.

Fusible and Infusible White Precipitate. The behavior of the compounds known as fusible and infusible white precipitate is worth special notice. These materials have been given the formulas $HgCl_2.2NH_3$ and $HgNH_2Cl$, respectively. They are white, soluble in acids, show the same reactions, and up to now could be distinguished only by their behavior when heated (fusion and decomposition) and the mercury-chlorine ratio as established by ultimate analysis. However, they behave differently toward nickel dimethylglyoxime solution. $HgCl_2.2NH_3$ reacts in a few seconds, and after several minutes is deep red. In sharp contrast, infusible white precipitate remains completely unchanged even after several hours' contact. This totally different behavior is in agreement with the formulation of these compounds as amino compound and ammoniate, respectively. The latter, as anticipated, shows a strong basic character, in contrast to the amino compound in which the NH_2 group functions as an acid residue. These compounds dissolve in an excess of warm acid, and the fact that both consume equimolar quantities of acid is not in contradiction with their behavior toward the equilibrium solution, since the latter merely reveals the rate of solution, which is conditioned by the character of the compound. This new means of distinguishing fusible and infusible white precipitate made it possible to prove that the latter in contact with ammonium chloride solution even at room temperature is quickly and extensively converted into the fusible salt:

$$HgNH_2Cl + NH_4Cl \rightleftharpoons HgCl_2.2NH_3$$

This conversion was first observed by Kane (*4*) when he treated $HgNH_2Cl$ with boiling ammonium chloride solution.

Organic Compounds. Care must be exercised in choosing the reagent solution when organic compounds are tested because, for instance, the copper solution should not be used when there is a possibility of the formation of copper double salts with organic bases, or of an oxidation by the cupric content of the reagent.

The following compounds, some of them basic materials not soluble in water, show an immediate positive reaction with the three equilibrium solutions: *p*-aminoacetanilide, *p*-aminophenol, atropine, benzidine, brucine, cinchonine, creatine, creatinine, dicyandiamidine, dimethylaniline, hexamethylenetetramine, naphthylamine (α and β), nicotine, nitron, 8-oxyquinoline, toluidine (*o*, *m*, *p*), phenylhydrazine, tetramethyldiaminodiphenylmethane, and 1,3,4-xylidine.

Comparative tests showed that *m*-aminobenzoic acid reacts very quickly, the para isomer slowly, and the ortho acid practically not at all. Sulfanilic acid does not react, because the acid character predominates in this amphoteric compound. On the other hand, its sodium and barium salts give a positive test immediately. The amide of sulfanilic acid reacts slowly. *m*- and *p*-nitraniline, nitroguanidine, and diphenylamine do not react; they are still acid despite their amino or imino groups.

Glycine and alanine, which are amphoteric and soluble in water, slowly give a rather weak positive test. *p*-Aminoacetophenone behaves similarly.

Egg albumin, keratin (from toothbrush bristles), and hide powder (pH = 5.5) react strongly.

No reaction was shown by caffein, phenylsemicarbazide, *p*-nitrophenylhydrazine, dinitrophenylhydrazine, isatin, and tetramethyldiaminodiphenyl ketone.

Filter Paper The constituents of equilibrium solutions are partially separated by capillary action if spots are placed on filter paper. A colored zone surrounds the spot. Nickel dimethylglyoxime solution works best in this kind of experiment. Different types of filter paper behave quite differently. The ring is particularly marked on qualitative papers, while on acid-washed quantitative papers (Schleicher und Schüll 589[2], 589[3]) it is exceedingly thin, and on Schleicher und Schüll 590 and 507 the intensity is intermediate. The whole spot is colored on thick absorbent paper, and the red appears first on the back side. The colored zones appear at the places of lowest acidity; the capillaries of the paper obviously hold back hydrogen ions. If neutral litmus paper is spotted with nickel dimethylglyoxime equilibrium solution a red circle is formed that extends beyond the zone of the precipitate. This demonstrates that the precipitation of this salt does not require complete neutralization, but only a decrease in the hydrogen-ion concentration, a conclusion quite in accord with the fact that the salt can be precipitated quantitatively from solutions containing acetic acid.

Bicarbonate Solutions. The addition of the nickel-equilibrium solution to bicarbonate solutions—e. g., calcium bicarbonate—produces a red precipitate. Hydrogen ion is taken from the equilibrium solution

$$Ca(HCO_3)_2 + 2H^+ \longrightarrow Ca^{++} + 2H_2O + CO_2$$

and an equivalent quantity of nickel dimethylglyoxime separates. The presence of bicarbonate in water containing carbon dioxide can be quickly established by this test. The development of a procedure for the quantitative determination of the temporary (bicarbonate) hardness of waters, based on reactions with equilibrium solutions, is now in progress.

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Potentiometric Determination of Vitamin C

Combined Use of 2,6-Dichlorophenolindophenol and Iodate

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THAT sodium 2,6-dichlorophenolindophenol is a specific oxidant for ascorbic acid in fruit extracts is generally accepted. Harris and Ray (*5*) have shown this to be true if titration is carried out in acid solution, but not so in the neutral, or nearly neutral, solutions used by Tillmans (*10*). Since the dye is red in the oxidized form in acid solution, it is often difficult or impossible to obtain the end point visually with precision in certain highly pigmented extracts. That the difficulties involved in the colorimetric method may be avoided by electrometric titration has been demonstrated by Kirk and Tressler (*7*).

A serious disadvantage, however, in the use of standard aqueous solutions of the dye is their instability. Frequent standardizations are necessary (*1*). A method which would eliminate this disadvantage and at the same time depend on the specificity of the dye for ascorbic acid seems desirable. Such a method involving the combined use of iodate and the dye has been developed.

Preliminary Experiments

In order to determine the effect of pH on the specificity of iodine as an oxidant for vitamin C, results were obtained with triiodide and with iodate (iodide previously added) on samples of various citrus juices over the pH range 0.3 to 3. The quantity of reducing substances found in these extracts with either of these oxidants was consistently higher, approximately 10 per cent, than that found with the indophenol dye. This is in agreement with the conclusions of previous investigators (*5*, *8*, *9*) so far as the nonspecificity of triiodide is concerned. That iodate, with iodide previously added, should be no more specific than triiodide is to be expected from the fact that in acid solutions iodate and iodide react practically instantaneously to form iodine. In fact, without previous addition of iodide, more iodate was reduced than triiodide by equal volumes of the same fruit juice. All titrations in this investigation were followed electrometrically (to be described later).

Other experiments were carried out to determine whether the dye might act as a selective alternate oxidation-reduction catalyst for the oxidation of ascorbic acid by iodine in citrus juices. Over the pH range 0.3 to 3 no catalytic effect was observed. However, it was noted that iodine did not oxidize the dye (reduced) at an appreciable rate even at those pH's where the oxidation of the dye is thermodynamically possible (*3*). It seemed probable, therefore, that the dye solution could be added to the extracts until the end points corre-

Table I. Typical Potentiometric Data with Grapefruit Juice

With Indophenol Dye			Followed by Iodate, Iodide Previously Added			With Iodate Alone, Iodide Previously Added		
Potentiometer Reading	Increment	Buret, Ml.	Potentiometer Reading	Increment	Buret, Ml.	Potentiometer Reading	Increment	Buret, Ml.
....	....	...	....	....	...	....	....	0.01
....	....	...	....	....	...	0.2317	....	4.01
....	....	0.00	....	....	0.00	0.2356	....	4.06
0.2212	....	8.83	0.2413	....	0.25	0.2373	0.0017	4.11
0.2298	0.0086	8.87	0.2413	....	0.28	0.2556	0.0183	4.16
0.2417	0.0119	8.92	0.2512	0.0099	0.33	0.2757	0.0201	4.20 e. p.
0.2573	0.0156	8.97	0.2752	0.0240	0.37 e. p.	0.2832	0.0075	4.25
0.2763	0.0190	9.02 e. p.	0.2851	0.0099	0.42	0.2912	0.0080	4.30
0.2903	0.0140	9.07	0.2907	0.0056	0.47	0.2953	0.0041	4.35

sponding to the oxidation of the vitamin C were reached, after which the titration could be completed with iodate in the presence of iodide. The total reducing capacity of another equal volume of the citrus juice could then be determined with the standard iodate solution alone. The difference between the equivalents of iodate reduced in the two titrations would thus yield the equivalents of vitamin C present. The determination would therefore be based solely on the standard iodate while making use of the specificity of the dye whose concentration need be known only very approximately.

Procedure and Apparatus

An ordinary student potentiometer, equipped with a reflecting walltype galvanometer with lamp and scale attachment (sensitivity, 0.014 microampere), was used. An agar bridge, saturated with potassium chloride, connected the 200-ml. lipless titration beaker with a saturated calomel half-cell. A platinum wire electrode completed the circuit with the potentiometer setup. Slow uniform stirring was maintained during each titration by means of a mercury-sealed stirrer. By placing the citrus juice in series with the titration beaker, one source of oxygen-free nitrogen served to blanket the juice during the analyses as well as to provide an inert atmosphere during titration, and to force portions of the juice into the pipet.

Procedure Adopted. Ten milliliters of the citrus juice are transferred to the titration beaker and sufficient 2 *N* hydrochloric acid and water are added to make the initial acid concentration of the resulting solution (approximately 100 ml.) between 0.1 and 0.5 *N*. The indophenol dye (Eastman No. P3463) solution, containing 0.5 to 1 mg. per ml., is then added until one drop of the dye solution remains in the oxidized form. Approximately 10 ml. of 0.1 *N* potassium iodide are then added, and the titration is completed with standard 0.01 *N* potassium iodate. The iodate solution must be added dropwise, allowing at least 5 seconds between each drop. Another 10-ml. portion of the citrus juice is titrated with the standard iodate alone at approximately the same acidity and iodide concentration. The main portion of the iodate may be added, in this case, at any convenient rate. Ten-milliliter burets, graduated to 0.05 ml., are used in all iodate titrations.

A simulated fruit juice, containing known quantities of ascorbic acid and glutathione, was used in the development of the above procedure. Equally satisfactory results were obtained at any pH over the range 0.3 to 3. It is somewhat simpler, however, to work at the higher acid concentrations (0.1 to 0.5 *N*) since readily measured volumes (via graduated cylinder) of common acid reagents may be used.

When the main portion of the residual iodate was added too rapidly after the dye end point was attained, more iodate was reduced than that equivalent to the glutathione present. This is due to the fact that the dye (reduced) is oxidized by iodate to a measurable extent, even with iodide present, unless the iodate is added slowly.

The equivalence point is recognized, without plotting titration data, when the largest change in e. m. f. occurs per drop (0.05 ml.) of oxidant solution added. In the titration of pure ascorbic acid solution or citrus juice with the dye, location of the end point with certainty by this procedure necessitates the inert atmosphere.

As is usually the case in potentiometric titrations, it is necessary to go slightly past the end point to be sure that the largest change has been registered. If the approximate volume of the oxidant required is determined by a rapid titration until a color change is noted, then, in the potentiometric determination, oxidant may be added to within several tenths of a milliliter of the approximate end point before recording potentiometric readings (color change not readily observable with dye solution over a week old). When within a few tenths of a milliliter of the end point, an e. m. f. balance is established after each subsequent drop of oxidant added. From 25 to 40 seconds were required for attainment of the maximum e. m. f. reading after addition of each drop. During this time interval, the potentiometer was continually adjusted to maintain zero deflection of the galvanometer, in order to obtain the reading before the drift downward in potential occurred. By this procedure the maximum change in potential per drop added was readily observed. The titration should not be interrupted during the addition of the last few tenths of a milliliter of oxidant; otherwise the drift in potential will make the results unreliable.

The sharpness of the potentiometric end point is assured if the platinum wire electrode is dipped into chromic acid cleaning solution and rinsed with water before each titration.

To illustrate the precision with which the end point can be determined, results of a typical titration on a sample of citrus juice are given in Table I.

Analytical Results

All citrus juices were filtered through a double thickness of cheese cloth. A drop of capryl alcohol was added to each total sample to prevent foaming caused by nitrogen bubbling through. The nitrogen prevented oxidation of the juices during analysis and also kept them uniform.

The results obtained by the combined use of indophenol dye and standard iodate and by the ordinary method, based on standard dye solution, are given in Table II. The dye solution was standardized by the usual procedure, involving the use of a known solution of pure ascorbic acid (Eastman No. 4640), as determined with standard iodate (or triiodide). Standardization was carried out potentiometrically. In every case 10.00 ml. of the citrus juice were used.

Discussion

Certain investigators (*5*, *6*) have reported that the rate of addition of the dye to either pure ascorbic acid solution or to citrus juice is a determining factor. Presumably the titrations were performed in the presence of air. In the inert atmosphere of nitrogen used in this investigation, the volume of dye solution required was found to be independent of the rate of its addition to either of these solutions. This suggests the possibility that in the presence of air the oxidation of ascorbic acid by oxygen may be induced during its oxidation by the dye. This possibility is also indicated by the observations of Harris and Ray (*5*) and of Fujita and Ebihara (*4*) that greater precision is obtained in the ordinary determination with the standard dye solution if the ascorbic acid solution is added to a measured volume of the dye solution. The conditions under which variations in the method of mixing the reductant and oxidant may change the extent of an induced oxidation by oxygen have been described by Bray and Ramsey (*2*, p. 2284).

Table II. Vitamin C Content of Citrus Juices

(Comparison of direct dye titration with dye-iodate method)

Citrus Juice[a]	Orange				Lemon			Grapefruit
	I	II		III	I	II		I
HCl normality	0.25	0.25	0.60	0.25	0.25	0.50	0.25	
Dye normality × 10^3	4.670	5.697	5.697	5.687	5.708	3.146	3.146	4.339
Volume, ml.	11.28	9.89	9.91	9.06	11.12	20.08	20.09	9.09
	11.25	9.90	...	9.08	...	...	...	9.07
Milliequivalents × 10^3, av.	52.60	56.37	56.46	51.58	63.47	63.17	63.20	39.40
Residual iodate, 0.01040 *N*								
Volume, ml.	0.51	0.55	0.55	0.56	0.40	0.42	0.43	0.39
	0.52	0.53	...	0.58	...	...	...	0.37
A. Milliequivalents × 10^3, av.	5.36	5.62	5.72	5.93	4.16	4.37	4.47	3.95
Iodate without dye								
Volume, ml.	5.65	6.00	6.01	5.54	6.56	6.50	6.50	4.18
	5.64	...	...	5.55	...	...	6.51	4.20
B. Milliequivalents × 10^3, av.	58.70	62.40	62.50	57.67	68.22	67.60	67.65	43.58
B minus A	53.34	56.78	56.78	51.74	64.06	63.23	63.18	39.63
Difference from dye titer, %	+1.40	+0.72	+0.57	+0.31	+0.93	+0.09	−0.03	+0.58

[a] Orange I, fresh navel; II, fresh Valencia; III, Valencia after refluxing in air for 0.5 hour. Lemon I, fresh; II, after standing one week out of contact with air (blanketed with nitrogen). Grapefruit, fresh. One milliequivalent of ascorbic acid = 88 mg.

Summary and Conclusions

This potentiometric method for the determination of vitamin C in citrus juices depends upon a stable potassium iodate solution as the only standard oxidant and, at the same time, makes use of the specific oxidation of ascorbic acid by 2,6-dichlorophenolindophenol. Standardization of the unstable dye solution is thereby eliminated.

The results obtained by this method (Table II) are in close agreement with those dependent on direct titration with an accurately standardized dye solution.

The method should be applicable to other biological fluids in which the indophenol dye is a specific oxidant for vitamin C.

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Elimination of the Water Wave in Polarographic Work

At Relatively High Indifferent Electrolyte Concentrations

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IT HAS been found (*1*) that the residual current obtained in relatively concentrated solutions of indifferent electrolytes, like alkali and earth alkali salts, is of the same order of magnitude as that obtained in more dilute solutions, when no electroreducible material is present. However, when a current flows through the cell owing to the reduction of some constituent in the solution, and the concentration of indifferent salt is greater than about 0.5 *M*, an abnormal wave occurs which is due to a reduction of water molecules:

$$H_2O + e \longrightarrow H + OH^-$$

This "water wave" starts at about −0.9 volt (*vs.* saturated calomel electrode), reaches a maximum at about −1.35 volts, and then decreases markedly as the potential is made more negative. This decrease is much larger than corresponds to the effect of the change of the drop time with the potential.

In analytical work the concentration of indifferent electrolyte in the solution is often greater than 0.5 *M*—e. g., in polarographic steel analysis. The occurrence of the water wave in such cases may be the source of large errors or misinterpretations, and should be eliminated, particularly when dealing with mixtures of reducible substances. When the solution contains only one reducible constituent which yields a diffusion current at potentials more positive than −0.9 volt, the water wave appears as a separate wave. On the other hand, when the solution contains a constituent which yields a wave at potentials more negative than −0.9 volt, the water wave overlaps with that of the reducible constituent. Consequently, an apparent diffusion current of the reducible constituent is found which is much larger than the true diffusion current.

In order to illustrate the effect, Figure 1 presents current-voltage curves of 0.0004 *M* zinc chloride in different concentrations of potassium chloride. These curves were obtained some five years ago by C. S. Miller in this laboratory with the manual apparatus at 25.0° using drop times of 1 and 2 seconds. The horizontal portion of curve 1 (in 0.05 *M* potassium chloride) corresponds to the diffusion current of zinc at a drop time of 1 second. Curves 2 and 3 have an abnormal appearance and show maxima. These are not of the same nature as the ordinary maxima which are often observed on current-voltage curves and are due to stirring effects. The maxima on curves 2 and 3 were found at potentials where the water current has a maximum value. The large decrease of the current on curves 2 and 3 after the maximum had been reached is mainly due to a decrease of the water current when the potential is made more negative. However, it is seen that the true diffusion current of zinc has not yet been attained at the potential where the potassium discharge starts. If we measured the "apparent" diffusion current at the

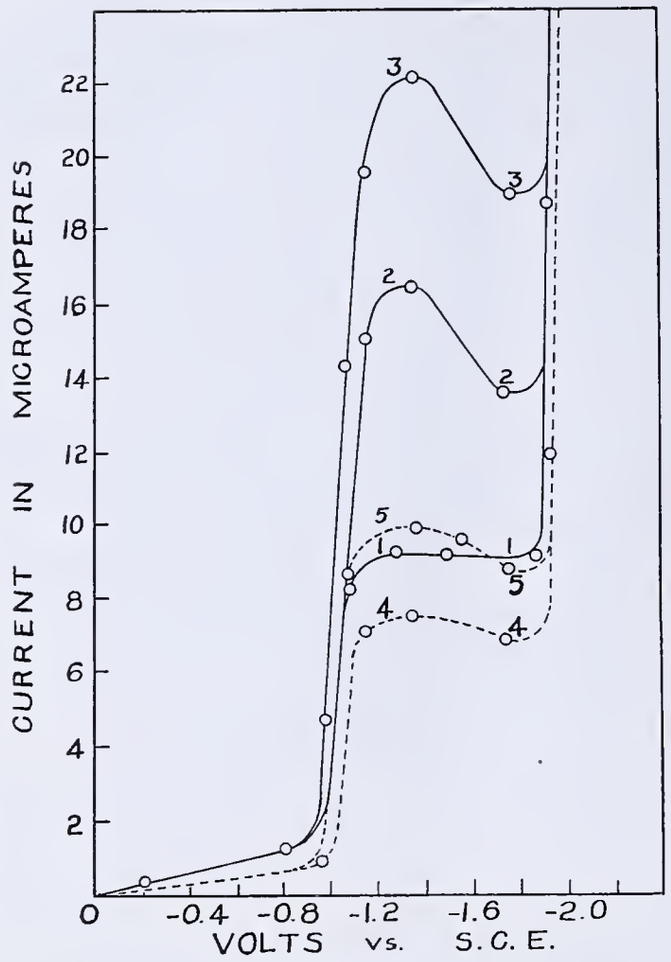

FIGURE 1. CURRENT-VOLTAGE CURVES OF 0.0004 *M* ZINC CHLORIDE IN POTASSIUM CHLORIDE

Concentration of KCl in curve 1, 0.05 *M*; curve 2, 0.5 *M*; curve 3, 1 *M*; curve 4, 0.5 *M*; curve 5, 1 *M*. Drop time in curves 1, 2, and 3 was 1 second, in curves 4 and 5, 2 seconds

maximum, we would find a value of 16.2 microamperes in 0.5 *N* potassium chloride and of 21.8 in 1 *N* chloride, whereas the true diffusion current is only 9.3 microamperes. The maximum becomes much less pronounced when the drop time is increased, as is evident from curves 4 and 5 in Figure 1. These curves were obtained with a drop time of 2 seconds.

Orlemann and Kolthoff (*1*) found that the water wave is proportional to the total current flowing, that it increases with increasing concentration of indifferent electrolyte in the solution, that it is of the same order of magnitude in solutions of potassium, sodium, and lithium salts, and that the water wave does not occur in solutions of tetraethylammonium chloride. Working with the same capillary the ratio of the water wave (i_{H_2O} at maximum value) to the total current i_t decreases when the pressure of the mercury on the capillary is decreased according to the expression:

$$i_{H_2O}/i_t = kP + \text{constant}$$

For example, in a solution of thallous chloride in 2 *M* potassium chloride they found a ratio of i_{H_2O}/i_t of 0.47 at a pressure of 100 cm. of mercury, of 0.24 at a pressure of 80 cm., and of 0.03 at a pressure of 50 cm. Thus, by working with a very slow-dropping capillary (drop time equal or greater than 5 seconds) the water current can be eliminated. In ordinary polarographic practice such slow-dropping capillaries are seldom used. Fortunately, it has been found possible to eliminate the water current completely by adding 0.01 per cent of gelatin to the solution. The effect of a number of capillary-active substances, including tylose, upon the water wave has been investigated. All the capillary-active substances affected the water wave, but only gelatin eliminated it completely.

TABLE I. EFFECT OF WATER CURRENT IN ANALYSIS OF MIXTURES OF THALLIUM AND ZINC AT 25° C.

(t = 3.60 seconds at −0.6 volt)

Curve in Figure 2	Concentration of KCl	Gelatin	i_{Tl^+}	$i_{Tl^+} + i_{Zn^{++}}$	$i_{Zn^{++}}$[a]	Error in Zn
	M	%	*Microamp.*	*Microamp.*	*Microamp.*	%
1	0.1	0.01	0	9.30	9.30	..
2	2	0.01	0	9.40	9.40	..
3	2	0	0	12.20	12.20	+30
4	1.0	0	8.00	20.50	11.90	+27
5	1.0	0.01	7.25	16.70	9.45	+ 1

[a] In all solutions concentration of zinc ions was 0.0016 *M* and concentration of thallium ions was of the order of 0.002 *M* in curves 4 and 5.

Therefore, when current-voltage curves are determined with the dropping electrode in solutions whose concentration of indifferent electrolyte is greater than 0.5 *M*, 0.01 per cent of gelatin should be added to eliminate the water current. This addition is especially essential when dealing with mixtures of reducible substances.

In order to illustrate the order of magnitude of the error which can be made when the water current is not eliminated, current-voltage curves of mixtures of thallium and zinc were determined under different conditions. Some of the curves are shown in Figure 2, and analyzed in Table I.

Comparison of curves 1 and 2 (Table I) reveals that the true diffusion current of zinc in 2 *M* potassium chloride is practically the same as in 0.1 *M* chloride. If the water wave was not eliminated in the 2 *M* chloride solution, an apparent diffusion current of zinc was found which was 30 per cent larger than the true diffusion current. Curves 4 and 5 refer

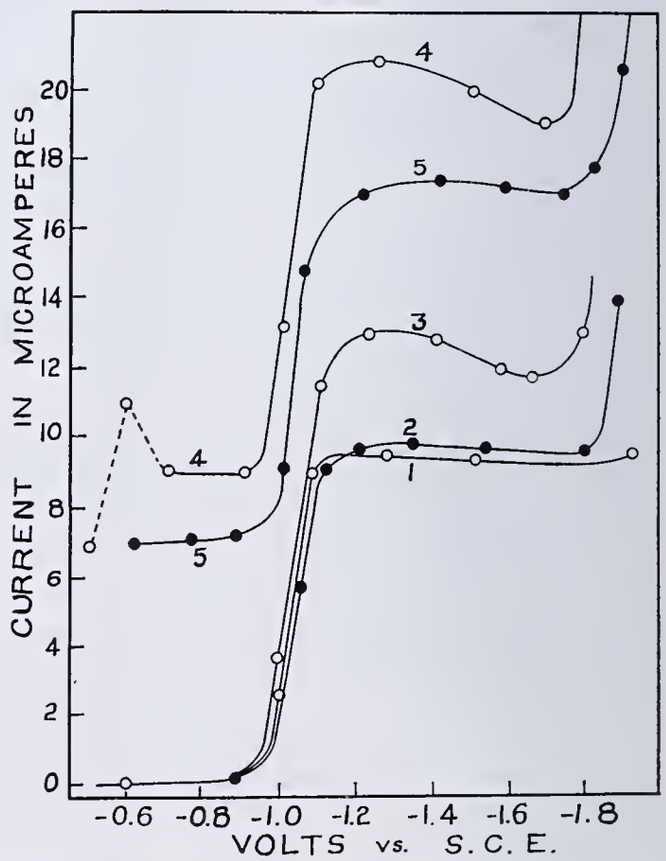

FIGURE 2. EFFECT OF GELATIN ON WATER CURRENT IN MIXTURES OF THALLIUM AND ZINC IN POTASSIUM CHLORIDE SOLUTIONS

Composition of solutions is given in Table I

to mixtures of thallium and zinc in only 1 *M* potassium chloride. If the water wave was not eliminated an error of 27 per cent in the zinc wave was found, the error being only 1 per cent in the presence of 0.01 per cent of gelatin. The error in the absence of gelatin would increase with increasing ratio of thallium to zinc, since the water current is proportional to the total current. It would hardly be possible to correct for the water wave by making calibration curves with known mixtures of thallium and zinc in the particular medium. The results in Table I show that such calibration curves are entirely superfluous, if 0.01 per cent of gelatin is added to the solution to be analyzed. The magnitude of the error due to the water wave depends upon the characteristics of the capillary, as mentioned above.

Summary

Large errors may be caused by the occurrence of a water wave in the polarographic analysis of solutions which have a concentration of indifferent electrolyte larger than about 0.5 *M*. The water wave and the abnormalities caused by it are completely eliminated by the addition of 0.01 per cent of gelatin to the solution.

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Specific Gravity of Petroleum Oils by the Falling Drop Method

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A method of densimetry which has shown many advantages in determinations with body fluids has been modified to adapt its use to measurement of the specific gravity of petroleum oils. Operation is rapid and easy, since the procedure depends only on the measurement of the time of fall of a drop of oil in an alcohol-water solution. The specific gravity is then determined by direct conversion of the time of fall from a calibration curve for the solution. This method has been found accurate to within ±0.0010 unit of specific gravity. Since only a few drops of the petroleum fraction are required, the determination may be made on the very small volumes of oils often obtained in analytical distillations or extractions.

OFTEN small volumes of petroleum oils are obtained by extraction or by fractional distillation, especially during the analysis of asphalt and road oil (*2*, *5*, *6*). To understand the nature of these fractions, characterization by physical constants is desired, values for density and refractive index being especially informative (*2*, *7*). Usually but little difficulty is had in obtaining accurate readings of refractive index; however, when only small volumes of oil are available, pycnometer determinations for density are difficult and time-consuming.

A method of densimetry which requires a small volume of fluid and less than a minute's time would be especially valuable. Such a method was described by Barbour and Hamilton (*1*) and later with variations by Guthrie (*3*) and by Kagan (*4*). Although the procedure described was devoted entirely to measurement of body fluids, the principle of the method, "timing the fall of a drop of body fluid of known size, through a definite distance in a mixture nonmiscible with the fluid" (*1*) would generally apply to any liquid.

Modifications of the procedure and equipment to permit the principle to be applied to petroleum oils are described in this paper.

Theory

When a small spherical body moves through a fluid so slowly that the latter flows past it in smooth streamlines, the relative velocity, V, between the body and fluid may be obtained from Stokes' law:

$$V = \frac{gD^2(d_1 - d_2)}{18\mu}$$

where μ is the coefficient of viscosity of the fluid, D the diameter of the sphere, d_1 the density of the sphere, d_2 the density of the fluid, and g the acceleration due to gravity.

V is a constant for a given particle having free movement and, if the distance of fall were fixed, would be inversely proportional to the time of fall of the sphere. Under the conditions of operation of the falling drop method, wherein the distance of fall, 30 cm., the diameter of the drop, D, and the properties of the falling drop solution are constant, V should be directly proportional to the density of the drop, d_1. Actually, over the range covered and with the apparatus described a direct ratio is not obtained, probably because of wall and turbulence effects. However, the results are reproducible as shown by calibration curves, and the accuracy of the measurement is not related to the lack of proportionality.

Equipment

Three glass tubes with a 1.3-cm. bore and 50 cm. long, obtained from a stock of Pyrex boiler gage glass, were marked with lines 30 cm. apart, extending all the way around the tubes, one line being 5 cm. from the end of the tube. Rubber stoppers were inserted to close the ends of the tubes nearest to the lines. As shown by Figure 1, the tubes were mounted in a 2000-ml. cylinder (8 cm. in inside diameter × 48 cm.) by means of a brass plate standing on three 1-cm. legs and with a cap on the top. The three holes fitting the glass tubes in the brass plate and cap were drilled with their centers on the apexes of an equilateral triangle and 3.5 cm. apart. In addition, holes were drilled in the cap for a thermometer, a 250-watt knife-blade heater, and an expansion-type thermoswitch. Copper tubing of 0.3-cm. (0.125-inch) diameter was run down the side of the cylinder to supply air for stirring the water bath. A leveling stand was provided by fastening the base of the cylinder into the case from a Bourdon gage, leveling screws being fastened to the rim of the case. The

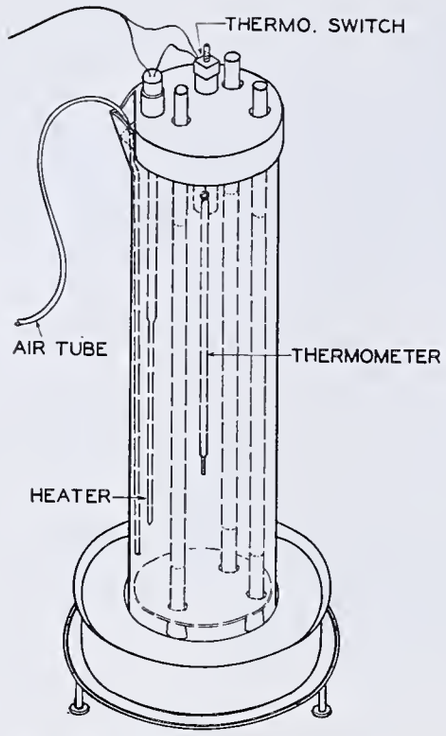

FIGURE 1. FALLING DROP EQUIPMENT

thermometer was suspended so that it could be used as a plumb bob to adjust the falling drop tubes in a vertical position.

A falling drop pipet calibrated to deliver two drops of exactly 0.01-ml. volume each was used to form the drop of petroleum fraction, a Guthrie pipet controller being used to regulate the delivery of the pipet. The time of fall of the drop was measured with an electric timer which could be read to 0.1 second.

Falling Drop Solutions

Solutions of ethyl alcohol and distilled water were mixed to cover the range in specific gravity of the petroleum fractions likely to be run. Before filling the tubes the solutions were heated to boiling under a reflux condenser to dispel any air present; otherwise bubbles collected on the falling drops.

The composition of the solutions used for the determination of the data in Table III and the range of specific gravity for which they could be used are listed in Table I. The range in specific gravity was obtained by referring to a graph similar to Figure 2 constructed for all five solutions and selecting the specific gravities corresponding to the time of fall from 7 to 25 seconds.

Method

After the oil had been drawn into the pipet to the upper mark, a standard drop was formed with the tip of the pipet under the surface of the solution in the tube. A slight jerk upward released the drop. The release was made 5 cm. or higher above the upper mark in the tube to allow a substantially constant velocity to be reached by the drop before the mark was passed. The time required for the drop to fall the 30 cm. between the marks was measured to the nearest 0.1 second by a timer. If the time of fall was not in the range of 7 to 25 seconds, a trial drop was formed in one of the other solutions, trials being repeated until the time of fall came within this range. An additional drop or drops were then released in the selected solution to have determinations agreeing to within 0.2 second.

When viscous oils and waxes were run, the temperature of the water bath was maintained constant at about 71° C. (160° F.) to permit the liquid to be easily expelled from the pipet. The pipet, filled to the upper mark with the sample which had been heated to a fluid condition on a lamp bank, was immersed in the alcohol-water solution to above the upper mark. After holding it thus for 3 to 5 minutes to allow equalization with the temperature of the water bath, the drop was formed and released underneath the surface of the liquid as before.

With such viscous oils, especially if they were resinous, it was difficult to shake the drop from the pipet, the drop sometimes breaking and part remaining on the tip. By rubbing a very small quantity of soap on the tip of the pipet, the interfacial tension was found to be changed so that the drop could be easily formed and released.

The pipet was cleaned of oil with carbon tetrachloride, gas then being blown through the pipet to evaporate the carbon tetrachloride.

Calibration

The falling drop solutions were calibrated by running three oils selected to have times of fall defining the range from 7 to 25 seconds. The specific gravity at 25° C./25° C. of the three oils was determined to within ±0.0005 unit with a 5- or 10-ml. pycnometer. The calibration curve was plotted on semilogarithmic paper as shown by Figure 2, the time of fall being plotted on the logarithmic scale.

After the curve was once established for a particular solution at a given temperature, but one run with a standard oil was necessary to check the calibration. If the concentration of the solution had changed but slightly, the time of fall of this standard oil was used to establish a new calibration curve parallel to the previous curve.

When the determinations were carried out at 71° C. (160° F.) the calibration was checked after each 2 hours of operation to determine if the loss of ethyl alcohol by evaporation had been sufficient to increase noticeably the density of the falling drop solution. The solution was renewed if the run with the standard oil differed markedly from the previous calibration—i. e., by as much as one second in falling time.

Since the coefficient of expansion of petroleum oils may be considered to vary only with specific gravity and not with source, the oils used in calibration need not be from the same crude as the unknown oils. An appreciable error arises in determinations with waxy oils, or with wax, due to the change in volume of wax upon crystallization, but this error may be eliminated by selecting similar waxy oils or waxes as the calibration standards. If the specific gravity data may be reported at temperatures above the crystallization point, calibration curves determined with wax-free oils can be used to determine the density of a hypothetical liquid at 25° C./25° C.; then the National Standard Petroleum Tables can be used to determine the correction needed to obtain the specific gravity at a temperature above the crystallization point.

TABLE I. SOLUTIONS USED

Solution No.	Per Cent Ethyl Alcohol by Volume	Specific Gravity of Solution at 25° C./25° C.	Range in Specific Gravity at 25° C./25° C. for Which Solution Is Suitable
1	98	0.793	0.836–0.877
2	75	0.852	0.873–0.911
3	70	0.865	0.885–0.925
4	60	0.889	0.920–0.950
5	45	0.923	0.953–0.993

Experimental

TEMPERATURE CONTROL. The variations in falling times with a change in temperature of 2.8° C. (5° F.) were determined for oils of known specific gravity. Three alcohol-water solutions representative of the usual concentrations employed were calibrated at normal operating temperatures as shown in Table II. These data, when used to construct a graph similar to Figure 2, show that a 2.8° C. (5° F.) change in temperature shifts the calibration curve by not more than 0.0010 unit in specific gravity. To have data accurate to within 0.0010 unit, the tolerance allowed in the thermostatic control could therefore be ±1.4° C. However, such a wide variation is not permissible if rapid temperature fluctuations occur because convection currents are set up in the alcohol-water solutions. Control within ±0.2° C. is not difficult to obtain and is recommended as desirable to eliminate such currents which interfere with the normal fall of the drop.

Table II. Variation of Falling Drop Time with Change in Temperature of Solutions

Ethyl Alcohol	Temperature		Time of Fall Trial 1	Time of Fall Trial 2	Time of Fall Av.	Specific Gravity at 25° C./25° C. by Pycnometer
% by vol.	° C.	° F.	Sec.	Sec.	Sec.	
98	28.9	84	10.2	10.3	10.2	0.8520
98	28.9		13.4	13.6	13.5	0.8414
98	28.9		19.1	19.3	19.2	0.8364
98	31.7	89	10.1	10.3	10.2	0.8520
98	31.7		12.9	13.1	13.0	0.8414
98	31.7		18.8	18.9	18.8	0.8364
75	68.3	155	10.4	10.3	10.3	0.8949
75	68.3		11.8	11.9	11.8	0.8873
75	68.3		17.3	17.5	17.4	0.8780
75	71.1	160	10.3	10.4	10.3	0.8949
75	71.1		11.6	11.8	11.7	0.8873
75	71.1		16.7	16.9	16.8	0.8780
40	68.3	155	8.8	8.9	8.8	0.9853
40	68.3		10.3	10.4	10.3	0.9795
40	68.3		16.2	16.4	16.3	0.9666
40	71.1	160	8.6	8.6	8.6	0.9853
40	71.1		10.0	10.1	10.0	0.9795
40	71.1		15.9	16.1	16.0	0.9666

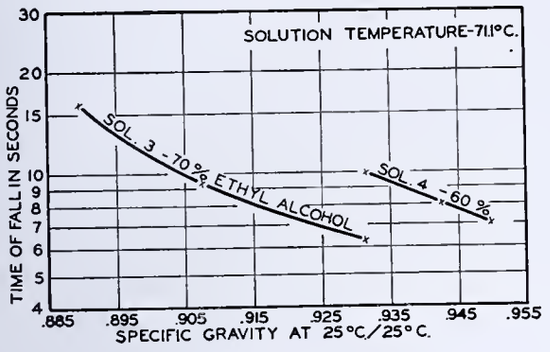

Figure 2. Calibration Curves for Falling Drop Solutions

Comparison with Pycnometer Determinations. The specific gravities at 25° C./25° C. of 37 oils ranging from 0.8364 to 0.9853 were obtained with a 5-ml. pycnometer, duplicate determinations being made which agreed for each oil within 0.0005 unit. The falling times of these oils were determined with solutions which allowed the drop to fall within 7 to 25 seconds, with one exception of 6.3-second time of fall. During the determinations the temperature of the water bath surrounding the falling drop tubes was controlled by the thermostat to within ±0.2° C.

The minimum, an intermediate, and the maximum time of fall for three of the oils run in each solution were plotted as calibration curves on semilogarithmic paper, as illustrated by Figure 2. In all, five different solutions were calibrated, fifteen of the oils being used as standards. The times of fall of the twenty-two remaining oils were converted to specific gravities at 25° C./25° C. by referring to the calibration curves. The pycnometer values were subtracted from the chart values to determine the differences in specific gravity by the two methods, as shown in Table III.

Accuracy

In the group of twenty-two oils, a difference greater than 0.0010 unit was found for four oils, only one determination showing a difference greater than 0.0020 unit. With fourteen oils a difference of 0.0005 unit or less was found. The average difference was 0.0006 unit.

From consideration of this series of runs completed in a routine manner, the accuracy of the method is believed to be within ±0.0010 unit of specific gravity. This is well within the deviation allowable in calculation of characterization factors of complex mixtures, such as petroleum fractions, from density and refractive index.

Range of Application

The lightest oil which could be run would have to be slightly heavier than ethyl alcohol, or with a specific gravity of about 0.79 at 25° C./25° C. If the viscosity is very low, liquids such as kerosene and light gas oil form drops which break off the pipet before the standard drop volume of 0.01 ml. is reached.

The upper range in specific gravity is limited by the oil being too dark for the meniscus in the pipet to be discernable. Viscosity is also a factor, some of the resins recovered from asphalts being light enough in color but too viscous to be handled at the temperature of 71° C., which is about the highest temperature found practicable for operation without excessive evaporation of the ethyl alcohol solutions. The higher alcohols, such as propyl and butyl, cannot be used because of their appreciable solvent power for petroleum fractions at such temperatures.

With the time of fall between 7 and 25 seconds, each solution of alcohol and water has been found suitable for a range of approximately 0.040 unit of specific gravity.

Table III. Comparison of Determinations by Pycnometer and Falling Drop Method

Solution No.	Temperature	Time of Fall Trial 1	Time of Fall Trial 2	Time of Fall Av.	Specific Gravity at 25° C./25° C. By pycnometer	By time of fall	Difference
	° C.	Sec.	Sec.	Sec.			
1	28.9 (84° F.)	10.3	10.1	10.2	0.8520	Calibration	.....
1	28.9	11.6	11.4	11.5	0.8464	Calibration	.....
1	28.9	21.5	21.5	21.5	0.8364	Calibration	.....
1	28.9	10.9	11.1	11.0	0.8491	0.8484	−0.0007
1	28.9	11.3	11.6	11.5	0.8477	0.8464	−0.0013
1	28.9	14.5	14.3	14.4	0.8415	0.8410	−0.0005
2	71.1 (160° F.)	9.2	9.3	9.2	0.8949	Calibration	.....
2	71.1	10.7	10.7	10.7	0.8873	Calibration	.....
2	71.1	14.8	15.1	14.9	0.8780	Calibration	.....
2	71.1	9.3	9.3	9.3	0.8936	0.8940	+0.0004
2	71.1	9.8	9.9	9.8	0.8895	0.8908	+0.0013
2	71.1	11.5	11.4	11.4	0.8847	0.8849	+0.0002
2	71.1	14.8	15.0	14.9	0.8780	0.8780	0.0000
3	71.1	6.3	6.3	6.3	0.9313	Calibration	.....
3	71.1	9.3	9.5	9.4	0.9075	Calibration	.....
3	71.1	15.9	16.1	16.0	0.8895	Calibration	.....
3	71.1	7.8	7.8	7.8	0.9182	0.9180	−0.0002
3	71.1	8.2	8.3	8.2	0.9150	0.9150	0.0000
3	71.1	10.2	10.5	10.4	0.9033	0.9032	−0.0001
3	71.1	13.8	13.6	13.7	0.8915	0.8940	+0.0025
3	71.1	13.9	13.8	13.8	0.8949	0.8939	−0.0010
4	71.1	6.9	7.0	7.0	0.9497	Calibration	.....
4	71.1	8.0	8.2	8.1	0.9421	Calibration	.....
4	71.1	10.0	10.0	10.0	0.9313	Calibration	.....
4	71.1	8.3	8.5	8.4	0.9398	0.9395	−0.0004
4	71.1	9.0	9.2	9.1	0.9356	0.9358	+0.0002
4	71.1	9.1	9.3	9.2	0.9349	0.9350	+0.0001
4	71.1	9.2	9.2	9.2	0.9341	0.9350	+0.0009
4	71.1	9.6	9.8	9.7	0.9316	0.9330	+0.0014
5	71.1	8.4	8.4	8.4	0.9853	Calibration	.....
5	71.1	12.1	12.3	12.2	0.9666	Calibration	.....
5	71.1	15.3	15.5	15.4	0.9585	Calibration	.....
5	71.1	8.7	8.9	8.8	0.9819	0.9820	+0.0001
5	71.1	9.0	9.2	9.1	0.9795	0.9800	+0.0005
5	71.1	9.1	9.3	9.2	0.9784	0.9794	+0.0010
5	71.1	10.3	10.4	10.4	0.9725	0.9730	+0.0005
5	71.1	13.4	13.6	13.5	0.9622	0.9626	+0.0004

Thanks are due W. E. Garris, Jr., for assistance in the laboratory determinations and in assembly of equipment; and to S. F. Crynes, pathologist at the City Hospital, Springfield, Ohio, for drawing the author's attention to the method and its possibilities in other than clinical determinations.

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Determination of the Drying Rates of Thin Films

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An apparatus, which can easily be constructed from equipment available in any laboratory, has been devised for measuring the drying rates of thin films. This apparatus and technique have been applied to a study of thin nitrocellulose films, the maximum dry weight of which was about 0.02 gram; the area was about 13 sq. cm. (2 square inches); the total solvent content was about 300 mg.; and the drying time was about 20 minutes. The drying rates of these selected films have been calculated and the results presented in graphs. The suggested apparatus and technique have been shown to give satisfactory and reproducible results.

IN THE past few years there has been a revival of interest in the evaporation rates of solvents and in the determination of these evaporation rates. Most of this work has been done on pure solvents, and there have been very nearly as many different types of apparatus employed as there have been investigators. Each new development has been in a sense a refinement of older methods in an attempt to obtain an easier operation and a greater reproducibility of results (*1*, *5*, *6*, *8*, *10*, *11*, *12*).

From an over-all viewpoint it has been the endeavor of this investigation to devise an apparatus that will minimize the effects of any local operating conditions and give consistent results. Although these results may not quantitatively be comparable to results obtained on other apparatus, at least they will be relative.

In the case of a pure solvent, the more accurate evaporation measurements show that a plot of the per cent solvent evaporated *vs.* time is a straight line; this means that a pure solvent has a constant evaporation rate. If one or more solvents are introduced into the original solvent and evaporation measurements are made, then the plot of the per cent volatiles evaporated is no longer a straight line. This means that the solution containing two or more solvents does not have a constant evaporation rate.

If instead of another solvent or other solvents, a nonvolatile component be added to the original solvent, it has been shown that the resulting solution again exhibits no constancy of evaporation or drying rate. Bogin and Wampner (*3*), Stewart and Dorsch (*9*), and Durrans and Davidson (*4*) have done much work on the drying rates of solutions of nonvolatiles in volatile solvents. Their published reports indicate that the presence of nonvolatile materials affects the drying rate of a solution and that this rate falls off as the solvent evaporates, finally approaching zero as the resulting mass nears dryness.

The determination of the drying rates of such solutions means the employment of some apparatus that will enable weight readings to be taken at various intervals of time during the drying process. Durrans and Davidson placed a quantity of their solution (nitrocellulose in a suitable solvent) in a shallow dish and passed air of a constant temperature over it. The sample was large enough and the amount evaporated in each time interval of sufficient magnitude, so that any evaporation occurring during the time of weighing probably introduced very little error.

However, the use of a large sample of any appreciable depth means that an actual thin film as ordinarily dealt with in the application of a protective coating is not simulated. Information on the drying rates of any sort of film-forming solutions, it seems, should be obtained when the solution is in the form of a thin film.

The determination of the drying rate of a thin film, however, brings forth difficulties not ordinarily encountered when larger and deeper volumes of solutions are considered. For example, the supporting base or tare on which the film is cast generally constitutes the major portion of the total weight of the whole system. Therefore, a very sensitive weighing de-

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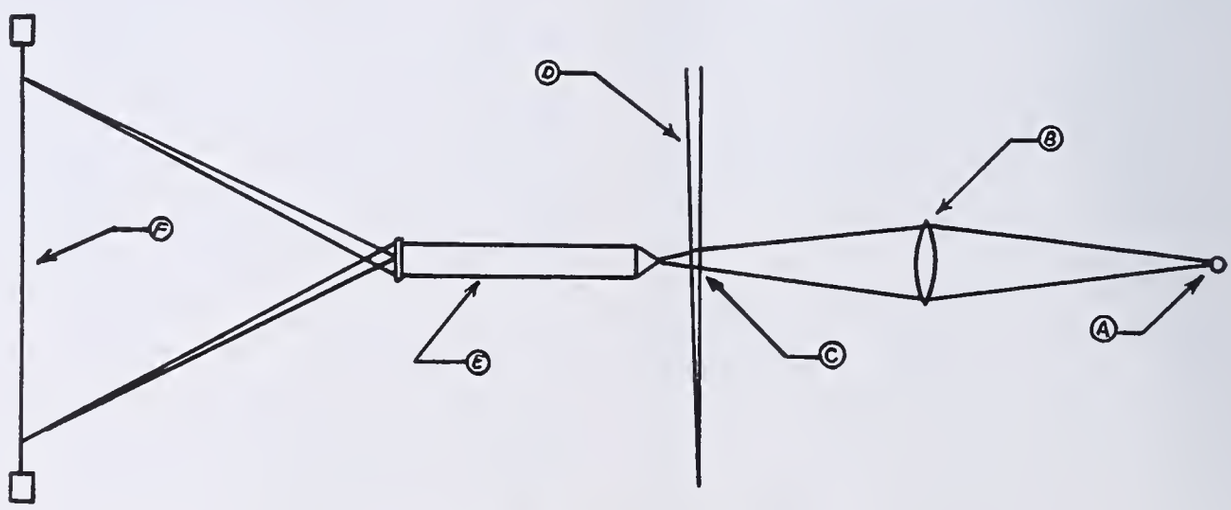


FIGURE 1. OPTICAL SYSTEM

A. Light source
B. Condenser lens
C. Pointer scale
D. Pointer
E. Balance lens
F. Projection screen

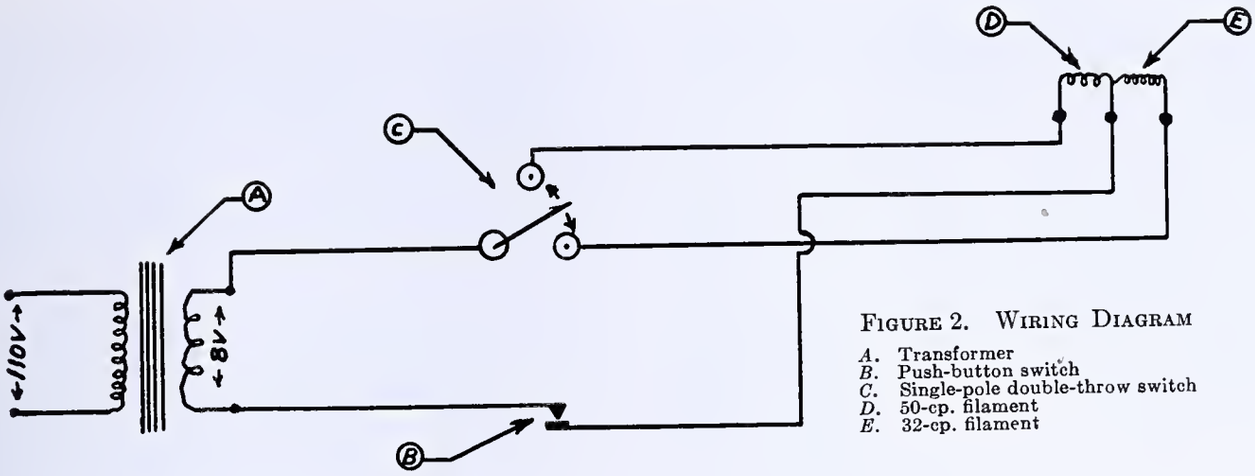


FIGURE 2. WIRING DIAGRAM

A. Transformer
B. Push-button switch
C. Single-pole double-throw switch
D. 50-cp. filament
E. 32-cp. filament

vice must be used to detect small changes in the weight of the film.

Apparatus

The requirement of sensitivity suggested the use of some standard analytical balance. However, using a chainomatic type of analytical balance, it was found that with very thin films which weighed but a few milligrams and contained fast-evaporating solvents, the balance operator could not keep up with the weight changes with any degree of accuracy. In an attempt to overcome this operational difficulty, the following apparatus was devised for determining the drying rates of thin films.

A Christian Becker (Type 508-A) air-damped analytical balance was used. The distinguishing feature about this balance, which makes it or one similar to it particularly adaptable to a study of the drying rates of thin films, is the scale attached to the pointer. This scale, similar to a microscopic stage micrometer, is ordinarily read by means of a low-power microscope attached to the body of the balance case. It is graduated in milligrams up to 100 mg. on each side of the zero mark. Each milligram is divided into two parts, so that it is possible to estimate to 0.1 mg. weighing with the same accuracy that a rider or chain can be read on the usual type of analytical balance.

The beam is air-damped, which under normal operating conditions prevents overshooting and provides a smooth response to weight changes in the pans. It is possible to read this balance over the range of a 200-mg. change in weight without changing the weights on the pan. The hairline used in conjunction with the pointer scale is an integral part of the eyepiece of the microscope attachment.

Since, in general, it is fatiguing to peer constantly through such a microscope, the objective lens was adjusted so that the scale could be projected onto a screen. In order to project the pointer scale it was necessary to illuminate it with a small concentrated beam of light. The light source employed was a double-filament automobile headlamp bulb having a 32- and a 50-candlepower filament. The 50-candlepower filament was used during operation, while the 32-candlepower filament was kept wired for use in case of failure of the stronger filament. The lens system and wiring diagram are shown in Figures 1 and 2.

The screen was mounted on one edge of the table that supported the balance and was so constructed that it could be swung to one side of the balance while weights were being changed inside the case. The back glass pane of the balance case was removable. This pane was raised slightly and underneath it was fitted a board which held in place a length of rubber tubing connected to an aspirator. Thus the air in the balance was gently exhausted at all times, but not rapidly enough to set up any stray air currents that would disturb the pans. This exhaust system was probably not necessary, since the loss of weight of any sample, which represented the weight of volatiles evaporated into the balance case, was never over 300 mg. This weight, if not removed during the run, would have exerted a negligible vapor pressure compared to that exerted by the solvent in the drying film. Figure 3 is a photograph of the complete assembly.

Besides facilitating reading of the pointer scale, the screen is also useful if the balance is kept in a constant-temperature room. In such a case, a small window may be put in the door of the room, and by placing the push-button switch shown in Figure 2 outside the door, readings may be taken without entering the room. The use of the push-button switch makes it unnecessary for the light to be on except when readings are being taken. Within limits, the scale as projected on the screen may be enlarged to any degree. It is, of course, projected upside down; however, in very little time the operator can become used to reading the inverted scale.

Experimental Procedure

A discussion of the experimental procedure followed during the investigation of some selected nitrocellulose solutions is probably the simplest manner in which to present the technique developed for use with the apparatus just described.

Solvent or solvent-diluent mixtures were made up at 25° C. by use of a 100-ml. buret. The desired portion of one component was thoroughly mixed with the other component and stored in a

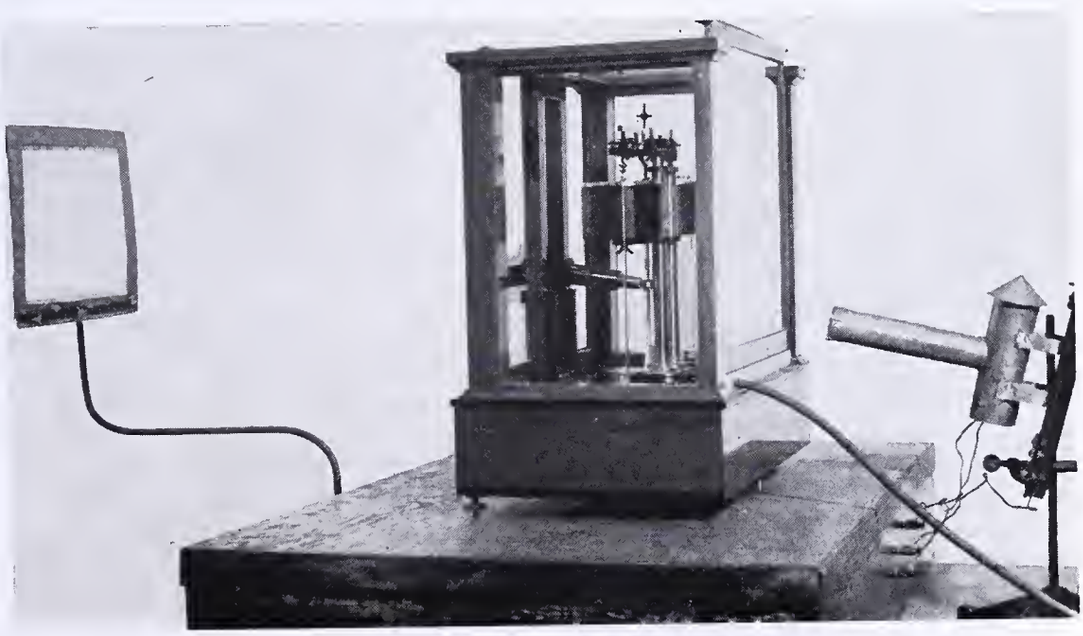


FIGURE 3. COMPLETE ASSEMBLY

stoppered glass bottle prior to weighing into the final solution. The nitrocellulose as received was wet with ethyl alcohol. The alcohol was removed by drying for 24 hours in a warm-air dryer. When dry, the nitrocellulose was placed in air-tight cans to await final formulation.

All nitrocellulose solutions were made up by weight into 0.5-pint standard friction-top cans. The desired amount of nitrocellulose was weighed into a can, and then by means of an analytical pipet the solvent or solvent-diluent mixture was quickly added to give the desired weight proportion. As soon as these operations were completed, a few grains of the spirit-soluble dye, Sudan I, were added to make the cast films more visible for area measurements.

The cans of nitrocellulose solutions prepared in this manner were tumbled for not less than 6 hours on a set of mixing rolls to allow complete dispersion of the nitrocellulose into the solvents. When complete dispersion was assured, the cans were set upright until all air bubbles disappeared. At this point the solutions were ready to be cast into thin films.

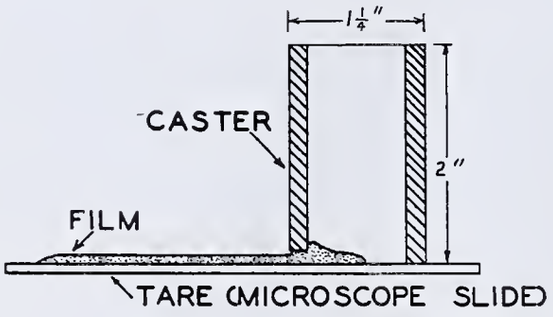


FIGURE 4. FILM CASTER

Standard 50- by 70-mm. glass microscope slides were used as supporting bases or tares on which the films were cast. The film caster was made from a 5-cm. (2-inch) length of 3.1-cm. (1.25-inch) thick-walled brass tubing. One end was carefully machined, and a 2.5-cm. (1-inch) casting channel was milled out to a depth of 0.5 mm. (0.02 inch). A schematic drawing of this caster in operation is shown in Figure 4.

The series of operations performed during a drying run may be outlined as follows:

A glass slide or tare was numbered and cleaned and the tare weight obtained. The tare was placed on a clean piece of paper adjacent to the balance, and the film caster was placed on the tare. By use of a short piece of glass tubing, a few milliliters of the solution were placed on the tare area inside the walls of the caster. The caster was immediately drawn over the surface of the tare, excess solution was thrown on the paper, and the tare was immediately removed from the paper and placed on the balance pan. The break in the film at the edge of the tare was clean and no solution ran back on the underside of the tare.

At the instant the film was prepared and placed on the balance pan, an electric stop clock was started. The weights on the pan of the balance were then rapidly adjusted so that they were approximately 100 mg. short of balance. The constant-temperature room was immediately darkened, the balance case closed, the projection screen swung into place, the aspirator turned on, and the balance room door closed. All the above operations between the film casting and the closing of the door of the balance room were made in less than 30 seconds. The first weight reading was taken at a certain noted time; subsequent readings were taken at equally spaced time intervals.

At the end of the drying run, which was marked by a small weight loss over a comparatively long period of time, the tare with the film on it was removed and placed in an oven at 40° C. for 1 hour. Then the sample was allowed to sit in the balance room overnight; after this interval, the final or "dry" weight was determined. This temperature of 40° C. was arbitrarily chosen; the only restriction was that it had to be low enough to prevent decomposition of the film. This had been determined previously by a series of drying tests on a given sample.

It was found that films subjected to this post-drying treatment always lost weight in the oven, and that a portion was regained upon exposure to the atmosphere. It was concluded from this that not only residual solvent but also some moisture was removed in the oven. Upon exposure to the air, this moisture was apparently regained until a constant or equilibrium weight was obtained. If this period of regain had been eliminated, any ensuing calculations of solvent content would have had a positive error equivalent to this regained moisture. It was decided that the least error would be incurred if the film were allowed to regain its moisture content. This weight after regain will be referred to as "dry weight". The area of the dried film was measured by means of a planimeter.

Except for intervals during which weights were being changed, the balance room was unoccupied. This aided materially in keeping conditions constant during the run and simplified control of the drying runs.

Table of Nomenclature

T = drying time, minutes
W = total corrected weight of tare and film, grams
V = weight of volatiles, grams
F = free volatile content of film, grams per gram of dry film
P = per cent, on wet basis, of volatiles in film
R = drying rate, milligrams of volatiles per square inch of dry film per minute

Treatment of Data

After a run was completed, the following original data were at hand: the tare weight of the glass slide; a series of time, T, and weight, W, readings; the final or "dry" weight of the dried film; and the dry film area. The values of weight listed in the tables are the corrected weights, W, at any time, T. These weights are the total weights of the film and tare. By subtracting the tare weight from the final or dry weight, the weight of the dry film is obtained. The weight of the dry film subtracted from any value of W, at time T, gives the weight of the volatiles, V, present in the film at time T.

The weights of the volatiles, V, at any value of T, divided by the weight of the dry film give the grams of solvent per gram of dry film, F, at time T. F is in reality the per cent volatiles in the film on the dry basis divided by 100. It was also desired to obtain the per cent solvent, P, in the film on the wet basis. Such values of P were calculated in two ways:

$$P = 100 \times \frac{F}{1 + F}$$

or,

$$P = 100 \times \frac{V}{V + \text{weight of dry film}}$$

Which of the above equations to use was determined by the accuracy of the values of F. If F were expressed to three or four figures, the first equation was used. However, at higher values of T, the values of F were rounded off to two figures and hence it was more accurate to use the second equation.

The slope of a W *vs.* T curve at any point T is the drying rate at point T. Therefore, one method of calculating the values of R, the drying rate, would have been to draw a W *vs.* T curve and construct tangents at various points. Results by this method could not have been checked very readily by anyone attempting to work with the data later on, because of the "personal element". Then, too, any values of R obtained by this method would have been affected by the thinness of the line tracing the W *vs.* T curve, the accuracy of the graph paper, and other factors not inherent in the original data.

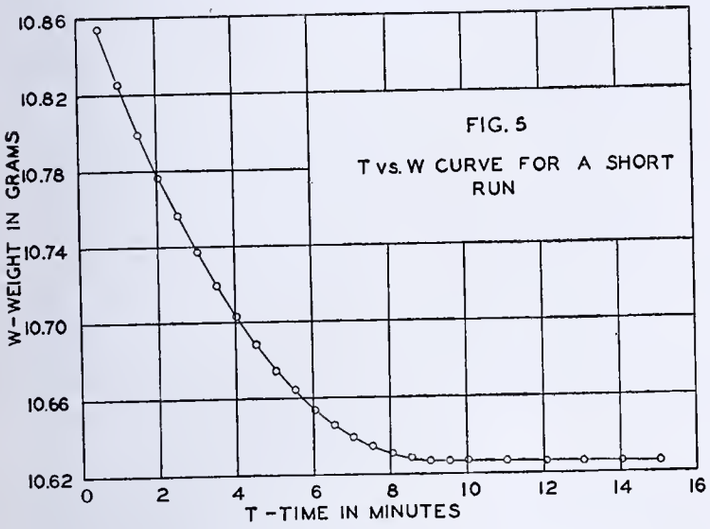
Another method of calculating R values, and the one which was used, was to take T and W readings at regular intervals. If these intervals were short enough so that the portion of the W *vs.* T curve included between them approximated a straight line, the following calculations would be justifiable:

	T *Min.*	W *Grams*	P %	Film Area *Sq. in.*
T_1	1.5	10.8179	97.5	2.17
T_2	2.0	10.8003	96.4	..
T_3	2.5	10.7853	95.7	..

$$R = \frac{10.8179 - 10.7853 \times 1000}{1 \times 2.17} =$$

15.0 mg. per sq. in. per min. at $P = 96.4\%$

Such an illustrated method of calculation is often employed in dealing with drying data. It is, of course, an approximation which assumes that the tangent to the W *vs.* T curve at T_2 is parallel to the chord drawn between T_1 and T_3. Since this method is an approximation, it was necessary to determine the following two points before it could be used: (1) Does the apparatus give T and W readings that in nearly all cases lie on a smooth curve? (2) Are the intervals at which T and W readings may be taken short enough so that the portion of the W *vs.* T curve they represent approximates a straight line?



An inspection of a number of W *vs.* T curves obtained using the apparatus showed these two conditions to be fulfilled. Figure 5 is an example of one of these curves, and represents the data from a run in which the weight and area of the dry film were 15.8 mg. and 13.55 sq. cm. (2.10 square inches), respectively. A consideration of this plot shows that all points lie on a smooth curve, and that approximately straight lines connect these points. Table I presents typical data and calculated results obtained for a drying run on a 5 per cent solution of 20-second nitrocellulose dissolved in ethyl acetate of C. P. grade.

Reproducibility of Results

In his recent book on industrial solvents, Mellan (*7*) lists the following factors as those which influence the rate of evaporation of a solvent from a solution: (1) Temperature of the substance, (2) temperature of the surrounding area, (3) rate of external heat applied, (4) conductivity of heat, (5) specific heat of the substance, (6) latent heat of evaporation, (7) number and type of molecular aggregates, (8) surface tension, (9) presence of dissolved substances, (10) atmospheric pressure, (11) presence of water vapor (humidity), (12) vapor pressure or pressures of the substance or substances under consideration, (13) amount and rate of temperature depression, (14) molecular weight of the substance, (15) method and speed of removal of vapors, (16) depression of vapor pressure, and (17) viscosity.

The factors are, of course, neither equal nor separate in effect, and since the quantitative determination of all is a complex and almost impossible task, it was decided to proceed as follows:

(*a*) Assume some were either constant or negligible; (*b*) maintain others constant; and (*c*) determine the rest quantitatively.

TABLE I. TYPICAL DATA AND CALCULATED RESULTS

(Run 11, 5% solution of 20-second nitrocellulose. Solvent composition, ethyl acetate 100%)

Weight of tare: 10.5473 grams Area of dry film: 3.02 sq. in.
Weight of dry film: 0.0264 gram Room temperature: 23.0° C.

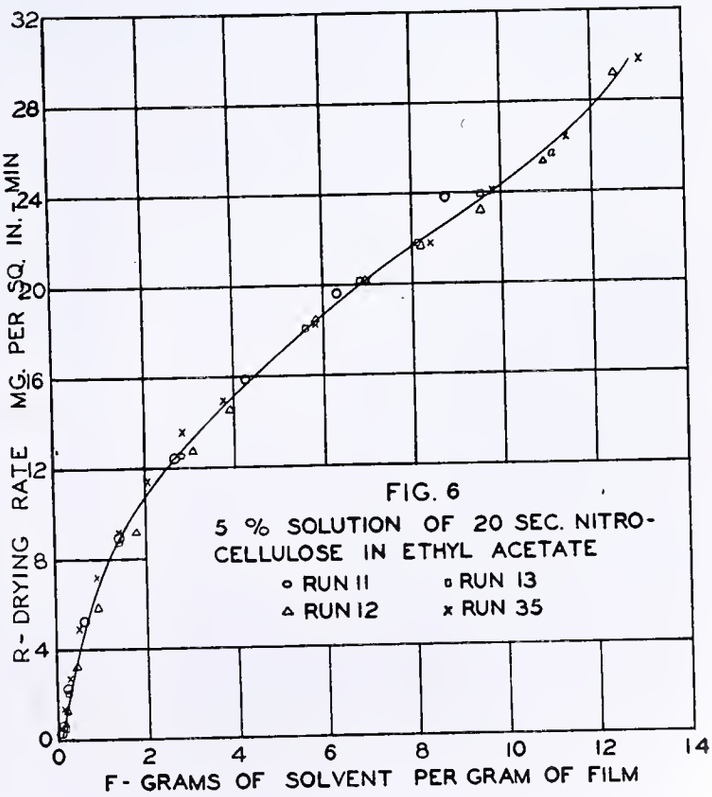
T	W	V	F	P	R[a]
1.0	10.8847	...	..	..	...
2.0	10.8057	0.2320	8.79	89.7	23.80
3.0	10.7412	0.1675	6.35	86.5	19.60
4.0	10.6874	0.1137	4.30	81.0	16.90
5.0	10.6452	0.0715	2.71	73.0	12.42
6.0	10.6124	0.0387	1.47	59.5	8.90
7.0	10.5914	0.0177	0.67	40.2	5.23
8.0	10.5808	0.0071	0.27	21.2	2.22
9.0	10.5780	0.0043	0.16	14.0	0.60
10.0	10.5772	0.0035	0.13	11.7	0.22
11.0	10.5767	0.0030	0.11	10.2	0.08
12.0	10.5767	...	..	..	...
13.0	10.5764	...	..	..	...
14.0	10.5762	...	..	..	...
15.0	10.5762	...	..	..	...
20.0	10.5757	...	..	..	...
30.0	10.5757	...	..	..	...
Dry	10.5737	...	..	..	...

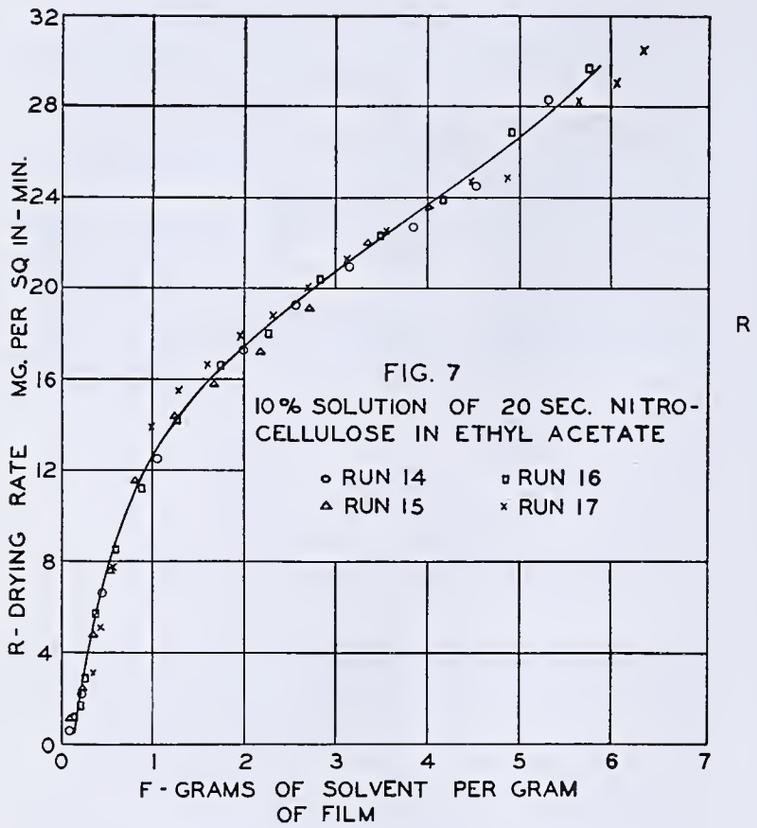
[a] All values of R calculated by 2-minute intervals.

Under (*a*) the following factors can be grouped: (3), (4), (5), (6), (8), (10), (11), (13), (14), and (16). By use of the constant-temperature room and by maintaining the material in the closed cabinet of the balance case, the following may be grouped under (*b*): (1), (2), (12), and (15). By careful formulation of the nitrocellulose solution the following remaining factors fall into group (*c*): (7), (9), and (17).

Of course some of the factors listed in any one group are closely related to factors in the other groups or in the same group; for example, the viscosity (17) of a nitrocellulose solution is a function of the type of nitrocellulose (7) and the amount in solution (9). The vapor pressure of the solution (12) is a function of the temperature of the solution (1), the nature of the solute (7), the amount of solute in solution (9), and the molecular weight of the solute (14).

When one considers all these factors, it is not difficult to understand why such a great deal of effort has been put forth in developing apparatus for the determination of evaporation and drying rates and why results obtained on one apparatus do not agree quantitatively with results obtained on another apparatus. However, it is reasonable to assume that




R - DRYING RATE MG. PER SQ IN - MIN.
F - GRAMS OF SOLVENT PER GRAM OF FILM
R
FIG. 7
10% SOLUTION OF 20 SEC. NITRO-CELLULOSE IN ETHYL ACETATE
o RUN 14
▫ RUN 16
△ RUN 15
× RUN 17

results obtained on any one apparatus would have the same interrelation that they would have had had they been determined on another apparatus. This assumption seems to have been borne out to some extent in the data in the literature; hence, the results presented here cannot be compared quantitatively to those obtained by other workers. This means that a drying rate of 15.0 mg. per square inch per minute for a certain solution at a certain per cent solvent concentration could not be reproduced on another apparatus unless all the prevailing conditions and the technique employed were exactly reproduced. However, if two different drying rates determined on the apparatus herein employed were found to have a ratio of five to one, then on another apparatus where similar conditions were only partially maintained, these two rates should still be in the same, or very nearly the same, ratio. In other words, the quantitative results obtained should not be considered absolute.

To determine the reproducibility of the data obtained on the apparatus employed, it was necessary to see if checks could be obtained on any one particular nitrocellulose solution. Therefore, a 5 per cent solution of 20-second nitrocellulose in ethyl acetate was made and triplicate runs were performed using this solution (runs 11, 12, and 13). The *F vs. R* curves for each of these runs are presented in Figure 6, which includes run 35 as a further check. This latter run represents another separately formulated batch of the composition used for runs 11, 12, and 13.

As a further check, three more runs were made on a 10 per cent solution of the same nitrocellulose in the same solvent (runs 14, 15, and 16). *F vs. R* curves for these runs are shown in Figure 7. In all cases the results of the check runs were in good agreement.

In some cases of drying, where diffusion of the volatile component through the drying film controls the rate of evaporation, the thickness of the material affects the rate of drying. The maximum thickness that could be laid down by the caster was 0.5 mm. (0.02 inch), which was the depth of the channel. However, it is generally accepted by users of these film casters that the thickness cast is one-half the channel depth or less. To check the effect of thickness a film was made by literally "piling-on" the same solution as was used for runs 14, 15, and 16. This film was dried and the drying data were plotted as run 17, Figure 7.

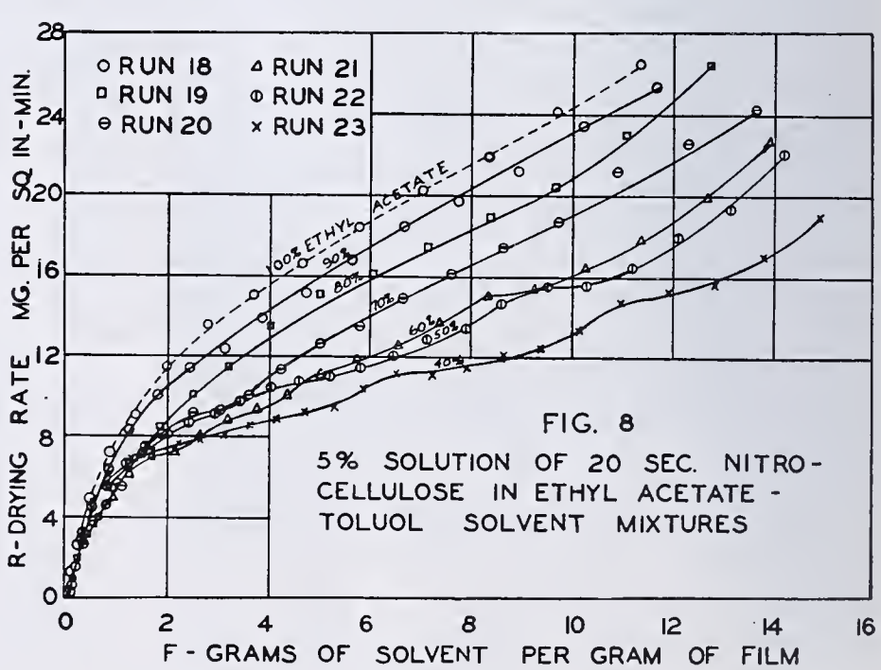
From the area and dry film weight, this film was calculated to be 6.04 times as thick as the film in run 15. This is a variation greater than the caster generally would give. When the values of F and R for run 17 are compared in Figure 7 with runs 14, 15, and 16, it is seen that the points check. These results show that variations in film thicknesses do not appreciably affect the drying rates under the conditions employed.

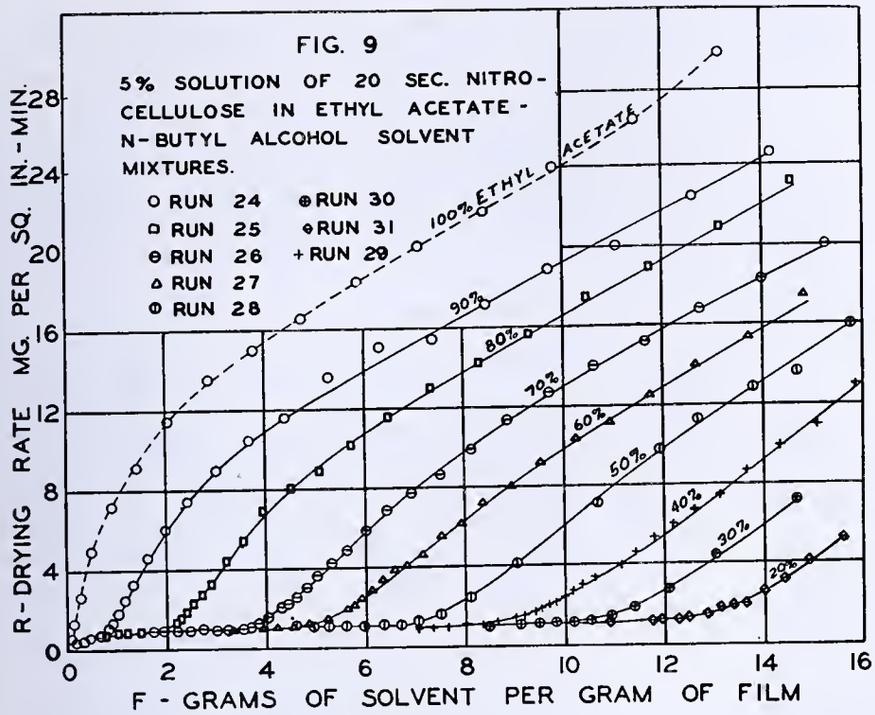
An inspection of the data (Table II) for these runs shows, however, that variations in film thicknesses have a considerable effect upon the total drying time. Run 16 lasted 12 minutes, while run 17 lasted 82 minutes. A comparison of these two figures illustrates one of the advantages of using thin films for studying drying rates. Nearly seven times as many runs like run 16 could have been made in the time necessary for run 17.

Experimental Results

Table II gives a summary of a number of drying rate determinations made using the apparatus and technique just discussed. These runs are graphically presented in Figures 8 to 12.

A comparison of runs 11, 12, and 13 with runs 14, 15, and 16 indicates that the more concentrated nitrocellulose solutions had the higher drying rates for any given value of F. This behavior indicates that the drying rate of a nitrocellulose solution is a function not only of the solvent content of the film after casting, but also of the original solution composition. Such a conclusion is logical, for otherwise, if the drying rate of a solution were a function only of the film's





solvent content after casting, then regardless of the original composition all solutions would behave alike. In other words, a 5 per cent solution film would dry until the composition became 10 per cent in nitrocellulose, then the film would assume the characteristics of a 10 per cent solution and dry accordingly. Such a behavior is shown not to exist.

Runs 18 through 23 were made on a 5 per cent solution of 20-second nitrocellulose in solvent mixtures of ethyl acetate and toluene. These runs were made to study the effect upon the drying rate of increasingly greater amounts of nonsolvent or diluent (toluene) in the presence of a solvent (ethyl acetate), and to show the effect of varying two volatiles that had different evaporation rates as determined on the pure solvents. According to Bogin (*2*), ethyl acetate has a rate of 525 compared to *n*-butyl alcohol as a standard of 100, while toluene on the same basis has a rate of 195.

The *F vs. R* curves for each of these runs were plotted and are shown in Figure 8. It is seen that as the composition of the slower evaporating liquid was increased, the rates of drying decreased. The curve for 100 per cent ethyl acetate (run 35) is also shown in Figure 8 for comparison.

The most important feature of this family of curves is the irregularity shown by the lower members. This irregularity is attributed to the fact that these samples blushed to a marked degree. Both the amount of blushing and the irregularity of the curves increased with increasing toluene content. The irregularity of the curves for the blushed samples indicates that when blushing occurs, drying equilibrium is upset in some manner. One explanation would be that the resistance to drying is decreased by the precipitation which causes the blushing. If such were the case, the drying rate would increase momentarily, and the rate would begin falling again until another area blushed and again the rate would increase momentarily. However, as the rate increased owing to blushing, it would at the same time tend to decrease owing to the drop in the solvent content (a decrease in *F*). The combination of these effects would tend to flatten out the drying rate curves at the points of blushing. Examination of the curves (Figure 8) shows no flattening; so this explanation is apparently not valid.

The irregularity in the drying curves of the blushed samples might, however, be explained from another viewpoint which is more acceptable. It may be reasoned that as soon as the film blushed, the blushed area became momentarily useless as an area from which drying could occur. If this were true, the area immediately available for drying would decrease as blushing continued. However, in the calculations of the rate values, the area was taken as the entire area of the dried film, blushed or clear. This use of an area larger than was really available for drying would tend to give rates too low. This means that the *R vs. F* curves would drop momentarily at the points of blushing. By inspection of the curves of Figure 8 this is seen to be the case.

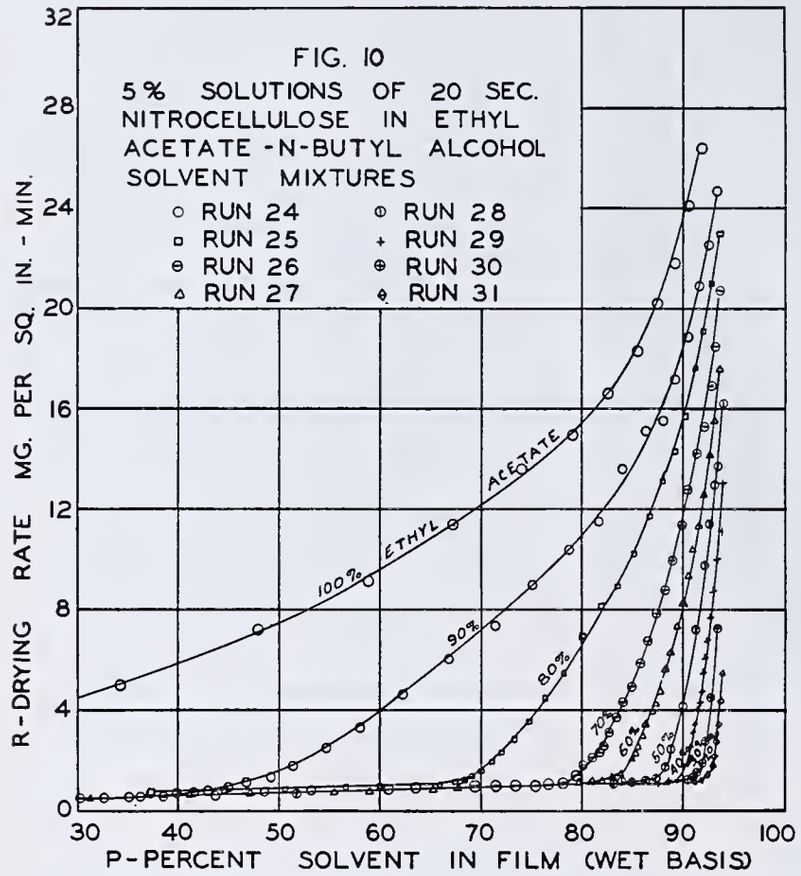
Runs 24 through 31 were made on a 5 per cent solution of 20-second nitrocellulose in solvent mixtures of ethyl acetate and *n*-butyl alcohol. The *R vs. F* curves for this series are shown in Figure 9. The drying rate curve for run 35 is included for comparison.

These runs were made to show the effect of increasingly greater amounts of a very slow-evaporating cosolvent in the presence of a very fast-evaporating solvent. The evaporation rate for *n*-butyl alcohol (*2*), on the basis of *n*-butyl acetate = 100, is 45, as compared to 525 for ethyl acetate. The solvent mixtures for this series consist of two solvents as contrasted to mixtures of a solvent and a nonsolvent (diluent) used in the ethyl acetate–toluene series just discussed.

By inspection of Figure 9 it is seen that as the butyl alcohol content increased, the drying rates decreased. It is interesting to note that all the curves seem to approach a

Table II. Summary of Drying Runs

Run No.	Nitrocellulose Viscosity	Solution Concentration	Solvent Composition			Wt. of Dry Film	Area of Dry Film	Length of Run
			Ethyl Acetate	Toluene	Butyl Alcohol			
	Sec.	*Wt. %*	*% by Volume*			*Gram*	*Sq. in.*	*Min.*
11	20	5	100	..	..	0.0264	3.02	11
12	20	5	100	..	..	0.0242	2.85	14
13	20	5	100	..	..	0.0232	2.87	14
14	20	10	100	..	..	0.0420	2.56	17
15	20	10	100	..	..	0.0446	2.68	15
16	20	10	100	..	..	0.0457	2.75	12
17	20	10	100	..	..	0.1004	1.00	82
18	20	5	90	10	..	0.0231	2.67	15
19	20	5	80	20	..	0.0158	2.10	15
20	20	5	70	30	..	0.0212	2.58	15
21	20	5	60	40	..	0.0169	2.16	16
22	20	5	50	50	..	0.0199	2.15	19
23	20	5	40	60	..	0.0187	2.27	17
24	20	5	90	..	10	0.0164	2.26	28
25	20	5	80	..	20	0.0157	2.16	40
26	20	5	70	..	30	0.0179	2.47	60
27	20	5	60	..	40	0.0140	1.92	60
28	20	5	50	..	50	0.0096	1.47	85
29	20	5	40	..	60	0.0139	1.91	100
30	20	5	30	..	70	0.0136	1.98	114
31	20	5	20	..	80	0.0164	2.29	130
32	0.25	5	100	..	..	0.0165	3.16	11
33	0.5	5	100	..	..	0.0173	2.71	13
34	4	5	100	..	..	0.0158	2.13	13
35	20	5	100	..	..	0.0138	1.74	11
36	70	5	100	..	..	0.0171	1.99	11



limiting value of R at about 0.8 mg. per square inch per minute. This leveling off of the drying curves in each case seems to indicate that there was a retention of solvent and that probably the solvent retained was the more slowly evaporating one—i. e., butyl alcohol. The upper portion of the curves, which are all similar, probably is the portion where the composition of the volatiles leaving the films is high in ethyl acetate.

Inspection of the R *vs.* F plots in Figure 9 might be misleading—for instance, in the case of run 24, it might be assumed that at a value of $R = 1$, the film was for all practical purposes dry. However, the same run plotted as P *vs* R shows that at a value of $R = 1$ the film contained very nearly 50 per cent solvent. Figure 10 is a plot of runs 24 through 31 on the basis of P. This gives a more comprehensive picture of the change in solvent content as the films dry.

An inspection of Figure 10 brought forth the interesting possibility that, since the curves were all similar excluding the lower portions, they might all be plotted as straight lines by the selection of proper coordinates. Figure 11 shows the result of such a construction and indicates well that the curves of Figure 10 were similar above the flattened regions.

Mathematically, the curves of Figure 11 state that:

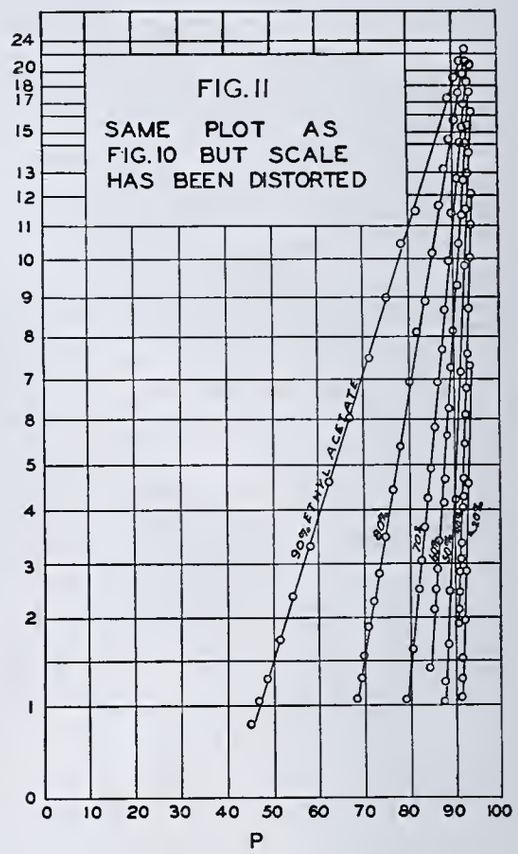
$$f(R) = a + bP$$

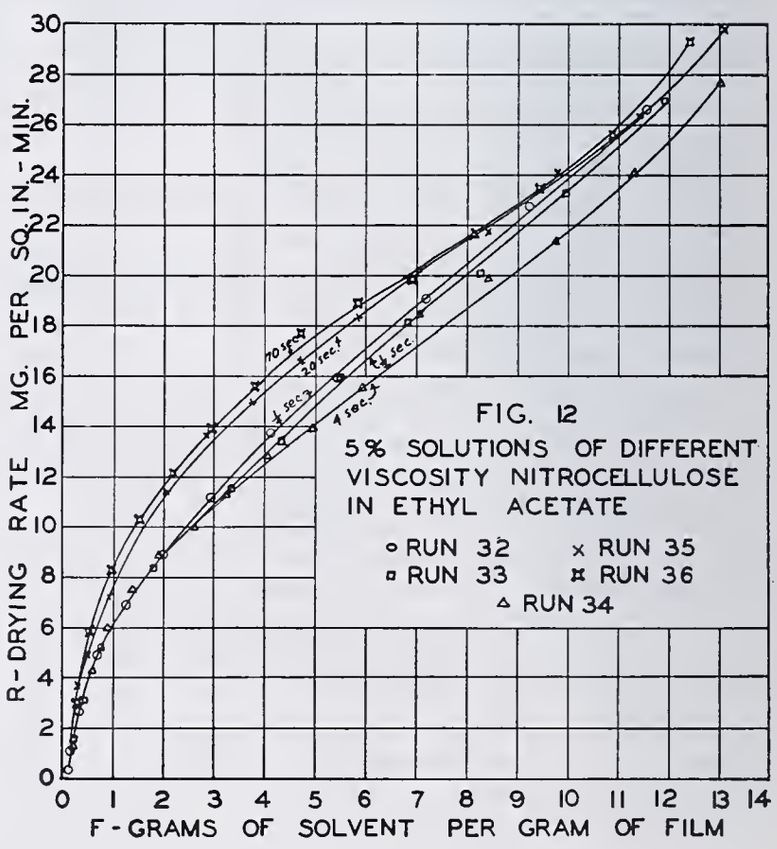
where a and b are constants different for each line. This means that some function of the drying rate, R, is a straight-line function of P, the per cent concentration of solvent in the film. This series of curves also shows that:

$$f(R) = a_1 + b_1P,\ f(R) = a_2 + b_2P, \text{ etc.}$$

This indicates that the mechanism of drying for each run is probably the same. Another important feature of this method of plotting is the fact that it affords an easy system for interpolation of intermediate data when two points have been determined.

Runs 32 through 36 were made on 5 per cent solutions of various viscosity nitrocelluloses in 100 per cent ethyl acetate, in order to show the effect of the viscosity of the nitrocellulose used on the rate of drying. The F *vs.* R plots are shown in Figure 12.





The curves for 0.25-, 0.5-, and 4-second nitrocelluloses lie close together, with the rates decreasing as the viscosity increases. However, the curves for 20- and 70-second nitrocellulose do not follow this order but appear above the aforementioned three curves. It is likely that the difference between the curves in Figure 12 is due not only to the differences in viscosities of the solutions, but also to the difference in nature of the nitrocelluloses used to get the different viscosities. To obtain a lower viscosity nitrocellulose, it is necessary to degrade the material to a lower molecular weight. In the case of solutions of the lower viscosity nitrocelluloses, the degradation has been carried out to a greater extent, and it is possible that the individual micelles of nitrocellulose begin to approach true molecular dimensions—i. e., they lose their colloidal nature to some extent. Therefore, in a given weight of a lower viscosity nitrocellulose, there would be more particles than in a given weight of a higher viscosity nitrocellulose. This would mean that the lower viscosity nitrocelluloses would tend to lower the vapor pressure of a solution more than the higher viscosity nitrocelluloses; hence, the rates of drying of the lower viscosity nitrocelluloses would be lower.

Conclusions

1. The apparatus that has been described can very easily be assembled from standard laboratory equipment.
2. The apparatus is particularly adaptable to the investigation of very rapidly drying solutions, and affords a means of studying their behavior when cast into thin films. The operation is simple, direct, and rapid, and the quantities involved are easily handled.
3. In instances where more slowly drying films are studied, the apparatus and technique described afford a means of making numerous drying runs in the time it has formerly taken to make one.
4. The apparatus has, by its proved operation in numerous drying rate determinations, demonstrated the ability to give satisfactorily reproducible results.
5. The apparatus and technique are applicable not only to the study of the drying rates of nitrocellulose solutions, but also to the study of the drying rates of any type of thin film, whether the drying process be purely evaporative, or a combination of evaporation and chemical reaction.
6. The drying rate of a nitrocellulose solution is a function of the type (viscosity) of nitrocellulose in solution.

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Presented by George Rieger as a partial fulfillment of the requirements for the M.S. degree in chemical engineering at the University of Minnesota, 1941.

Construction and Operation of a Polarograph

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This paper presents the experience of the authors with a polarograph of conventional design constructed locally from materials normally available in this country. The details of the construction of the instrument and of a modified electrode assembly are given briefly with illustrative material. Some notes on operation are included, together with typical records taken with the instrument.

THE Nejedly model of the Heyrovský-Shitaka polarograph (*1*) has certain advantages in spite of the inconvenience of taking records that have to be developed photographically. Among these are the ability to duplicate the type of record most prevalent in polarographic literature, ability to check and test the various sources of error in the measurements, flexibility of operation, ability to take records with increase or decrease of applied voltage, etc. The instrument also lends itself, in the form here described, to the plotting of curves manually, either roughly during the continual variation of applied voltage, or more accurately by intermittent operation.

At the time when the authors' project was initiated it was not possible to secure an instrument of the standard type with twenty turns of the bridge wire. Owing to the increasing interest in this field of research it was thought that others might profit from the experience here described.

A number of preliminary sketches were prepared and a general statement descriptive of the features desired was drawn up. The determining factors in the design are the amount of resistance desired in the potentiometer wire and the size of the photographic paper. The specifications were for a resistance higher than average—namely, about 30 ohms—and for a paper of rather large size, the final choice being 6 × 9 inches. On the basis of sketches with no exact dimensions, and after a brief inspection of an instrument of the Nejedly type through the courtesy of O. H. Müller at the Cornell University Medical School, New York, W. K. Grove (machinist, Frick Chemical Laboratory) was able to construct, from materials then readily obtainable, an instrument that has given excellent performance over a period of a year. Valuable ideas regarding materials and design were given by H. Boyd (assistant curator, Frick Chemical Laboratory), and numerous original improvements in details of design were made by Mr. Grove as the work progressed.

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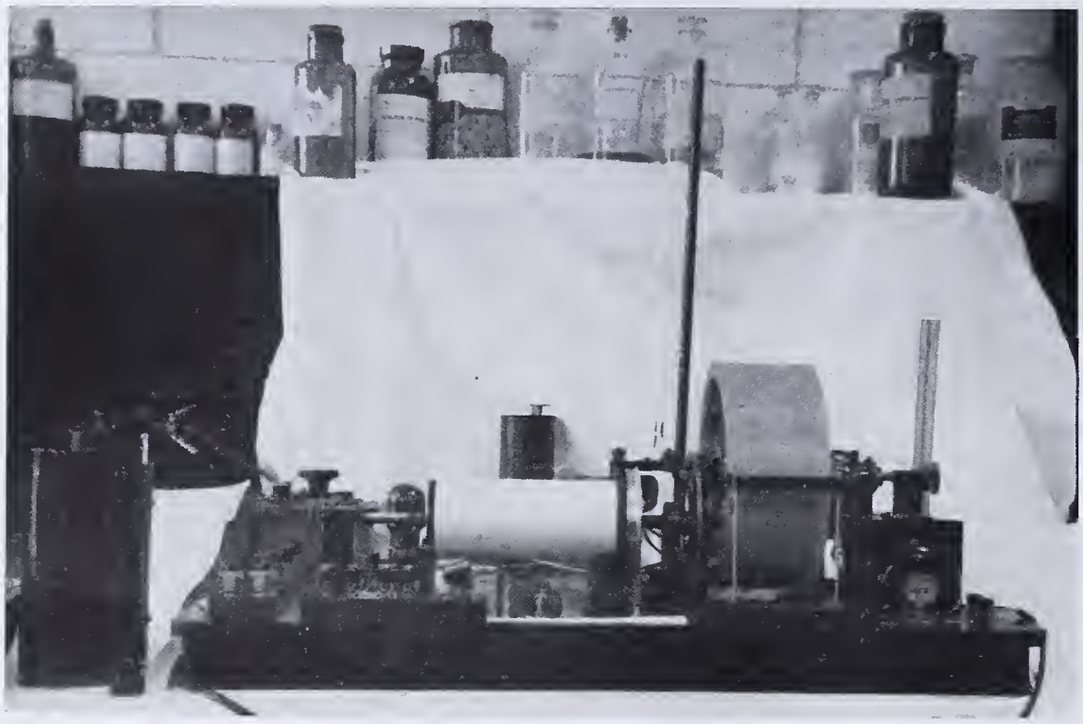

FIGURE 1. GENERAL APPEARANCE OF INSTRUMENT

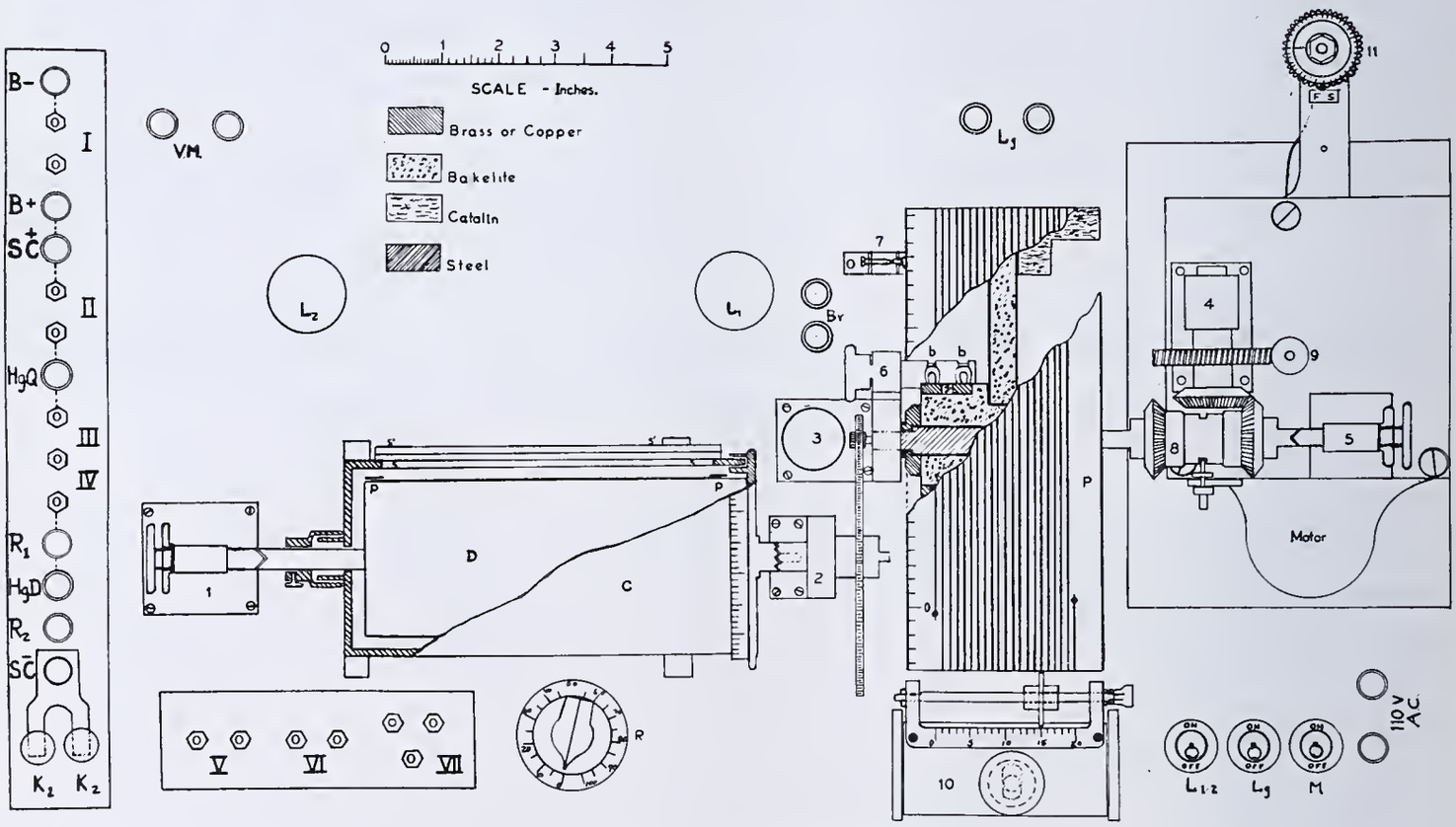

FIGURE 2. PLAN OF POLAROGRAPH

Electrical connections:
- *B*−, *B*+. To 4- or 6-volt cell
- *SC*+, *SC*−. Standard cell
- *HgQ*, *HgD*. To quiet and dropping mercury electrodes
- R_1, R_2. To shunt and galvanometer
- K_1, K_2. Tapping keys
- *R*. A 100-ohm rheostat
- I. Plug connection to close battery circuit
- II. Connects quiet mercury electrode to battery
- III. For anode potential measurement
- IV. For setting potential against standard cell
- V, VI. To short 16-ohm resistances ahead of or after potentiometer wire
- VII. Reversing device to make dropping electrode either anode or cathode
- *Br*. Binding posts to brushes
- L_1, L_2. Ordinate lamps
- L_g. Posts to galvanometer lamp
- *V. M.* Binding posts to voltmeter
- *M*. Switch for motor
- Lamp switches are near motor switch, lower right.

Mechanical features:
- 1, 2, 3, 4, 5. Bearings for camera drum, potentiometer wheel, and driving gears
- 6. Holder of Catalin for brushes *b*, *b*
- 7. Contact device for ordinate lamps, L_1, L_2
- 8. Reversing gears
- 9. Worm drive
- 10. Support for potentiometer contact wheel
- 11. Speed regulator for motor
- *C*. Camera
- *D*. Drum for photographic paper
- *P*. Potentiometer drum
- *b*, *b*. Brushes, multiple bearing surfaces
- *p*, *p*. Pins to hold thin metal strip that fastens bromide paper
- *s*, *s*. Slit of camera
- *s'*, *s'*. Adjustable jaws of slit

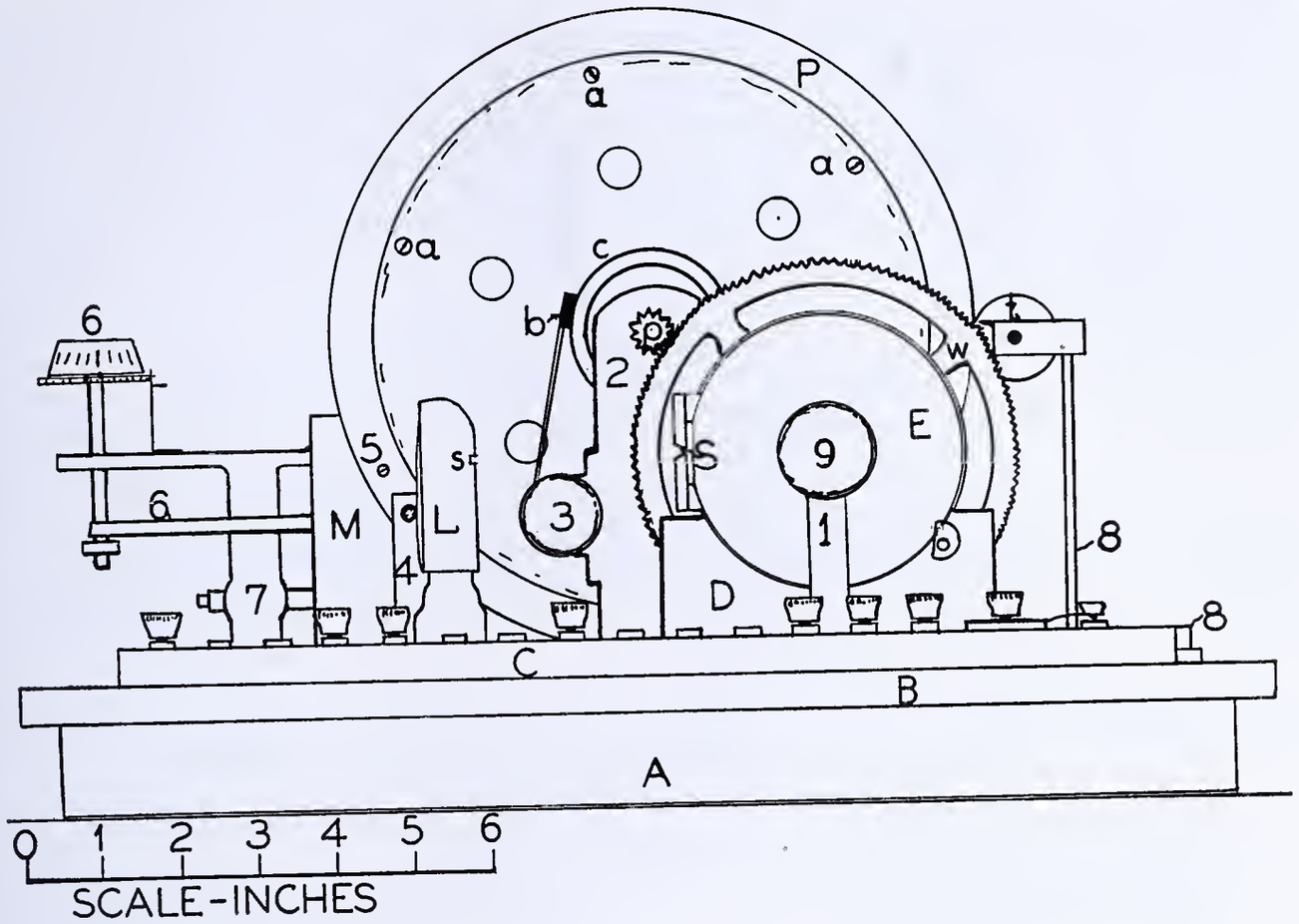

FIGURE 3. ELEVATION OF INSTRUMENT

A. Base formed of Bakelite strips at end and of iron pieces at sides
B. Bakelite floor
C. Hard-rubber strip with binding posts, etc.
D. Bakelite camera support
E. Camera
L. Ordinate lamp and housing
M. Motor housing
P. Potentiometer drum
S. Camera slit
a, a, a. Screws to hold Catalin drum fast to Bakelite disk
b. Brush with multiple contact
c. Copper commutator ring
s. Slit in housing of ordintae lamp
t. Contact wheel to potentiometer wire
w. Spur gear to drive camera drum
1. Camera bearing
2. Potentiometer bearing
3. Brush holder
4. Contact for ordinate lamps
5. Screw that operates 4
6, 6. Speed regulator
7. Bearing of motor
8, 8. Base of support for contact wheel

Plan and Materials

The general appearance of the instrument is indicated in Figure 1. The finish of the instrument is, in the main, black. The drum, brush holder, and other Catalin parts are red. The brass gears are unpainted, but the remaining exposed metal is painted. The camera is at left; paper on drum as for manual recording.

The plan of the instrument, drawn to scale, is shown in Figure 2. The more or less standardized relative arrangement of parts has been followed, but the choice of a high resistance and a large record has made the instrument rather larger than others of similar type (26.75 × 16 inches maximum height 10.25 inches).

The parts, other than small items of hardware and odds and ends of rod of brass and steel that are apt to be found in a well-equipped machine shop, were as follows:

Bearings, thrust type, to be used at points 2 and 3, Figure 2.

Gears, miter [3 for reversing gear (8, Figure 2) Boston, Mass., Gear Works].

Gear, brass spur wheel, 48 pitch, 0.125-inch face, 5 inches, with 240 teeth to turn camera drum (Philadelphia Gear Works).

Lamps for ordinates, 7-watt, with Bakelite housings (F. W. Woolworth & Co.).

Pinion rod, 12 teeth, for potentiometer axle (near 3, Figure 2, Philadelphia Gear Works).

Motor, Majestic, phonograph type, 20 watts (0.2 ampere at 100 to 110 volts) with speed regulator.

The following materials were especially purchased and needed to be cut, machined, or mounted:

Bakelite for base and substage motor mount (16 × 36 inches, 0.5 inch thick).

Bakelite rod, 2 inches round, 3 inches long, for potentiometer axle.

Brass rod, 4 inches round, 0.75 inch long, to form end of camera drum with scale.

Brass tube, 3.5 inches round, 0.125-inch wall, 7.25 inches long, for camera.

Brass tube, 2.75 inches round, 3 inch wall, 7 inches long, for camera drum.

Catalin cylinder 4 inches long, 1.25-inch wall, 8.25-inch diameter, for potentiometer.

Catalin rod, 2 inches round, 0.375 inch long, for commutator spacer.

Catalin rod, 0.563-inch diameter, 1.375 inches long, for brush holder.

Copper tubing, 2 inch diamter, 1.88-inch wall, for commutator rings.

Manganin wire, No. 24 B. & S. gage, 45 feet, for potentiometer (33 ohms).

Hard rubber for binding posts, plug sockets, and tapping key mounting, 2 × 24 × 0.5 inch.

Steel drill rod, 0.5 inch, 20 inches long for axles.

The elevation of the instrument from the camera end is shown in Figure 3.

THE CAMERA. The camera and drum and a light trap attached to the axle at the left of the camera (Figure 2) are de-

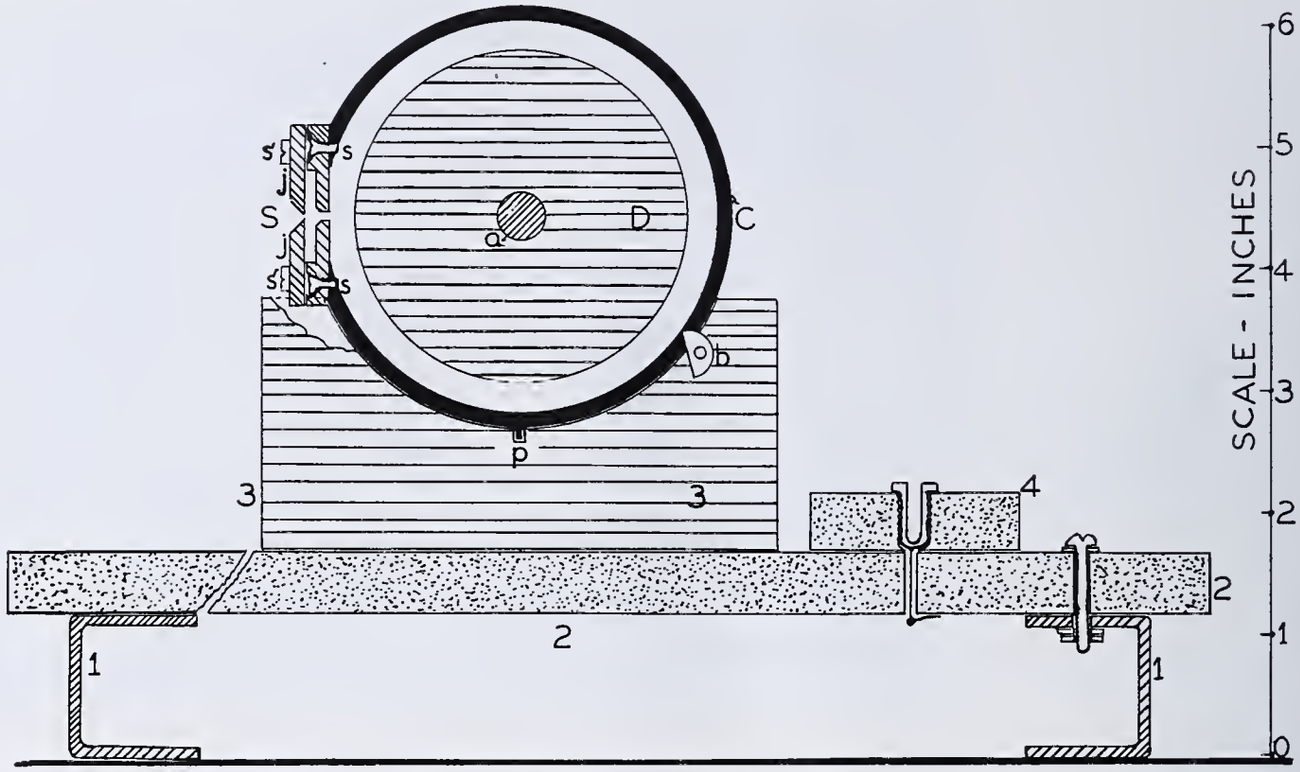

FIGURE 4. CAMERA AND CONSTRUCTIONAL FEATURES

1, 1. Metal support for sides of instrument. At opposite end of base a substage mounting for motor is made by fitting a piece of 0.5-inch Bakelite inside upon lower parts of metal
2, 2. Bakelite base, 0.5 inch
3. One of two Bakelite supports for camera
4. Hard-rubber support for electrical connections
C. Camera
D. Camera drum
S. Slit
a. Axle
b. Button to hold camera in place
j, j. Adjustable slit jaws
p. Pin to locate camera properly
s, s. Two of eight screws to hold slit to camera
s', s'. Two of four screws for adjusting jaws of slit

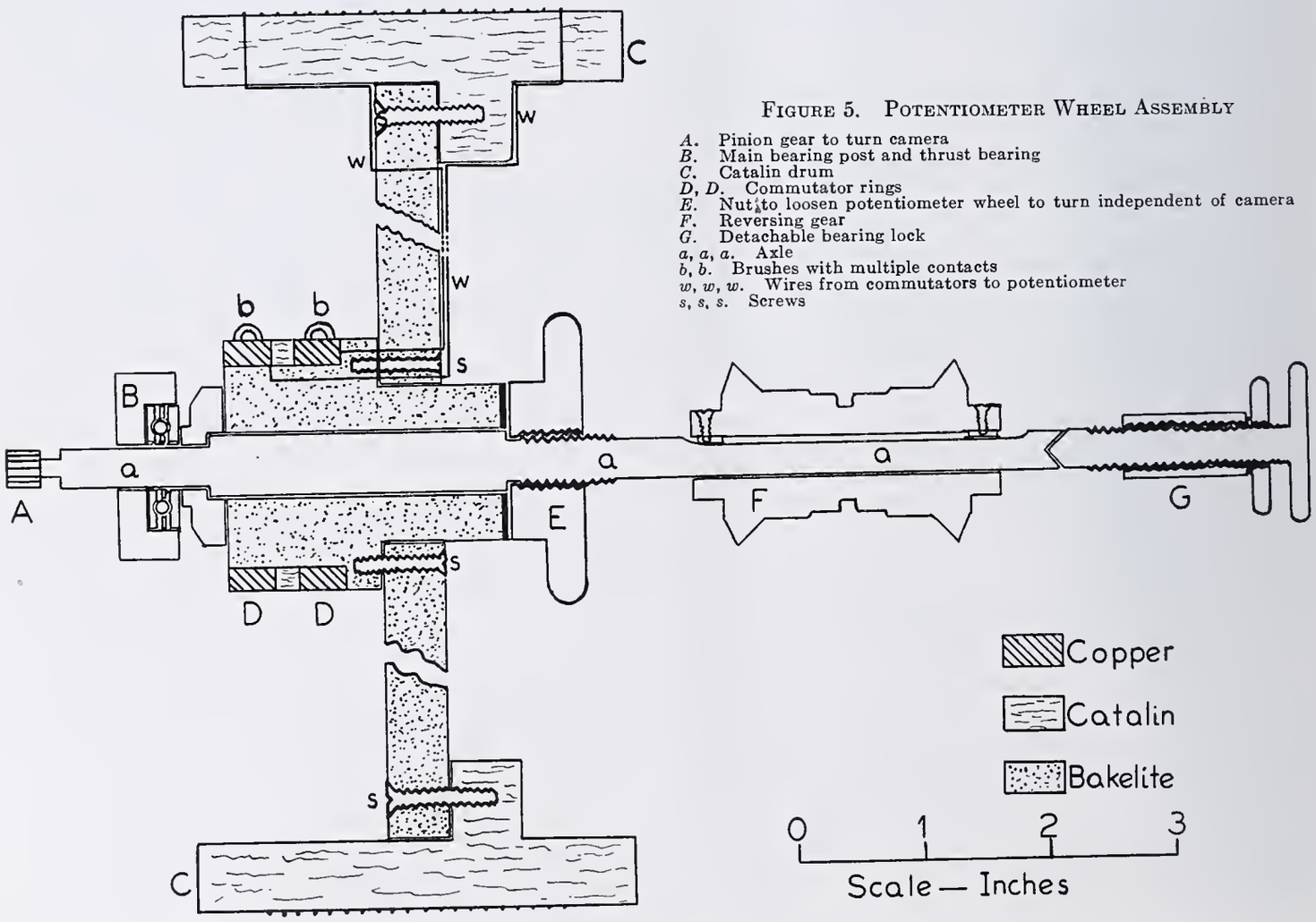

FIGURE 5. POTENTIOMETER WHEEL ASSEMBLY

A. Pinion gear to turn camera
B. Main bearing post and thrust bearing
C. Catalin drum
D, D. Commutator rings
E. Nut to loosen potentiometer wheel to turn independent of camera
F. Reversing gear
G. Detachable bearing lock
a, a, a. Axle
b, b. Brushes with multiple contacts
w, w, w. Wires from commutators to potentiometer
s, s, s. Screws

tachable as a unit by unscrewing the end bearing (1, Figure 2) and loosening button *b*, Figure 4. The details of the camera slit and several other constructional features are shown in Figure 2. The metal parts are painted black to avoid reflections, and since the metal strip that closes the slit occasionally dislodges paint or dust, a thin piece of paper is drawn along the slit to clear it as soon as it is opened.

The width of the thin metal strip that holds the bromide paper in place by fastening it against pins *p,p* (Figure 2) represents loss of available space for record. This is the chief defect in design thus far noted and is to be remedied by cutting a slot in the drum for one end of the paper. The other end may then be held by a much thinner strip or a wire.

The authors prefer a contrast paper, although normal paper gives good records. [P. M. C. No. 2 contrast paper, Eastman Kodak Co. The normal paper has the same letter and number designation (P. M. C. No. 2 normal).]

MANUAL RECORDING. With the camera housing removed and a strip of 6-inch paper on the drum it is very easy to take a rough preliminary record with the drum moving by putting dots on the image of the galvanometer filament at equal voltage intervals. When several curves are plotted in the same region pencils with different colored leads are used. More accurate records may be taken by stopping the drum to record points.

POTENTIOMETER DRUM. Details of the construction and assembly will be evident from Figures 2 and 5.

The wire is mounted in a slight groove machined on at 0.125-inch pitch per turn. A scale of 100 equal divisions is marked on the drum, and a pointer attached to the contact wheel support makes it possible to read the applied voltage accurately. The divisions are approximately 0.25 inch apart, so that interpolation is very reliable. The uniformity of the wire was tested relative to the whole wire taken as unity by reading the voltage at turn 20, then at turn 1, then at turn 20, etc.

The errors for the various fractions of the wire were, in per cent of the total length:

Fraction	0.05	0.10	0.15	0.20	0.25
Error	+0.05	−0.03	+0.04	−0.06	−0.08
Fraction	0.55	0.60	0.65	0.70	0.75
Error	−0.10	−0.07	−0.12	−0.06	−0.08
Fraction	0.30	0.35	0.40	0.45	0.50
Error	−0.07	−0.07	−0.11	−0.10	−0.07
Fraction	0.80	0.85	0.90	0.95	1.00
Error	−0.07	−0.01	−0.03	−0.07	Std.

The first interval includes extra resistance, because the contact with the copper lead is made below the face of the drum. In fact, this resistance is of the order of about 0.08 per cent of the total resistance. Aside from this slight error, which can easily be corrected for, the wire is uniform; this is

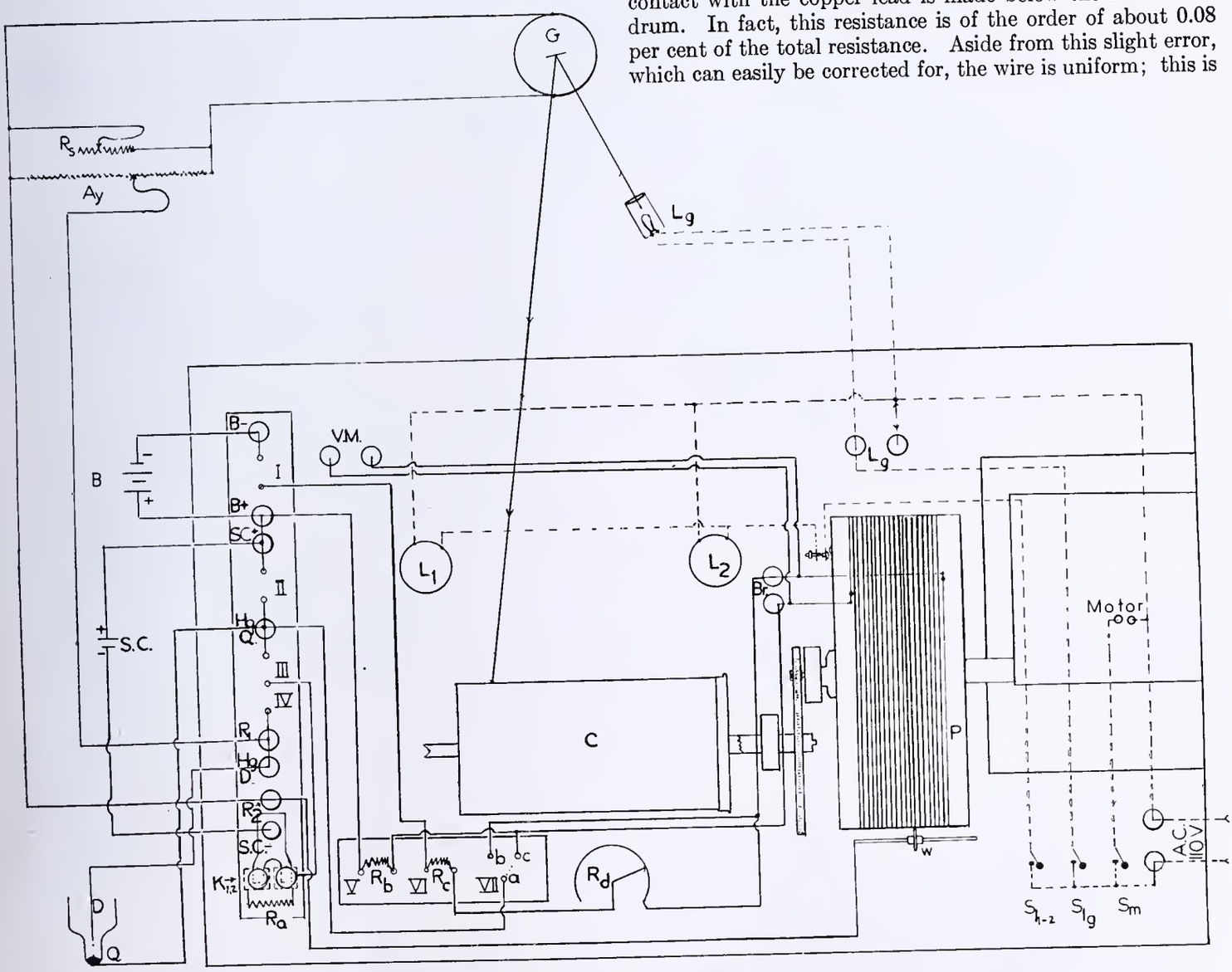

FIGURE 6. WIRING DIAGRAM

Binding post connections and plug connections I to VII are as listed for Figure 2.

G. Galvanometer
Ay. Ayrton shunt, 10,000 ohms total resistance
Rs. Shunt resistance (4-dial 10,000-ohm box used for convenience)
B. Battery
S. C. Standard cell
D. Dropping electrode
Q. Quiet electrode
Ra. 10,000-ohm protective resistance
Rb. 16 ohms
Rc. 16 ohms
VII, *a—c.* Connection for dropping cathode; *a—b* for dropping anode
Rd. 100-ohm rheostat

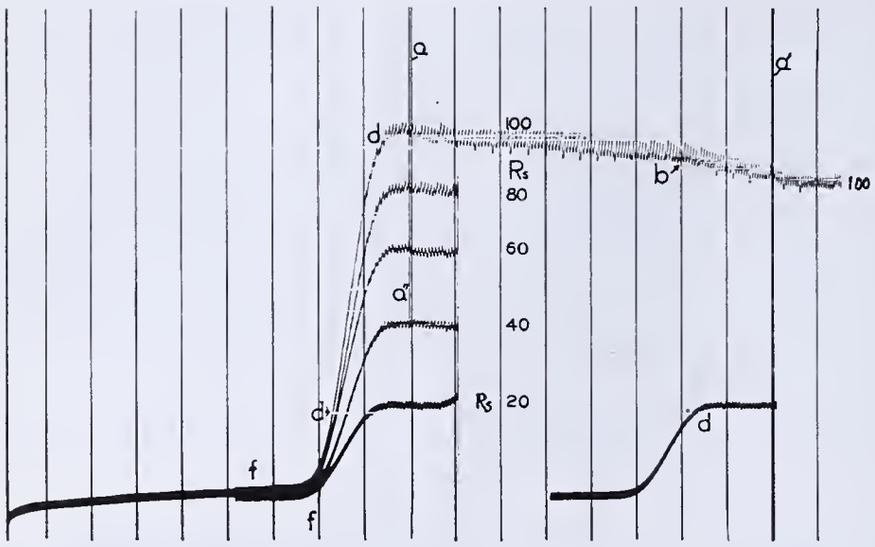

FIGURE 7. RECORD TAKEN WITH PORTABLE 2420-D GALVANOMETER

0.001 *M* cadmium chloride in 0.1 *M* KCl solution, at various sensitivities
f. Fogging due to imperfect lens action of lamp bulb
a, *a'*. Ordinates put on with decreasing e. m. f. *a''* ordinate next to *a* is masked off at bottom
b. Undesirably wide galvanometer record at increased sensitivity
d, *d*, *d*. Streaks due to dirt on slit
This was the first polarogram one observer took with the instrument, and it was taken before some of the essential points of manipulation had been found for this instrument

the reason that the errors are prevailingly negative for the other intervals.

Constant care is necessary to maintain good contacts, and the authors adopted, after some experience, the routine of a daily cleaning of potentiometer wire and contact wheel and commutator surfaces by allowing them to turn against a clean cloth moistened with carbon tetrachloride, followed by light greasing with vaseline.

Contact troubles are minimized by turning the potentiometer drum by motor rather than by loosening the clutch nut (*E*, Figure 5) in order to prepare for a succession of records. It is much simpler to move the contact wheel that bears against the potentiometer wire by loosening the support (10, Figure 2; 8, Figure 3).

ORDINATE LAMPS. Two 7-watt lamps, operating on the 110-volt alternating current line, were installed.

Each has a Bakelite housing and a switch at the base. A fine slit was cut in each Bakelite housing at the proper height (*s*, Figure 3). A small piece of aluminum was added as a reflector for each lamp. It was found that one lamp was adequate and that if the one near the drum (L_1, Figure 2) was used the ordinates were relatively fainter at the opposite end where critical parts of the records are apt to be located (Figures 9 and 10). There is a slight play in the gear mechanism, so that ordinates are flashed on in the direction in which records are taken, and in general only once for any part of the paper. In putting on a series of exposures, the critical ordinates are flashed on when the particular curve to which they apply is being taken. If ordinates are put on in both directions on a single sheet of paper, part of one series is masked off by covering a part of the slit (Figure 7, ordinate *a''*).

ELECTRICAL CIRCUITS, GALVANOMETER, SHUNT, AND GALVANOMETER LAMP. The general wiring diagram is shown in Figure 6. The alternating current circuits are shown in dotted lines.

Galvanometer. Preliminary Experiments. At low sensitivities a portable box type of galvanometer (Leeds & Northrup 2420-d of rated current sensitivity 5×10^{-8} ampere per mm. on glass scale 18 cm. from mirror, 310 ohms coil resistance, external critical damping resistance 2000 ohms, and a 3-second period) gave satisfactory records. The lamp was remounted with provision for adjusting the focus and the ground-glass piece with scale was removed. The sensitivity was doubled by placing the mirror 36 cm. from the paper on the drum of the polarograph. No lens was used, since the bulb of the lamp serves the purpose, although rather imperfectly, so that there is fogging at the side of the galvanometer trace (Figure 7).

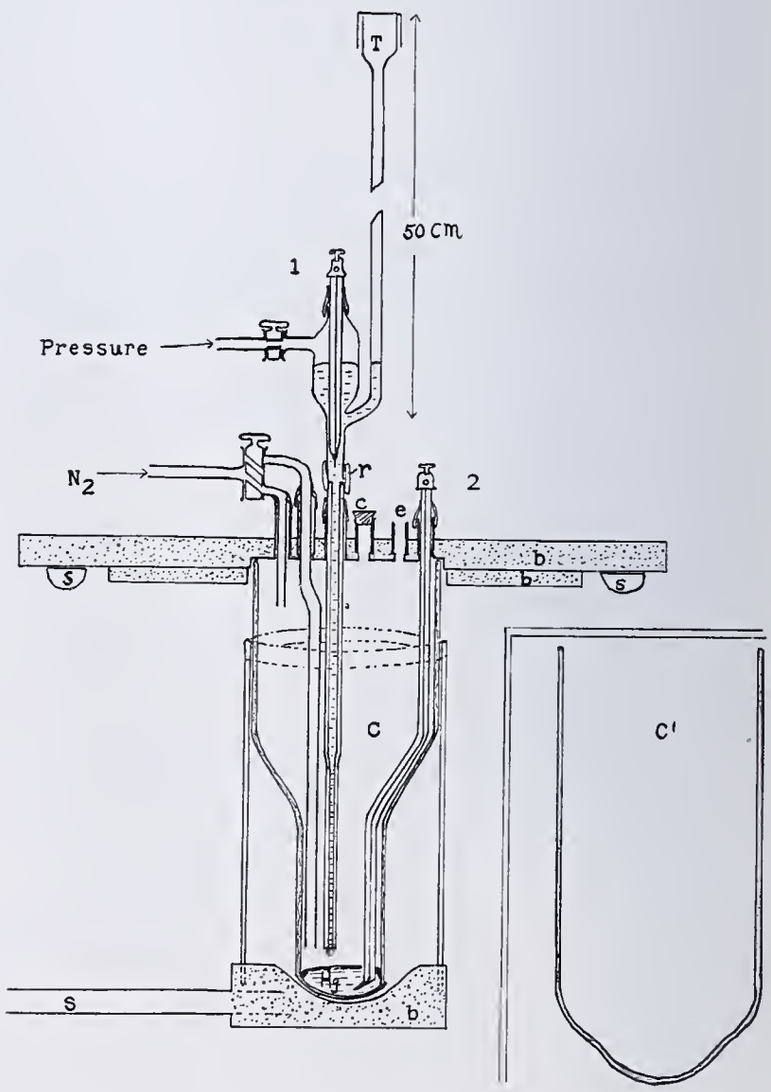

FIGURE 8. DROPPING ELECTRODE ASSEMBLY

C, *C'*. Interchangeable Pyrex glass cells of different capacity, but requiring same amount of mercury for quiet electrode. Outer diameter 4.5 × 10 cm.
b, *b*, *b*. Bakelite supports held in place by steel rods
s, *s*, *s*. Steel supports leading to heavy ring stand with two up right rods
1. Dropping electrode arrangement, with tube *T*, pressure inlet and electrode connection. Connected to capillary by *r*
2. Connection to quiet electrode
N_2. Washed nitrogen led either through or above solution
c. For introduction of microburet or pipet
e. Outlet for nitrogen and for introduction of side arm of half-ce for potential measurements

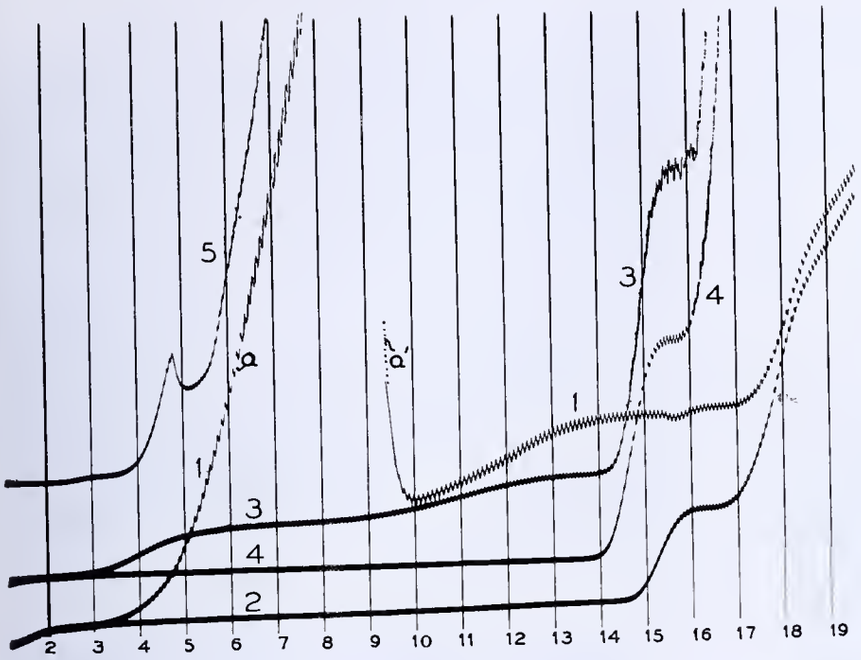

FIGURE 9. MAXIMA DUE TO AIR OR OTHER CAUSES

1. Effect of oxygen, maximum a, a'
2. Same as I after removal of oxygen
3. With air present and 4 are for same solution. Air was removed before taking 4
5. Small maximum not due to air

The instrument follows all these changes very well, although the descending branch, a', of the oxygen maximum on 1 can barely be seen on the original photograph.

The period of this glavanometer is so short that when attempts were made to get waves high enough for accurate measurement at lower concentrations the galvanometer trace was so wide that exact measurement was difficult.

An available Type R (Leeds & Northrup) galvanometer of rated sensitivity 9×10^{-11} ampere per mm. at one meter, 582 ohms' coil resistance, 19,000 ohms' external critical damping, and 14-second period, was tested. The instrument tended to lag badly behind the drum and was tedious to use; with adequate care good records were obtained.

Finally, upon advice of members of the research staff of Leeds & Northrup a Type P galvanometer was acquired to use with the instrument. This has a rated sensitivity of 9×10^{-10} ampere per mm. at 1 meter, coil resistance 1060 ohms, period 14 seconds, and external critical damping resistance 10,000. The use of this instrument was advised because it gives a linear record on flat paper, through a fortunate balancing of electrical and geometric characteristics. This point was tested by finding the variation of deflection with applied potential through known resistances at various shunt settings and there was no systematic trend in units of deflection per millivolt applied at any point along the recording drum. As far as the authors are aware, this is the most economical choice of a galvanometer for a recording instrument of this type that has been made.

The Ayrton shunt had sensitivity settings of 0.0001, 0.0005, 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, and 1. A few selected settings of the other shunt, *Rs* (Figure 6), were found in order to give intermediate sensitivities. The Ayrton shunt was one made by the Shallcross Manufacturing Co., Philadelphia, Penna.

Galvanometer Lamp. An economical assembly sold as a light source for photocells was used as a source of material (Herbach and Rademann Co., Philadelphia, Pennsylvania). This was made into a transformer unit, and the lens and a galvanometer lamp (0.5 ampere at 3.5 to 4.0 volts) were mounted in a locally made housing with concentric brass tubes for focusing.

DROPPING ELECTRODE AND ACCESSORIES. The authors have modified somewhat the conventional assemblies to produce an arrangement with interchangeable cells that should be easy to thermostat, although they have not done so. The cells have widely different capacities, so that volumes of 1 to 2 or 90 to 100 ml. may be handled, the latter being convenient when it is desired to run a series of experiments with substances added between polarograms. The assembly is shown diagrammatically in Figure 8. The pressure head is believed to be the design of the research staff of Leeds & Northrup, although similar general assemblies have been described by others (*2*, p. 242).

The various tubes led through the Bakelite mounting pass through short pieces of wider glass tube that are cemented with methyl methacrylate polymer applied in solution. These tubes fit in holes bored about three-fourths of the way through the Bakelite. The cells are held in place by stout wires mounted in the Bakelite support shown at the bottom of the diagram, and soldered to a metal collar that holds the cell rather rigidly in upright position. In this way damage to capillary and other tubes is avoided. The tubes that pass down through the Bakelite are fastened to the supporting tubes by short pieces of rubber tubing. The assembly is rather easy to take down for repairs and was designed to be suitable for measurements or titrations.

CAPILLARIES. The authors have drawn their capillaries following closely the directions given by Heyrovský (*1*). With the mercury pressure head as shown in Figure 8 there is little chance of contamination of the mercury, since it passes through only one joint that is sealed with rubber pressure tubing and here the contact is almost glass to glass. Apparently the useful life of a capillary might well be as long as it can be protected from mechanical shock or chemical etching. The reservoir holding the bulk of the mercury is made broad enough so that there is almost no change in level

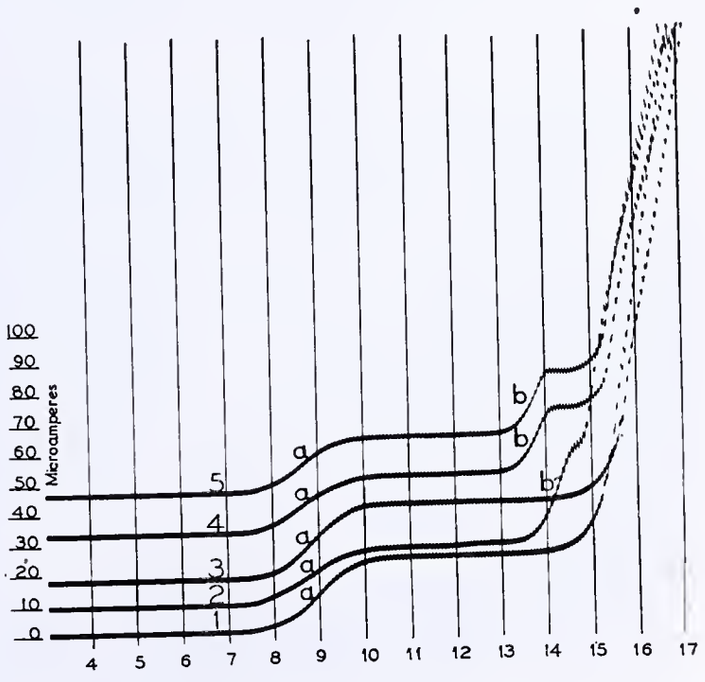

FIGURE 10. TYPICAL PERFORMANCE, SUCCESSION OF WAVES

Inflections at a, a, a correspond to maleic acid; at b, b, b are due to phthalic acid.
In 1 and 3 no phthalic acid is present.

in tube T (Figure 8) while a single curve is being traced. Readjustment of level is made when needed.

Capillary Constant. The simple method of characterizing a capillary by weighing the amount of mercury delivered in air at measured mercury pressure in a given time (number of drops and drop time noted) as described by Müller (*3*) was used. The constant $k = P/m$ is found by noting the mass of mercury delivered per second, in milligrams at various mercury pressures in centimeters. Then for solutions it is only necessary to note P and the drop time in order to be able to calculate $m^{2/3}t^{1/6}$ for the capillary under the particular conditions.

Current. The number of millimeters on the photographic record that corresponds to 1 microampere can best be determined by noting with a resistance in series, in place of the mercury electrode cells, the deflection produced by a known applied e. m. f. Then with a second known resistance the e. m. f. that will produce the same deflection is found. This method, described by Kolthoff and Lingane (*2*, pp. 227–9) is exceedingly simple and checks well with the current estimated from the rated characteristics of the instruments (within 1 per cent for this galvanometer).

Typical Performance

Figures 9 and 10 are typical polarograms taken with the instrument. Ones rather heavily exposed were selected in order to reproduce well.

Costs

The cost of materials for the mechanical instrument shown in Figure 2 was between $54 and $55. The galvanometer, lamp, shunt resistance, and cell assembly are not included in this figure. The chief cost in the instrument is the machining and design, and the machining cost without experience or detailed drawings, for a single instrument, may be estimated at between five and six times the cost of the material. The design, preliminary and other testing experiments, construction of dropping electrode assembly, etc., required approximately one month of the research time of each of the authors, this time being intermittent on account of teaching and other duties. There can be no saving as compared with a fairly priced manufactured instrument that is produced in quantity. The only questions are the availability of a particular type of instrument, and whether local conditions at the moment justify payment in wages for it rather than cash outlay for a finished product.

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Rotary Viscometer for Determination of High Consistencies

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The design, construction, and operation of a new rotating cylinder viscometer are discussed. Its application to rheological studies is illustrated by results for asphalts over a range of 0.001 to 1000 megapoises. Novel features of the instrument comprise corrections for both top and bottom end effects and a special design whereby the viscosity is measured at a constant mean rate of shear. The apparatus is particularly adaptable for measurement of equilibrium consistency, and studies of complex flow and the elastic properties of materials having relatively high viscosities.

COMPLETE evaluation of the rheological properties of a material possessing a high consistency and manifesting complex flow characteristics can be accomplished satisfactorily only with an apparatus in which the rate of shear–shearing stress relation can be determined at several different rates of shear. To evaluate a thixotropic material the instrument must be capable of shearing the sample at a constant rate in the same direction for an unlimited length of time.

The rotating cylinder viscometer, in the various forms in which it has found extensive application, fulfills the requirement of shear in a given direction for an unlimited time. However, most rotating viscometers are designed so that the sample is tested under a constant shearing stress. This is not satisfactory for evaluating the consistencies of materials manifesting thixotropy because, as the structure within the sample is destroyed by the mechanical working, a progressive decrease in consistency occurs with a concomitant increase in rate of shear under the constant applied shearing force. Thus, with the usual type of rotating viscometer, it is difficult to compare the consistencies of different complex (non-Newtonian) liquids at the same rate of shear.

Csagoly (*3*) proposed a rotating cylinder viscometer operating at a constant rate of shear for use in the study of the flow properties of asphalts having viscosities of 0.5 to 50 poises. However, his apparatus was not rugged enough to permit testing the asphalts at service temperatures where the consistencies are greater than one million poises (one megapoise). In the instrument described below, Csagoly's design has been improved and amplified to give a viscometer of rugged construction and simple operation capable of measuring an extremely wide range of consistencies—e. g., 0.001 to 1000 megapoises. In addition, a smaller sample (about 20 ml.) is required for test.

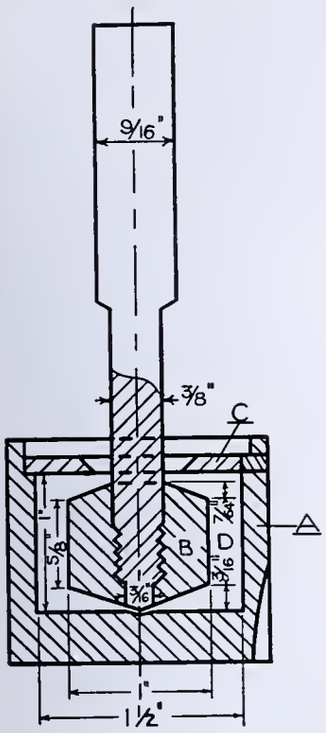


FIGURE 1. DIMENSIONAL SKETCH OF ROTOR AND STATOR

Design of Viscometer

The viscometer proper is shown in Figure 1. The space, D, between the rotor, A (outer chamber), and stator, B (inner cylinder), is filled with the material to be tested. As the outer chamber rotates at a constant angular velocity, the torque required to prevent B from rotating is measured. The top and bottom of the stator are cones of such an angle that the mean rate of shear is essentially the same at the ends as in the cylindrical part of the annulus. A method of correcting for end effects was developed by Mooney and Ewart (*5*) for the bottom; in the present design a similar correction for the top has also been included. The lid, C, has the advantage of preventing elastic asphalts from pulling away from the inner cylinder under the shearing action. The viscometer proper is fabricated from rolled brass except for the shaft which is of stainless steel. The brass surface of B and the inner surface of A are knurled in order to minimize slippage at the metal-liquid interface when materials of high consistency are being tested.

Figures 2 and 3 show the complete assembly. The outer cylinder of the viscometer proper (rotor A) is centered in the water bath, E, by means of a chuck in which the rotor makes a sliding fit. The double-walled water bath, E, is mounted on turntable F which contains a gear reducer. This is driven by a constant-speed motor, H, connected through a gear reducer, I, and a series of gears, G, capable of being shifted readily in order to obtain different angular velocities for the rotor.

B is connected by the friction coupling, J, to the vertical rod, K. A pair of bevel gears, L, transmit the torque to the horizontal shaft, M, and the indicator lever, N. The torque required to keep the stator from rotating varies, depending on the consistency of the material being tested and the rate of shear employed. A range of torque is covered by using weights of various sizes at different distances from the center of M. A wide range of consistency may be measured conveniently by using different pairs of bevel gears, L, of different gear ratios, by using a single pair of bevel gears and a variable series of driving gears, G, or by a combination of both arrangements. Scale O is calibrated to measure the deflection of the indicator which is directly proportional to the consistency. The indicator arm is a brass rod and is counterbalanced as shown. Shafts K and M are supported by ball bearings. The apparatus is mounted on a reinforced iron plate, and the upper assembly is supported by 2.5-cm. (1-inch) angle irons. Temperature of the water in E may be controlled by a suitable thermostatic arrangement.

Operation

In making a consistency determination on a material such as asphalt, rotor A and stator B are heated well above the softening point of the sample before the latter is poured into the viscometer. In practice a special holder (Figure 4) is used to fix the rotor and stator in proper alignment during the heating and filling operations. The annular space, D, is filled with the sample which has been heated until fluid. If the rotor lid, C, is put on immediately, the asphalt may pull away from some part of the stator as it cools and contracts. To prevent this the viscometer is filled almost to the top and allowed to cool to approximately the test temperature. A small additional amount of hot material is then poured into the viscometer and the hot lid put in place. Any excess of the sample comes out at the clearance between the stator shaft and rotor lid and is removed with a small spatula.

Since most asphalts harden rapidly with time (*6*, *8*), it is essential that evaluations of such materials be made at the same age if values are to be compared. Normally, a determination on an asphalt is started at one hour after pouring. The viscometer should be in the constant-temperature bath for at least 20 minutes of this hour. The gears are selected to give the rate of shear desired, the necessary weight is located at the proper position on the indicator arm, N, and the motor is started. As the outer cylinder rotates the indicator arm moves upward until a torque

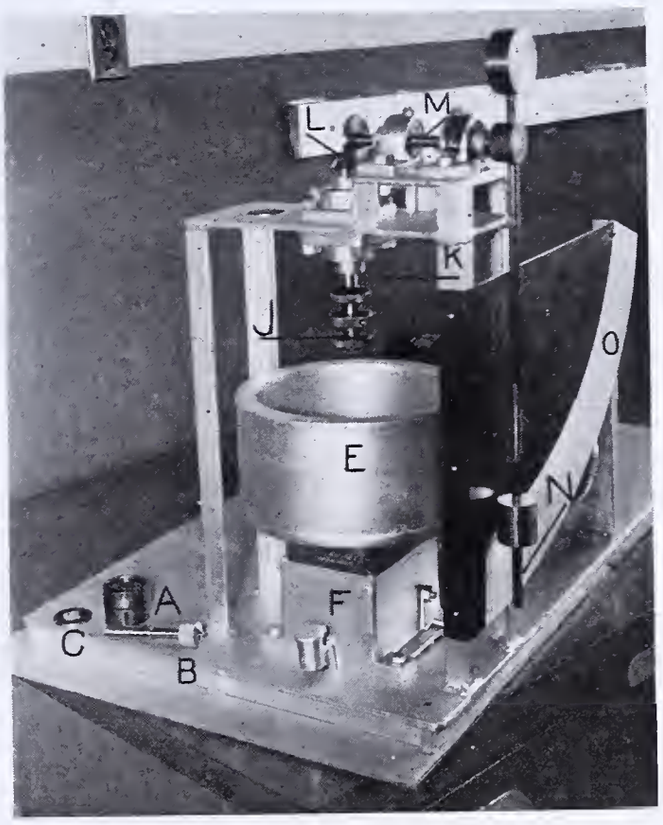


FIGURE 2. ROTARY VISCOMETER ASSEMBLY

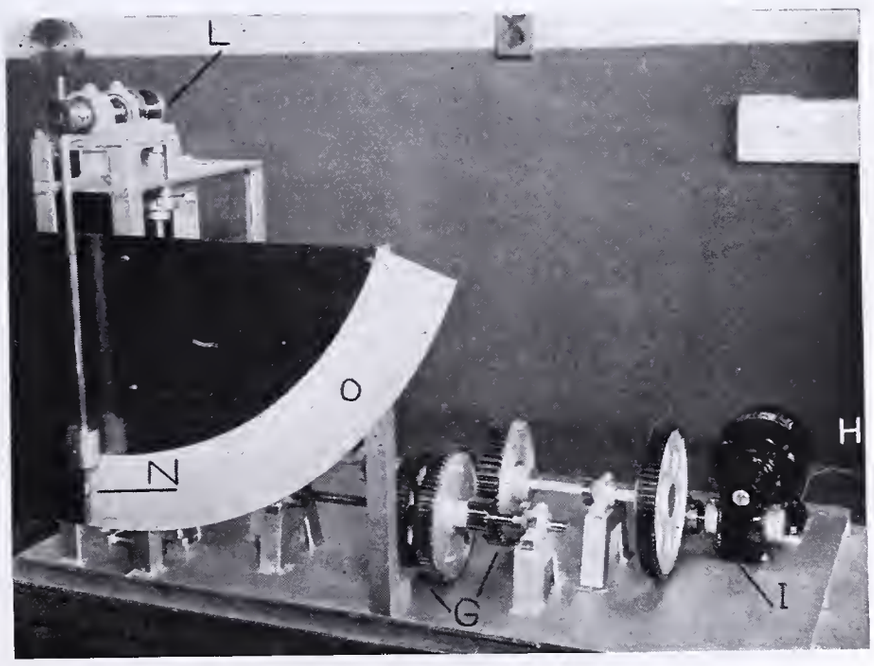


FIGURE 3. ROTARY VISCOMETER ASSEMBLY SHOWING DRIVING MECHANISM

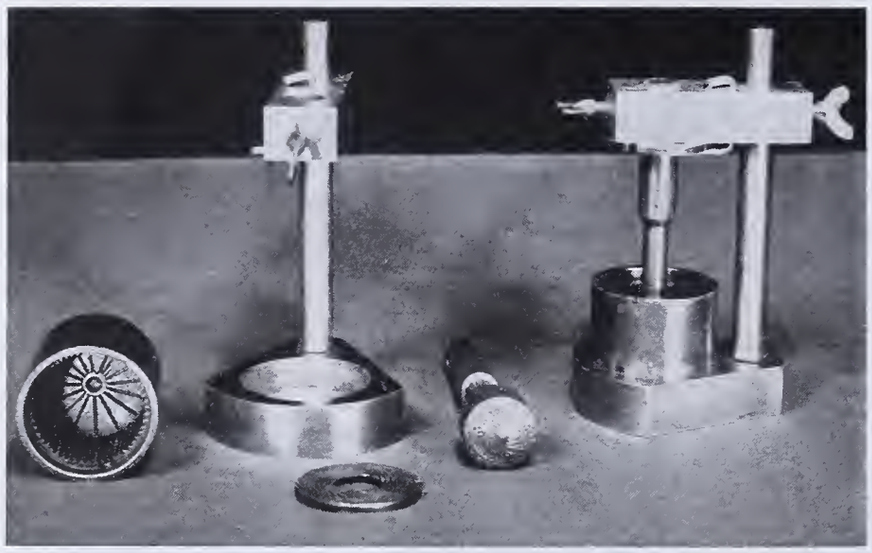


FIGURE 4. ALIGNMENT DEVICE FOR HOLDING VISCOMETER DURING FILLING

sufficient to prevent the stator from moving is attained. The time required to make a determination, after the sample has been poured and brought to a constant temperature, will vary, depending on the nature and consistency of the material and the rate of shear employed. For asphalts having viscosities of 0.001 to 10 megapoises from 5 to 40 minutes will be required.

Calculations

The viscosity or consistency is calculated by the equation:

$$\eta = K(T/\omega) \quad (1)$$

where η = viscosity or consistency, poises
T = torque required to prevent the stator from turning, dyne-centimeters
ω = angular velocity of rotor, radians per second
K = a constant determined by the dimensions of the viscometer, cm.$^{-3}$

Mooney and Ewart have developed the equations for evaluating the constant, K, for a rotating viscometer having an inner cylinder with one conical end. For an instrument with both ends of the inner cylinder conical, the equations for evaluating K are the same except that the correction for end effect is doubled. With this modification Mooney and Ewart's equation becomes

$$K = (b^2 - a^2)/[4\pi h_0 a^2 b^2(1 + 2r_0/3h_0 \sin\theta_0)] \quad (2)$$

where a = radius of inner cylinder, cm.
b = radius of outer cylinder, cm.

$$r_0 = [2a^2b^2/(a^2 + b^2)]^{\frac{1}{2}} \quad (3)$$

$$\theta_0 = (\alpha + \beta)/2 \quad (4)$$

where α = angle of cone of inner cylinder (69°)
β = angle of cone of outer cylinder (90°)

$$h_0 = h_c + (b - a) \times (1 - \cos\theta_0)/\sin\theta_0 \quad (5)$$

where h_0 = mean length of sample
h_c = length of cylindrical section of inner cylinder

The value of K for an instrument having the dimensions given in Figure 1 is 8.79×10^{-3}.

The equation for K is obtained from the equations for the mean shearing stress, F, and the mean rate of shear, S.

$$F = T/[2\pi r_0^2 h_0(1 + 2r_0/3h_0 \sin\theta_0)] \text{ dynes per sq. cm.} \quad (6)$$

$$S = (a^2 + b^2)\omega/(b^2 - a^2) \text{ reciprocal seconds} \quad (7)$$

The equation for the mean rate of shear, S, was developed, assuming that the material being tested exhibits simple (Newtonian) flow—i. e., $\eta = F/S$.

Since the indicator arm is counterbalanced, the displacement of the added weight is the only torque resisting rotation of the stator (neglecting friction which can be made negligible). Thus, T is easily calculated from the weight and its position on the indicator arm, the bevel gear ratio, and the indicator reading. A convenient chart may be developed for a particular instrument which shows the range of consistency covered by each combination of angular velocity, bevel gear ratio, weight, and its position on the indicator arm. For each combination a constant, C, may be determined which, when multiplied by the indicator reading, gives the consistency in poises.

The rotary viscometer was checked against the falling coaxial cylinder (7) and the inclined tube falling ball (Hoeppler) (4) viscometers, using a number of asphalts possessing essentially simple flow and ranging in consistency from 0.0011 to 2 megapoises at 25° C. (77° F.) with the results shown in Table I.

In order to evaluate the low viscosities of certain of the asphaltic residua in the rotary viscometer it was necessary to revolve the outer cylinder at about 100 revolutions per hour. This was much faster than the speeds used with the harder asphalts, which were usually evaluated with the outer cylinder turning 0.35 to 7.0 revolutions per hour.

The data in Table II on asphalts of various consistencies and origins indicate the degree of reproducibility obtained with the rotary viscometer at a given temperature and rate of shear. Based on a large number of determinations the standard deviation is about 3 per cent.

The above checking values were obtained at the same sample age (one hour). An asphalt, if held at a fixed temperature, will show increasing consistency with elapsed time, as is indicated by the data in Table III. Such variations in measured consistency, caused by the colloidal nature of the material tested, should not be attributed to lack of precision of the apparatus.

TABLE I. COMPARISON OF RESULTS OBTAINED WITH DIFFERENT VISCOMETERS

Source of Asphalt	Ring and Ball (1)	Efflux Viscosity at 98.9° C. (210° F.) (2)	Viscosity at 25° C. (77° F.) Rotary viscometer	Falling coaxial cylinder viscometer (7)	Inclined tube falling ball viscometer (4)
	° C. (° F.)	Seconds	Megapoises		
Vac reduced California	49.4 (121)	...	2.00	1.91	.....
Vac reduced Venezuela	47.2 (117)	...	1.37	1.30	.....
Mexican residuum	38.9 (102)	...	0.220	0.225	.....
Gulf Coast residuum	..	100	0.0108	0.0108	0.0108
Gulf Coast residuum	..	142	0.0070	0.0078	0.0075
Mixed residuum	..	146	0.0057	0.0057	0.0060
Gulf Coast residuum	..	115	0.00260	....	0.00266
Gulf Coast residuum	..	75	0.00110	....	0.00110

TABLE II. REPRODUCIBILITY OBTAINED BY ROTARY VISCOMETER

Source	Process	Viscometer No.	Ring and Ball ° C. (° F.)	Mean Rate of Shear Sec.$^{-1}$	Equilibrium Consistency at 25° C. (77° F.) Megapoises
Mexican	Vacuum	2	38.9 (102)	0.032	0.224
		3			0.220
Venezuela	Vacuum	1	47.2 (117)	0.032	1.17
					1.17
Northeast Texas	Air-blown	1	58.9 (138)	0.032	4.7
		2			4.9
		3			4.7
Northeast Texas	Air-blown	2	100 (212)	0.0032	210
		2			200

If the rate of shear is changed for duplicate runs on a material having flow properties essentially the same as those of a simple liquid, the consistency values check closely. This is illustrated by the data in Table IV, obtained on asphalt samples one hour after being poured.

However, when similar data are obtained for asphalts exhibiting complex flow characteristics the apparent consistency in poises, at a particular temperature and sample age, increases as the rate of shear decreases. This condition, which is illustrated by the data in Table V for certain asphalts aged one hour, makes necessary the use of an arbitrarily selected rate of shear.

Data obtained under the above conditions are a means of evaluating deviations from simple flow. It is evident that the steam-reduced Mexican asphalt possesses complex flow characteristics to a less extent than the air-blown Gulf Coast asphalt.

TABLE III. INCREASE OF VISCOSITY WITH AGE OF SAMPLE

Source	Process	Ring and Ball ° C. (° F.	Age of Sample Hours	Mean Rate of Shear Sec.$^{-1}$	Equilibrium Consistency at 25° C. (77° F.) Megapoises
Gulf Coast	Air-blown	59.4 (139)	0.5	0.0032	15.9
			1.0	0.0032	17.6
			17.0	0.0032	19.6
			115	0.0032	22.0

TABLE IV. EFFECT OF RATE OF SHEAR FOR SIMPLE LIQUIDS

Source	Process	Ring and Ball ° C. (° F.)	Mean Rate of Shear Sec.$^{-1}$	Equilibrium Consistency at 25° C. (77° F.) Megapoises
California	Vacuum	49.4 (121)	0.032	2.00
			0.0032	2.09
			0.0016	2.17
Pressure still residuum	Steam	46.1 (115)	0.032	2.70
			0.0032	2.70
			0.0016	2.72

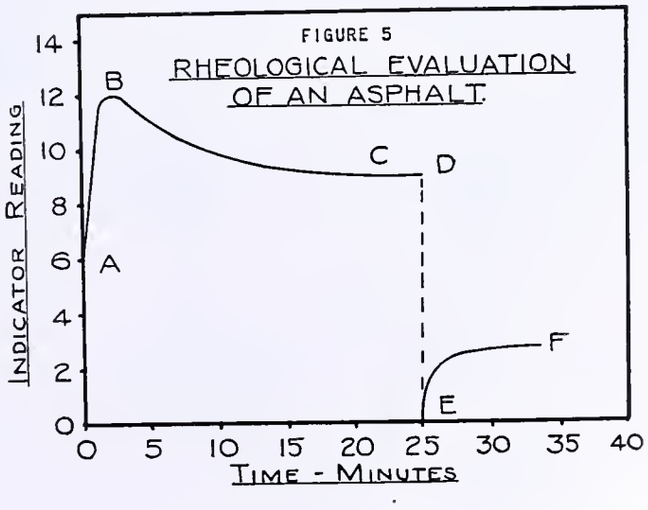
In evaluating the consistency of a complex liquid, there is usually a rate of shear above which "slippage" takes place. The phenomenon of slippage is probably caused by a greater breakdown in structure in the few layers of liquid around the stator than in the other parts of the sample. Theoretically, as the outer cylinder rotates, the annulus of material being tested rotates in layers concentric with the rotor and stator. With the size of viscometer illustrated in Figure 1, the shearing stress at the stator surface is b/a—i. e., 1.5—times that at the rotor wall. Thus, for a material showing considerable complex flow characteristics, the initial rate of shear at the stator is more than 1.5 times that at the rotor. Therefore, a sample with a large amount of structure will be broken down fastest at the stator where the rate of shear is highest. This effect is cumulative, and, in time, almost all of the shearing may be occurring in a few boundary layers near the stator with very little shearing in the outer layers of the annulus. If this type of slippage is taking place it is evident that no constant reading or equilibrium consistency will be observed. The effect on the measured consistency of this localized breakdown of structure can be minimized by making the ratio of stator radius to rotor radius as large as practical considering ease of filling the viscometer and by using low rates of shear. With the size of viscometer shown in Figure 1 no appreciable slippage was encountered in any of the asphalts tested at the two lowest rates of shear used (0.0016 and 0.0032 second^{-1}). However, at a rate of shear of 0.032 second^{-1} slippage started to occur in certain asphalts at consistencies between 10 and 50 megapoises.

TABLE V. EFFECT OF RATE OF SHEAR FOR COMPLEX LIQUIDS

Asphalt	Process	Ring and Ball ° C. (° F.)	Mean Rate of Shear Sec.$^{-1}$	Equilibrium Consistency at 25° C. (77° F.) Megapoises
Mexican	Steam-reduced	54.4 (130)	0.032	3.8
			0.0032	5.6
			0.0016	6.3
Gulf Coast	Air-blown	59.4 (139)	0.032	7.4
			0.0032	22
			0.0016	29

Figure 5 illustrates some of the deviations from simple flow exhibited by complex liquids when evaluated in the rotary viscometer at a constant mean rate of shear (0.032 reciprocal second). This figure is a plot of the indicator readings against time for an air-blown Northeast Texas asphalt of 58.9° C. (138° F.) ring- and- ball softening point.

The initial period, *AB*, of increasing consistency represents the extension of the elastic elements within the asphalt and is called the elastic fore effect. The period, *BC*, of decreasing consistency is caused by a breakdown of structure with shearing. The curvature in this period will be accentuated by an increase in rate of shear. At the essentially constant value *CD* the consistency is called the equilibrium consistency and represents a balance between the thixotropic breaking down and building up of structure within the asphalt. This equilibrium value is reproducible (if slippage does not occur), and, according to available data on asphalt, it is independent of any previous shear. At point *D* in Figure 5 the weight was removed, the indicator set at zero reading, and the motor stopped. The movement, *EF*, of the indicator measures the elastic recovery or elastic aftereffect of the asphalt in attaining



equilibrium after all imposed shearing stress has been removed.

The type of data shown in Figure 5 illustrates the application of the rotary viscometer to rheological problems: The equilibrium consistency in absolute units at a given constant rate of shear may be determined; the degree of complex flow exhibited by a material may be evaluated from the equilibrium consistencies at two or more rates of shear (*9*); and the elastic properties may be studied. Many of the above flow properties may be measured in other types of viscometers. However, in applied rheology no other single apparatus combines the simplicity, general adaptability, and theoretical advantages of a constant mean rate of shear rotary viscometer.

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Automatic Method for Cleaning Tar Distillation Flasks

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THE apparatus described can save appreciable time in cleaning flasks containing residue from tar distillations. It consists essentially of a container in which the solvent is converted from the liquid to the vapor phase. The vapor, conducted by a tube to the inverted tar flask, condenses on the walls and dissolves the residue, and the liquid is returned to the original container by a second tube. The residue in the flask is thus acted upon at all times by pure solvent.

A solvent that is effective with coal tar is *m*-xylene. Some free carbon is left on the walls of the flask being cleaned, but this can be removed by rinsing with nitric acid.

The cleaning operation should be carried on in a hood with a good draft, in order to prevent any vapors from coming in contact with the flame.

The arrangement shown in Figure 1 can be used when cleaning only one flask at a time.

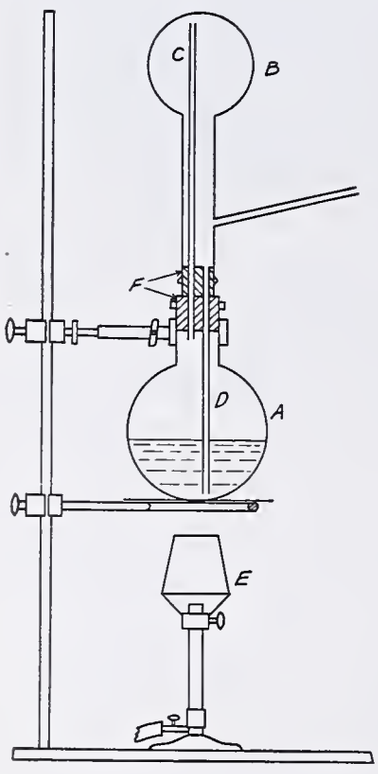

FIGURE 1. APPARATUS FOR CLEANING ONE FLASK
A. Solvent container, wide-mouthed round-bottomed flask
B. Flask being cleaned
C. Vapor tube
D. Solvent-return tube
E. Bunsen burner with chimney
F. Cork stoppers

If it is necessary to clean many flasks simultaneously, a more convenient apparatus can be constructed (Figure 2). For cleaning long-necked and short-necked flasks, space can be saved by alternating long and short vapor tubes, as shown.

By standing sheets of asbestos from leg to leg of the supporting frame, contact between vapor and flame can be minimized.

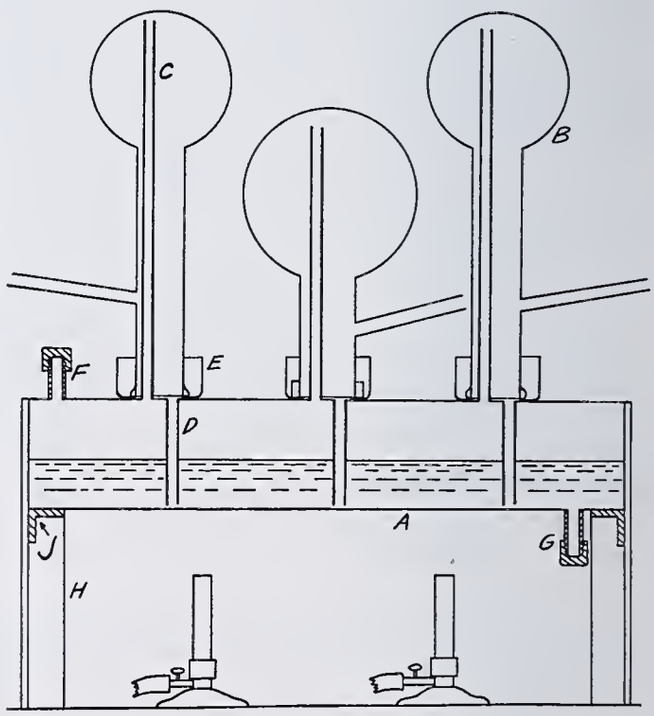

FIGURE 2. CROSS SECTION OF APPARATUS FOR CLEANING FLASKS SIMULTANEOUSLY
A. Rectangular box of sheet iron, welded construction, containing solvent
B. Flasks being cleaned
C, D. Vapor- and solvent-return tubes of brass, brazed into corresponding holes of box
E. Body of 4-ounce can with 1.5-inch hole in bottom, secured to *A* with hard solder, so as to enclose *C* and *D* and keep return solvent from straying
F. 0.25-inch iron pipe, closed by cap, for admitting fresh solvent
G. 0.25-inch iron pipe, closed by cap, for removing spent solvent
H. Vertical members, and *J*, horizontal members of 1 × 1 × 0.125 inch angle iron, welded for support of apparatus

Apparatus for Distillation of Corrosive Liquids

As Used for Purification of Chlorosulfonic Acid

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IN CONNECTION with some of the authors' work (*1*) small known quantities of chlorosulfonic acid of high purity were desired. Since a detailed procedure for the preparation of pure chlorosulfonic acid is not readily available in the literature, this method may be of interest.

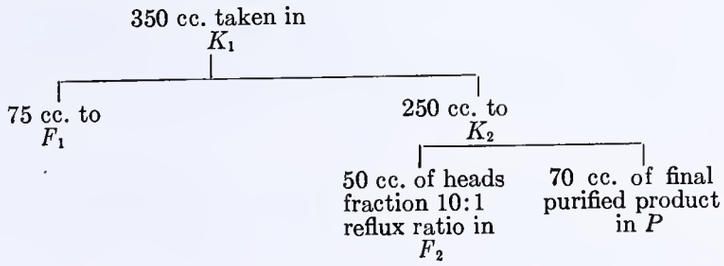
Sanger and Riegel (*4*) state that chlorosulfonic acid is best prepared by bubbling dry hydrogen chloride gas through strong oleum until the gas is no longer absorbed. To purify the chlorosulfonic acid, they say it should be distilled in a current of hydrogen chloride gas and the fraction between 145° and 160° C. redistilled. They mention that chlorosulfonic acid dissociates, when boiled, into hydrogen chloride gas and sulfuric acid, and into sulfuryl chloride and sulfuric acid.

As commercial chlorosulfonic acid is readily available, the preparation step may be omitted. In the apparatus shown in Figure 1, twenty-six ampoules containing known weights of chlorosulfonic acid were prepared. The purity obtained is indicated by the analyses shown for total acidity, gravimetric sulfur, and chloride by the method of Roberts (*2*, *3*).

	Acidity *Milliequivalents/g.*	Cl Content %	S Content %
Calculated	25.75	30.43	27.50
Found	25.92	29.61	27.87

The apparatus used consisted of two all-glass, helix-packed rectifying columns sealed together in tandem. The first of these, R_1, was made from a 25-cm. length of 12.5-mm. glass tubing packed with glass helices (2.5-mm. single turn) and was mounted on a 500-cc. flask, K_1. The column carried a simple stillhead, H_1, comprising a reflux finger and a thermometer well, T_1. The second still, R_2, was similar but had a 46-cm. packed length surrounded with a silvered vacuum jacket, J. Stillhead H_2 on the second column provided reflux control without contamination of or by the ground-joint lubricant. The parts of this stillhead shown in Figure 1 (right) were thermometer well T_2, reflux and total condenser C_2, reflux drop counter Q, and control valve B, keyed to "wrench" G. Condenser C_2 was designed so that only a small annular space remained between the inner cooled finger and the outer jacket. This effectively prevented any vapors from reaching the only greased joint, G.

The condenser length was about 18 cm. Other parts of the detailed sketch are drawn to the same scale. Control valve B was essentially a Fresenius stopcock with a long stem, keyed at the end to fit a socket in G. The cock was so designed that a 180° turn from normal position would drain the tube above it. This valve was lubricated by the distillate only, while the joint at G was greased in the usual fashion.



Three hundred and fifty cubic centimeters of commercial chlorosulfonic acid (du Pont) were placed in K_1 after drying the apparatus. With the system sealed, an absolute pressure of about 30 mm. was maintained, as measured on manometer M, by exhaustion through a large-capacity liquid nitrogen trap, N. Approximately 75 cc. of the acid were distilled into receiver F_1. Without discontinuing the distillation, F_1 was sealed off at constrictions A and A' and 250 cc. of the acid were collected in the bottom of K_2. (The connections from A' to K_2 and E' to P had slight upward slopes and were made of tubing of sufficiently large diameter to prevent formation of a siphon.) After sealing the tube from A' to K_2 and sealing the vacuum lead at D, a heads fraction of about 50 cc. was distilled from K_2 into F_2 at about a 10 to 1 reflux ratio. The final purified product, about 70 cc., was then collected in P after sealing off F_2 at E and E'.

In the bottom of P were inverted small weighed ampoules, S, with capillary tips. On readmission of dry air into the system, these ampoules filled automatically, leaving a layer of product in the bottom of the receiver in which the capillary tips of the ampoules remained immersed. Breaking open the receiver and removing the ampoules one by one to a flame for sealing completed the operation. The layer of chlorosulfonic acid in the receiver protected the contents of the ampoules until ready for sealing, so that the exposure of the final product to the atmosphere was a matter of a few seconds through at least 30 mm. of very fine capillary.

Twenty-six ampoules containing chlorosulfonic acid in accurately weighed quantities ranging from 0.5 to 2.2 grams were thus obtained in a single preparation.

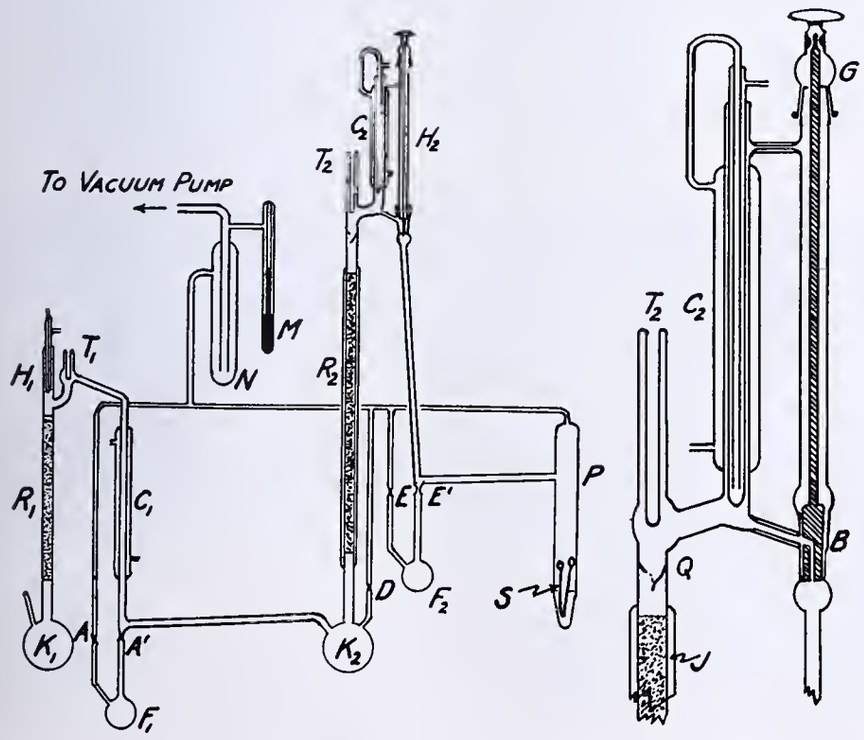


FIGURE 1. DIAGRAM OF APPARATUS

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A New All-Glass Mill

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THREE methods are currently in use for preparing test inks in studies of pigment, ink, and paint properties. The oldest and perhaps most widely used method is hand mulling, but it has the deficiencies of, first, the poor dispersion obtained, particularly with hard pigments; and secondly, contamination which invariably occurs, especially due to steel rubbed off in the spatula pickup. The first factor is important when working with hard pigments, the second when working with whites or light colors. Finally, it is difficult to disperse by this method inks which, though finally workable to a flowing ink, are extremely dry on first mixing.

For tests involving larger quantities of ink, three-roller mills of laboratory size are available. Use of one of these mills usually gives excellent dispersion, but contamination invariably ensues with hard white and light-colored pigments, unless the roll setting is so loose that efficient dispersion is not attained. Furthermore, the amount of pigment required for such a test is often not available in the pigment research laboratory.

Automatic mullers now on the market involve the same source of contamination as hand mulling—steel rubbed off during spatula pickup. The writer is not familiar with the efficiency of this type of mill in other respects.

Principle of Mill

All these deficiencies were kept in mind when the new mill was designed. The grinding surfaces are entirely glass, and are so disposed that pickup off these surfaces is not necessary. Good dispersion is achieved by using grinding pressures of the order of 50 to 300 pounds per square inch, as compared with 1 to 5 pounds per square inch in hand mulling.

The new apparatus which achieves these conditions consists essentially of two truncated cones, one of which is fitted inside the other, together with a plunger feed mechanism which forces crudely mixed ink into the annular space between the two sections, one of which is rotated with respect to the other. The ink flows out through the annular space and is collected at the end opposite the feed mechanism. By using conical grinding sections, high grinding pressure is attained while the outside force impressed is of moderate proportions.

Construction

An ordinary ground joint supplies the essential part of the mill For the model constructed in this laboratory, a special set was designed. The females differed from the regular stock models, in that they were constructed of heavier glass and in the elimination of the usual bead at the base of the ground section. The males were standard solid stoppers, with the small end beveled off to a depth of about 0.125 inch. The dimensions are those of the standard 15/35 ground-glass joint. The cost was only 40 cents more for each pair than for a pair of the same size out of stock.

The female part was used as supplied. The male part was grooved at the laboratory, in order to provide a smooth feed of unground ink through the mill (Figure 1). Various types of grooves were tried; the type finally adopted involves cutting a spiral which begins at the center of the small end of the stopper, the sense of the spiral being such with respect to the direction of rotation, that crudely mixed ink is forced from the center towards the wall of the joint. This spiral groove is continued smoothly along the wall of the stopper, again in the sense which on rotation forces material through the joint. This "feed-in" section is run along for about 0.5 inch, at an angle of about 45°, then its direction is changed to make a 15° angle in the opposite sense. The leading edge of this "feed-back" groove is sharp, but the trailing edge is tapered and smoothly rounded. The ink is thus forced forward along the groove by the feed-in section, and backward by the feed-back section, with the result that it passes into the grinding section between the two ground-glass parts. This passage is assisted by the tapered edge of the feed-back groove. The grooves were cut with a small brass wheel, using a Carborundum-water mixture.

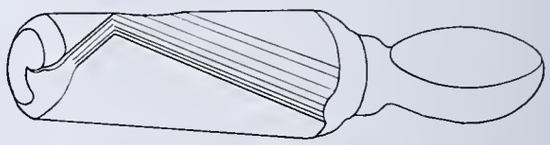

FIGURE 1. SLOTTED GROUND STOPPER

In operation, the crudely mixed ink is placed in the tubular section of the female joint, and the plunger is inserted (inset, Figure 2). The plunger consists of a mushroom-shaped rubber head, the stem of which fits into a steel cylinder and is held there by a flush setscrew. The female section is held in a rubber-lined clamp, while the male section is rotated by means of 0.05-horsepower motor, the drive shaft of which is geared down to a speed of 600 r. p. m. Speed of rotation is held at about 150 r. p. m. by operating off a variable transformer at about 60 to 75 volts. In the model constructed, the force on the plunger and on the ground stopper was supplied by oil-filled pneumatic cylinders, operated through reducing valves from the laboratory air line. The drive motor is fastened directly to one of the pistons, and made to move on castors. Thus the grinding force is transmitted through the motor. The pressure in each pneumatic cylinder is measured by means of a pressure gage covering the range 0 to 60 pounds per square inch; the pistons are 1 inch in diameter. The air lines are so arranged that the pressure in the feed cylinder can never be greater than the pressure in the cylinder supplying the grinding force. Thus the plunger can never accidentally be made to push out the stopper completely. A photograph of the mill appears in Figure 2.

Operation

With the stopper in place, the crudely mixed ink is placed in the tubular section of the female part. The plunger is inserted, and a small quantity of ink is forced into the ground-glass section for lubrication. The assembly is then clamped into the machine as shown in Figure 2. The grinding pressure is set at about 30 pounds per square inch, the feed pressure is simultaneously set to 20 pounds per square inch, and the motor is turned on. The flow of ink is observed and the pressures are adjusted to promote a slow steady delivery of ground ink. The machine was tested and has been used at a delivery of about 0.4 gram (6 grains) per minute for the first pass, and about 0.6 to 0.8 gram (8 to 12 grains) per minute for the succeeding pass. When the inks are very heavy, forming a dry cake when first mixed, it is convenient to have a small handle on the piston of the feed cylinder, by means of which the feed rate can be manually speeded up on the first pass. Since the grinding apparatus is transparent, the point at which the ink is completely fed out is easily observed. Quantities of 1 to 8 grams (15 to 120 grains) of ink can be conveniently handled.

When the crude mix contains large, hard aggregates there is some tendency for the ink coming through first to be richer in vehicle. For this reason, the ink is always repassed once after the original grind. Tests have shown that even in an extreme case, the composition of the ink after the second pass is not more than 1 per cent different from the original composition.

The mill will operate efficiently on inks over a very wide range of viscosity, and will conveniently handle mixtures which are too heavy or too dry to mull by hand without excessive work.

The temperature to which the ink is heated by the grinding has not been measured. The author's tests have not shown the heating to have an appreciable effect on the

finished ink. Where this might arise, a water-jacketed female section can be employed.

Characteristics of the Mill

Freedom from contamination was tested by grinding portions of a base crudely mixed ink, using various methods, and comparing the brightness of the resulting ink. For an average laboratory-produced pigment in linseed oil, results are listed in Table I. Results for a very hard white laboratory-produced pigment are shown in Table II. In connection with the "hardness" of these pigments, it has been observed that a commercial "soft" titanium dioxide pigment, when mulled in a linseed oil 3 × 65 on a smooth glass slab, was generally contaminated to the extent that its reflectance was 0.5 to 4 per cent too low. Contamination encountered with "hard" laboratory pigments may be expected to be greater under the same conditions.

Table I. Reflectance Tests

(Typical laboratory-produced titanium dioxide pigment, 70% pigment-vehicle ratio in refined linseed oil vehicle)

Conditions of Dispersion	% Reflectance of White Ink
3 × 65 mulls, ground porcelain slab	83.3
3 × 65 mulls, smooth glass slab	90.15
All-glass mill	94.45

Table II. Tests on Very Hard White Pigment

(70% pigment-vehicle ratio in refined linseed oil vehicle)

Method of Dispersion	Brightness	Texture	Apparent Tinting Strength
4 passes on 4 × 8 inch roller mill	Very gray	Smooth	100
3 × 65 mulls unweighted muller, smooth glass slab	Intermediate between other two	Very coarse	95
2 passes on all-glass mill	Very bright	Smooth, slight evidence of some aggregates	100

Efficiency of grinding was estimated by means of texture tests and by determining the strength developed in a hard grinding colored pigment. Results indicative of the texture obtainable are listed in Table II, as are strength determinations on the same white.

The most exacting test, in so far as strength development is concerned, was carried out with a hard grinding iron blue, a commercial product dating back to 1936. A preliminary mix at a pigment-vehicle ratio of 45 per cent was prepared, and mixed further by two very loose passes over a laboratory 4 × 8 inch three-roller mill. Samples of this ink were then developed by each of three methods—namely, by the 4 × 8 inch three-roller mill, by mulling with a 15-pound weighted muller, and finally on the new mill. The results are listed in Table III. A sample of the same brand of pigment, produced five years later, was apparently of improved quality with respect to hardness, for it was developed to equal strength by both the three-roller mill and the new all-glass mill. "Strength" refers to tinting strength, and was a measure of the amount of a standard white ink required to tone the color down to match an arbitrary standard. Strengths are referred in Table III to that of the ink made by passing five times over the three-roller mill as 100.

Table III. Tinting Strength Tests

(Very hard grinding iron blue at 45% pigment-vehicle ratio in refined linseed oil)

Method of Dispersion	Apparent Tinting Strength
8-inch three-roller mill, 1 pass	80
2 passes	96
5 passes	100
15-pound weight muller, 2 × 100 strokes	80
4 × 100 strokes	90
New glass mill, 2 passes	90
4 passes	94
6 passes	95

Speed of delivery is, of course, variable to suit the demands of the operator. Operating with 1.5 grams (23 grains) of ink, it has been found convenient to make the first pass in about 3 or 4 minutes, and the second in about 2 minutes. Including time consumed in loading and cleaning, an ink can be prepared in 10 minutes or less. This compares favorably with hand mulling under any conditions, and to advantage when a very heavy ink is encountered, which is difficult to work in. On the other hand, a roller-mill grind of a small quantity of ink takes about 10 minutes, when the time required to clean the mill is considered. When a very thin ink is encountered on the three-roller mill, the new all-glass mill shows up to advantage as far as the time factor is concerned.

Discussion

Tables II and III show that the mill lies between the muller and the roller mill, with respect to grinding efficiency, and is clearly much closer to the roller mill than to hand mulling. Nearly all the commercial pigments will be developed to essentially the same strength by the new glass mill as by the three-roller mill.

Examination of Tables I and II shows that the new all-glass mill is superior to other methods of dispersion, when freedom from contamination is considered. This makes it very useful for white and light pigments.

With respect to time consumed, the mill is as fast as or faster than either of the other methods considered.

The mill is superior to hand mulling in every respect. It is superior to the three-roller mill with respect to freedom from contamination, but yields slightly poorer dispersions when very hard grinding colors are encountered. The ability to produce excellent dispersions on samples which are too small to be handled by the laboratory roller mill further expands its field of usefulness.

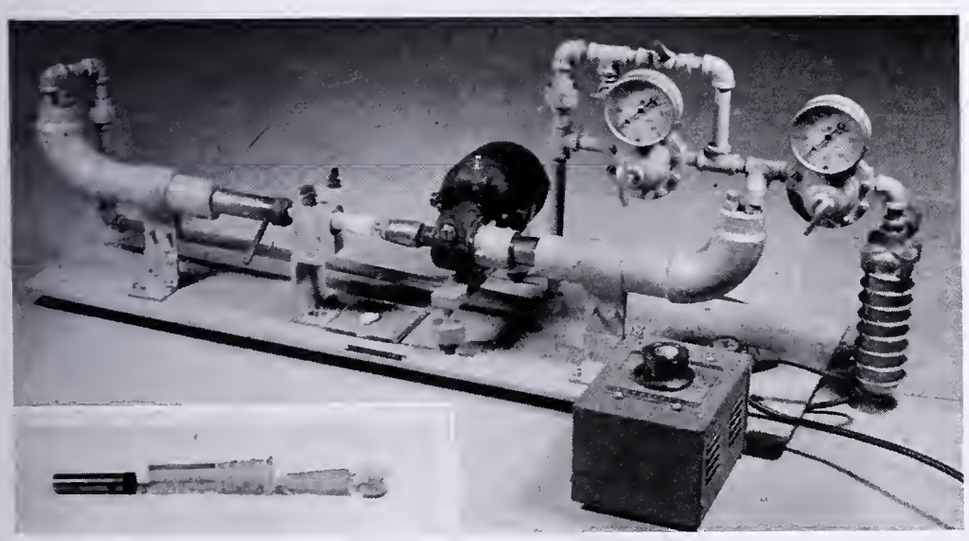

Figure 2. All-Glass Mill
Inset. Grinding sections and plunger disassembled

Summary

A new mill has been developed, by means of which inks and paints over a wide consistency range can be thoroughly ground without contamination. This new apparatus consists essentially of two truncated glass cones, one of which is fitted inside the other, together with a plunger feed mechanism which forces crudely mixed ink into the annular space between the conical sections, one of which is rotated with respect to the other. For example, the outer section of a ground-glass joint is fitted with a plunger at the small end, and ink forced by a rotating appropriately slotted stopper. The efficiency of this mill has been tested relative to that of a laboratory 4 × 8 inch three-roller mill as well as hand milling. Advantages are excellent dispersion and freedom from metallic contamination, with whites and light colors.

A Dialyzing Concentrator

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A simple apparatus for simultaneous dialysis and concentration has been devised. The rates of evaporation of water from 5 per cent sucrose solutions and from distilled water through Visking casings were observed under various conditions. By use of two fans and a steam unit heater to blow air past eight Visking casings, a maximum rate of evaporation of 1.5 liters of water per hour was obtained.

A NUMBER of methods for dialyzing have been reported (*1*, *2*, *7*, *8*, *10*, *12*), and procedures have been devised for concentration by removal of solvent through membranes (*3*, *13*, *15*). Only a few techniques have been employed which permit simultaneous concentration and dialysis and in general these have been expensive or complicated. The need for a simple procedure which would permit dialysis and concentration of 20 to 30 liters of kidney extract a day led the authors to investigate the adaptability of some methods previously reported.

Simms' method (*11*) was rapid but required strong collodion bags and could be adapted only with difficulty to the authors' larger volumes of material. Wet Visking casings of 1.4 or 1.875 cm. ($^{18}/_{32}$- or $^{24}/_{32}$-inch) diameter filled with water burst when from 200 to 300 mm. of mercury pressure were applied and so could not be substituted directly in a modified Simms' apparatus. The authors have employed a maximum pressure equal to that of 140 mm. of mercury in their apparatus. Thalhimer's method (*14*), employing corn sirup in dialysis, introduced considerable amounts of carbohydrate into the extracts, which was undesirable. Similar methods employing ammonium sulfate or other salts were objectionable for like reasons. Ultrafiltration through membranes on Seitz filters has been useful in industry (*9*), but was not feasible in the authors' laboratory.

They found that Kober's pervaporation technique (*4*, *5*, *6*) could be readily adapted to their laboratory equipment, and the apparatus pictured in Figures 1 and 2 has proved capable of handling materials without requiring much attention. Since few observations on rates of concentration and dialysis by any of these methods have been reported, the authors have measured rates and temperatures with their equipment under varying conditions which may afford indications of the value of this type of apparatus.

Apparatus

The apparatus consists of a reservoir, from a bottom outlet of which the material to be concentrated and dialyzed flows by gravity through glass and rubber tubing into eight Visking casings arranged in parallel, each 165 cm. (5.5 feet) long. These hang before a steam unit heater (with its fan) and one or more fans. The lower ends of the casings, which are expanded by pressure of the contained fluid, dip into a jar of water. Tap water is led through coils in the reservoir to keep the material cool, and thence through a rotameter (Fischer & Porter Co., Philadelphia, Penna.) to measure its rate of flow. From there it runs into the dialyzing jar, overflows onto a tray, and runs into the trough of the laboratory bench. To gather the data in Table I, wet- and dry-bulb thermometers were placed beside the casings directly in front of the unit heater, and a thermometer was placed inside one

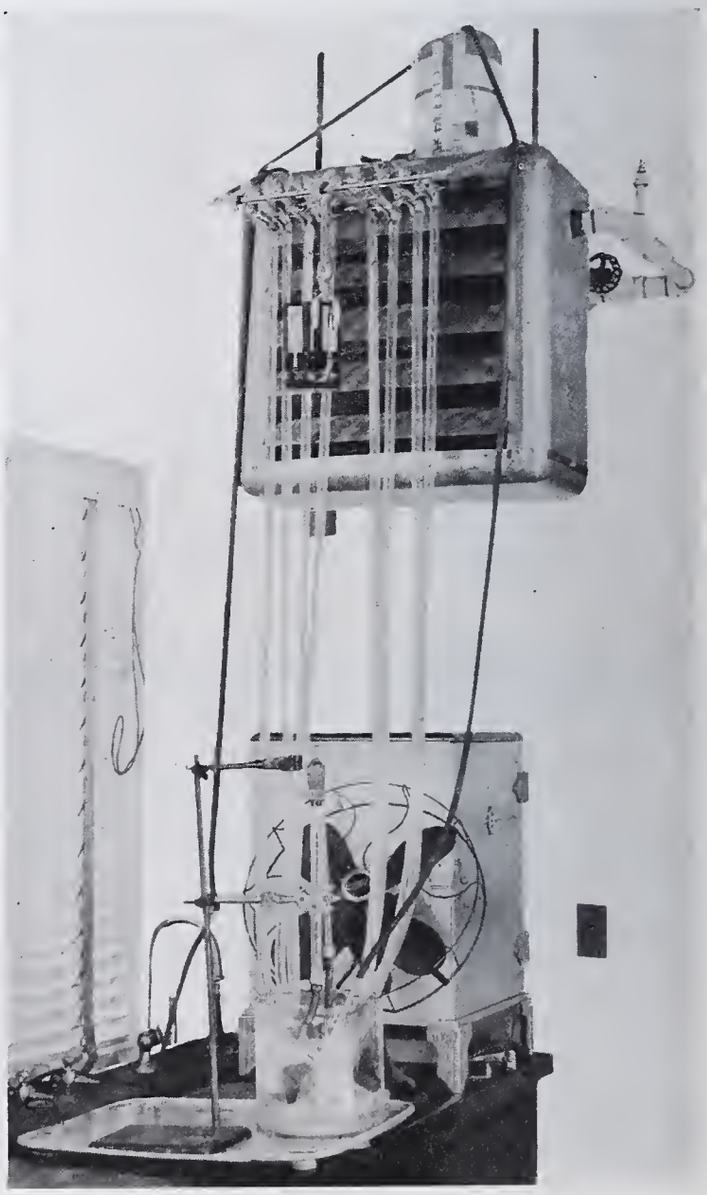

FIGURE 1. APPARATUS IN OPERATION

TABLE I. DATA ON DIALYSIS

Starting Material	Diameter of Casings	Temperature in Reservoir	Temperature in Casing	Temperature in Front of Unit Heater	Wet-Bulb Temperature	Temperature above Jar	Temperature in Jar	Tap Water Flow Rate	Fans	Heater	Duration of Experiment		Evaporation Rate	Final Sucrose Concentration		Sucrose Dialyzed Out
														Casings	Reservoir	
	Inch	°C.	°C.	°C.	°C.	°C.	°C.	*L./min.*			*Hours*	*Min.*	*L./hr.*	*G./100 ml.*		*G./hr.*
Distilled H_2O	18/32	24.5	18.5	29.5	17.8	28	21	0.99	..	..	1	20	0.23	..	..	..
	18/32	24.5	17.5	29.5	16	28.5	21	0.99	2 on	..	2	50	0.74	..	..	..
	18/32	27.5	28	64	28	35	22	0.99	2 on	On	1	10	1.29	..	..	..
	18/32	29	30	64	30.6	40	25	0.57	2 on	On	4	..	1.14	..	..	..
	18/32	25.5	19.5	29.5	18	29	22	0.54	2 on	..	1	..	0.57	..	..	..
	18/32	24.5	20	29.5	18	29	22	0.54	3 on	..	1	40	0.57	..	..	..
	18/32	29	22	29	20.6	29	22.5	0	..	..	16	..	0.12	..	..	..
	18/32	32	26	32	24	31	26	0	..	..	12	40	0.17	..	..	..
5% sucrose	18/32	24.5	18.5	30	17	28	21.5	0.99	..	..	2	10	0.17	4.68	4.85	11.5
	18/32	24.5	17.5	26.5	17	28	21.5	0.99	2 on	..	2	40	0.54	6.49	4.97	13.5
	18/32	27.5	27.5	64	28.5	32	22	0.99	2 on	On	3	..	0.95	9.21	4.97	13.3
5% sucrose	24/32	23.5	22	27	20.6	25	22	0.99	..	..	2	10	0.12	4.38	5.0[a]	18.9
	24/32	24.5	17.5	27.5	16	25	22.5	0.99	2 on	..	1	20	0.65	5.28	5.0[a]	23.3
	24/32	28	28	64	29	34	24.5	0.99	2 on	On	2	40	1.34	7.53	5.0[a]	25.9
Distilled H_2O	24/32	23	20	29	19	28.5	22	0.99	..	..	2	40	0.29	..	..	..
	24/32	23.5	18	28	16	27.5	21	0.99	2 on	..	3	30	0.76	..	..	..
	24/32	27	28	64	28	34	22	0.99	2 on	On	2	50	1.47	..	..	..

[a] Estimated figure.

of the casings at the same level. Thermometers were also placed in the dialyzing jar, 30 cm. (1 foot) above it, in the reservoir, and on the wall 180 cm. (6 feet) to one side of the apparatus.

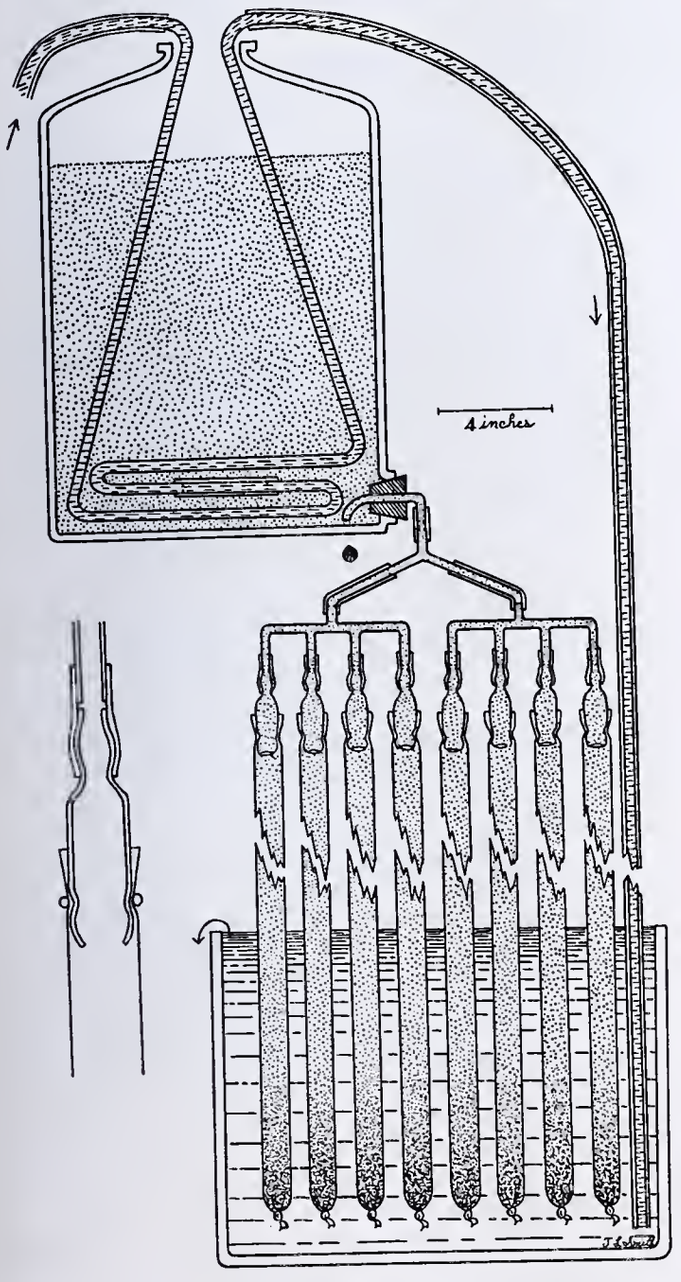

FIGURE 2. RESERVOIR, COOLING COILS, TUBE CONNECTIONS (WITH DETAIL), AND TUBES WITH LOWER ENDS DIALYZING

Table I shows that evaporation from the casings proceeded slowly without the use of fans. The rate of evaporation was tripled if two fans were allowed to blow air past the casings, provided humidity and room temperature remained about the same. The use of steam in the unit heater practically doubled the rate of evaporation over that when only two fans were used, but produced increased temperature within the casings. The presence of sucrose in the casings always decreased the rate of evaporation. The rate of dialysis depended to a considerable extent on the surface of casings immersed in the dialysis jar, and could be increased by using longer casings.

Among the advantages of this type of apparatus in the authors' laboratory are its low cost, its automatic operation, its high rate of concentration, the readiness with which it may be adapted to handle more or less material, the removal of dialyzable materials simultaneously with concentration, the gradual passage of nondialyzable material from the upper heated zone of concentration to the lower cooler zones of the casings (provided the nondialyzable material has a higher specific gravity than water), the continual supply of the most dilute material to the zone where the evaporation rate is highest, the ease of removing the material from the casings when operations are completed, and the small amount of equipment which needs cleaning. No trouble with foam, spray entrainment, or overheating such as may occur in vacuum distillations has been encountered with this apparatus. Disadvantages encountered may be: decreased evaporation rates when high humidity prevails, breakage of casings, collection of dust on the outside of the casings facing the fans, collection of some solids on the inside of the casings if the material is overconcentrated, and the slight heating of the material within the casings when steam is run into the unit heater.

Acknowledgment

The authors are indebted to Fred Blanchard for technical assistance in gathering these data.

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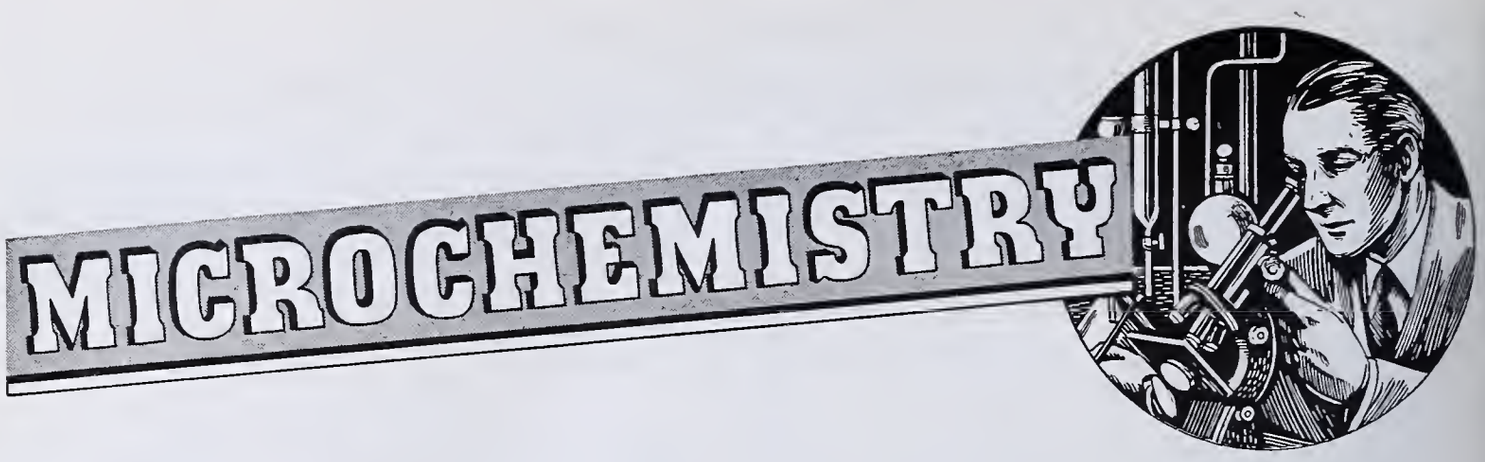

Photoelectrometric Particle-Concentration Analysis

Estimation of Anthraquinone-Beta-Sodium Sulfonate in Presence of Alpha-Isomer and of Anthraquinone-1,8-Disulfonic Acid in Presence of 1,5-Isomer

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THE microscopical quantitative analysis of heterogeneous mixtures (*1*) by methods such as simple estimation, comparison with known standards, or counting, while generally not affording the precision of most chemical analyses, is sometimes preferable to chemical methods because it is economical of time and materials. Sometimes the lack of pronounced chemical differences between the constituents of a mixture makes chemical methods of analysis impossible. Resort must then be had to physical methods, including microscopical methods. Microscopical observations are often fruitful in working with isomers which can be differentiated only by staining behavior, crystal form, refractive index, or other optical properties.

Of the various microscopical methods, counting methods are the most precise. A counting method has been reported from this laboratory for the analysis of anthraquinone-1,8-disulfonic acid in the presence of the 1,5-isomer (*2*). This was an extension of the classical methods of particle counting to the counting of crystals which have been precipitated as salts of the original substances. The precise salt which was to be precipitated was chosen in order to take advantage of differences in the optical properties, so as to be able to count one substance without interference by the others. In this paper a method is reported whereby the tedious and sometimes impossible task of counting is eliminated and instead the light due to the birefringence of the constituent to be estimated is measured, after suitable means have been taken to minimize light from the other constituents.

Photoelectrometric Principle

In specimens consisting of a mixture of anisotropic particles and amorphous or isotropic particles, the light which is transmitted by the specimen when placed between crossed Nicol prisms, and which is measured by a photosensitive element connected with a galvanometer, is proportional to the quantity of anisotropic material present, within definite limits of concentration. As in counting methods, a standard curve is first prepared for the analysis in question by analyzing a number of mixtures of known composition. Slides of samples being analyzed are prepared under standardized conditions, and the concentration of the constituent being estimated is read from the calibration curve by applying the galvanometer reading. The method is most useful for low concentrations of anisotropic material in the sample, which give the greatest precision and accuracy.

This principle was first tested on several mixtures of different concentrations of potato starch suspended in glycerol in order to see whether a regular relationship could be obtained between the light transmitted and the concentration of potato starch particles. This worked out as expected.

An attempt was then made to determine anthraquinone-1,8-disulfonic acid in the presence of the 1,5-isomer by means of precipitation with barium under conditions similar to those reported for the counting method (*2*) but using a photoelectric measurement instead of counting. A linear relationship was found to hold between the galvanometer reading and the concentration of 1,8-isomer, but the slope of the curve relating the galvanometer reading to the percentage of 1,8- was not favorable for a good precision. The point was proved, however, that one could carry over the counting method involving precipitated crystals to the photoelectrometric technique just as well as with preformed particles such as potato starch.

A method was nevertheless worked out for 1,8- in the presence of 1,5- by utilizing the manganese salt instead of the barium salt. This could not be done by precipitating the manganese salt of the 1,8- from aqueous solution because it was too soluble. It was necessary to dry the mixture of manganous acetate and disulfonic acids. The manganous acetate precipitates anthraquinone-1,8-disulfonic acid as anisotropic needles and the 1,5-isomer as an amorphous material in a manner similar to the action of barium chloride. The manganous acetate has, moreover, the advantage of drying to a transparent isotropic film when exposed to the air. (The authors are indebted for a knowledge of this useful property of manganous acetate to a private communication to this company from E. M. Chamot.) Thus, the excess manganous acetate present, which is added to a drop of the anthraquinonedisulfonic acid, will not interfere. The light transmitted by the 1,8- crystals gives galvanometer readings which are related by a linear function to the percentage of the 1,8- within certain limits of concentration.

A method of photoelectrometric particle-concentration analysis is described for the estimation of one constituent of a mixture which involves measuring, by means of a photosensitive cell and a sensitive galvanometer, the intensity of the light transmitted by that constituent. The value of the concentration is read from a standard curve. In general, the method should be capable of adaptation to any mixture of which one constituent can be made luminous and the other constituents invisible or of low luminosity by any means, such as by crossed Nicol prisms, selective staining with the use of color filters, or fluorescence. The technique may be used to replace counting particles and is less tedious, less subject to personal errors, and adaptable to mixtures where counting would be difficult and to routine analysis.

Specifically, the method has been applied to three problems: (1) to counting starch particles, as an illustration of its use with a classical example of a counting method for already formed particles; (2) to the estimation of anthraquinone-beta-sodium sulfonate in the presence of the alpha-isomer, in which case the beta-sodium salt does not itself serve as the source of transmitted light, but rather the precipitated crystals of the barium salt, which are luminous between crossed Nicol prisms, whereas the barium salt of the alpha-isomer forms an amorphous background; and (3) to the estimation of anthraquinone-1,8-disulfonic acid in the 1,5-isomer by means of a precipitation with barium, previously reported for use with a counting method, as well as by means of a precipitation with manganese. The effect of impurities upon the results has been studied.

The principle was next applied to the estimation of anthraquinone-beta-sodium sulfonate in the presence of the alpha-isomer. (Since both sodium salts occur as anisotropic plates, they could not be used directly for this method of analysis.) The barium salts were found to possess characteristics suitable for differentiation of the isomers. The barium salt of the beta-isomer appears as bright anisotropic plates, occurring singly and in clusters, whereas the alpha-barium salt is so much more insoluble that it appears as an amorphous precipitate under controlled conditions discussed below. It becomes a simple matter to measure the light transmitted by the beta-salt by means of a photosensitive element and a sensitive galvanometer.

Nomenclature, Theory, and Possible Developments of Method

The authors propose to apply the term "particle-concentration analysis" to that class of methods of analysis which is based upon an actual estimation of the density of distribution of the particles of one substance in the presence of others. These methods are to be differentiated from other methods (whether physical or chemical) which, on the one hand, involve an actual separation of one constituent from another, followed by weighing or some other means of estimation; or, on the other hand, do not require such a separation but depend upon measuring the concentration of one constituent by means of properties which are independent of any consideration of the presence of discrete particles of this constituent. In other words, the criterion for application of the term "particle-concentration analysis" lies in the fact that it is the concentration of discrete particles which is being determined.

Counting methods, either of preformed particles or of precipitated crystals, illustrate one type of method of particle-concentration analysis. By another method, described in this paper, the particle-concentration is determined by photoelectrometric means. In developing this method, no attempt was made to find conditions which would give the highest possible precision and accuracy, but rather to get a precision and accuracy which would be satisfactory for the purposes for which the methods were devised. It may, however, be desirable to consider in a general way what sources of error are inherent in the photoelectrometric method and to what extent the method could be improved.

Certain errors of technique or apparatus may arise, the correction or elimination of which should increase the accuracy.

For example, any lack of planeness in the slide or cover glass would cause local variations in the thickness of the layer examined which would lead to variations in the light transmitted. To attain the highest accuracy the glass surfaces would have to be tested or more fields examined. With needle crystals there is a possibility that the shear in the suspending medium as it spreads beneath the cover glass might cause a tendency toward parallel orientation, at least in some regions, which would give nonuniform readings. An examination of some of the authors' photomicrographs does not indicate that this has occurred in this work, but with long needle crystals this tendency might manifest itself. Possibly the easiest way to compensate for such a tendency would be to take the average of two readings in 45° positions of the stage. Another error would arise if there were a marked difference in refractive index between the particles and the medium. Even isotropic material might then appear bright between crossed Nicol prisms because of the scattering and depolarization of light at its surfaces. This would be a more important factor the finer the material. This effect could be completely eliminated if the refractive indices could be matched precisely. (The authors are indebted to C. W. Mason for the discussion of the points in this paragraph.)

Elimination of errors of apparatus or technique would lead to only a limited improvement in the attainable accuracy. The limit of improvement would be set by the uncertainty attached to the mean values upon which the standard curve is based as well as by the uncertainty of the unknown. This uncertainty is caused by the randomness of such factors as orientation of crystals, particle size, and particle distribution. Theoretically, this uncertainty can be reduced to any desired extent by averaging more and more values. Actually, one soon reaches a practical limit which is set by the fact that the uncertainty of the mean varies inversely as the square root of the number of values used in calculating it, so that the number of determinations necessary for a desired precision soon becomes prohibitive for an analyst to perform. There would seem to be a possible opportunity for the use of instrumentation in this respect, for if an automatic system could be devised (mechanical, electronic, or otherwise) it might be possible within a brief period of time to get a mean value from an enormous number of individual values and so attain a markedly enhanced precision.

Some other possible applications of the photoelectrometric methods suggest themselves. If one type of particle can be selectively stained, it should be possible by means of suitable color filters to measure that type to the exclusion of others. There may be differences in the fluorescent properties of the various particles which could be utilized. There may also be a possibility of measuring these by means other than photoelectrometric—possibly photographically or photochemically. Considerably farther in the realms of speculation would be the possibility of utilizing properties other than optical ones

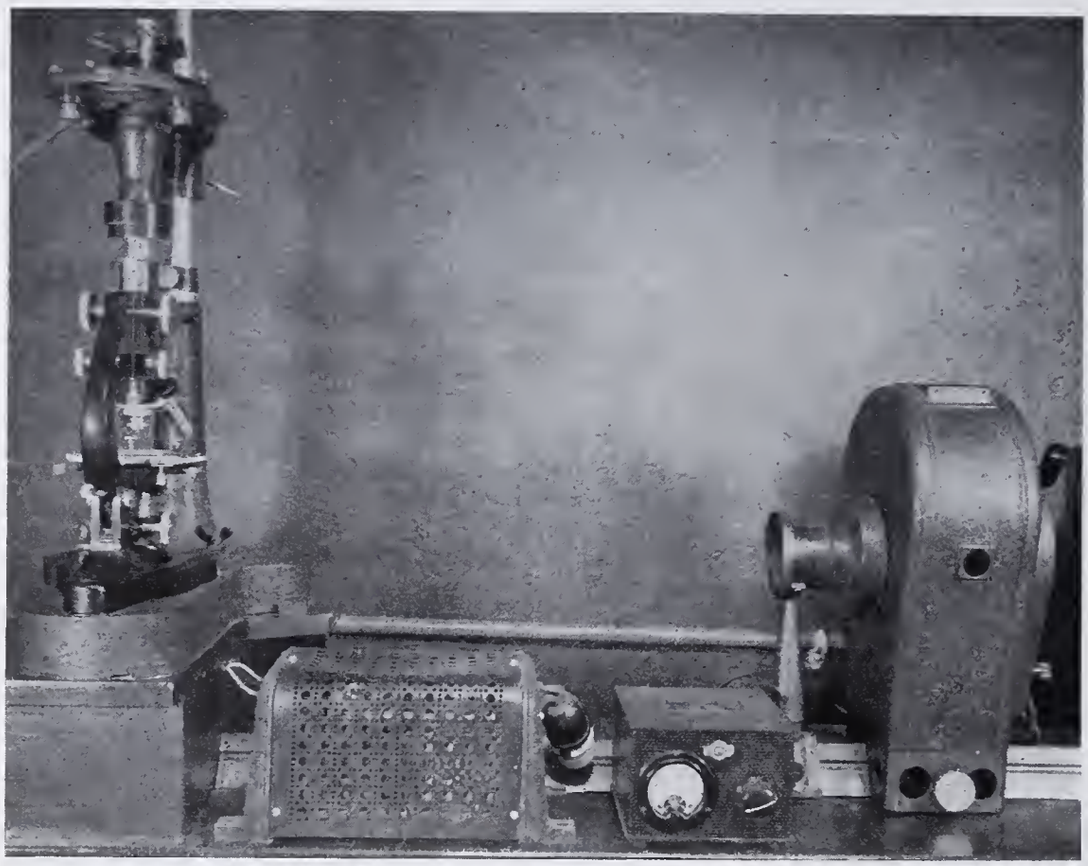

FIGURE 1. PHOTOELECTROMETRIC APPARATUS

for this purpose, as, for example, differences in magnetic permeability.

Apparatus

A Leitz petrographic microscope (Figure 1) (6 × P2 objective, 10 × p ⊕ ocular, no condenser, concave mirror) is set up on a Zeiss optical bench with a Leica rotating copying attachment to hold the self-generating, dry-disk photosensitive element. The cell is connected to a Leeds & Northrup Type R galvanometer (0.00013 microampere per mm.). The light source is a 30-watt, 6-volt lamp with a spiral filament, the amperage of which is regulated by means of a 6-ampere, 6-volt Leitz rheostat to about 4.3 amperes. A Raytheon voltage regulator (input 95/130 volts, output 115 volts, 60 watts) was used to cut down voltage fluctuations to a minimum. The light source center is set 79 cm. from the mirror center. A condenser lens is placed directly in front of the light source at such a point as to focus the filament on the microscope mirror.

In order to make it possible to reproduce standard lighting conditions, a standard starch slide, made up in Hyrax (Paul Ruedrich, manufacturing chemists), was prepared and a particular field was marked off by means of a scratch on the underside of the slide which is set on the cross hairs of the ocular. This apparatus was used merely because it was available; it could undoubtedly be simplified by using less expensive equipment and making it more adaptable to routine analysis. A fixed light source below the microscope stage as well as a microscope with fixed tube length, fixed magnification, and polaroid plates instead of Nicol prisms would be more practical where extensive use would be made of such a method as described here for the examination of one type of material.

Estimation of Potato Starch

The applicability of the photoelectrometric principle was first tested by means of potato starch suspensions in glycerol. Three concentrations were prepared and measured in a Howard mold-counting cell. Readings were made on different preparations of the same samples on three different days. Each time, the apparatus was dismantled and then reassembled. The measurements (Figure 2) thus indicate the variations which can be expected from all sources in using the apparatus.

Estimation of Anthraquinone-Beta-Sulfonate

CHEMICALS. The ordinary reagent grade of barium chloride dihydrate was used.

Anthraquinone-alpha-sodium sulfonate had been purified through the monomethylaniline salt which had been recrystallized to a constant melting point of 211.5° — 212.3° C. (corrected) and could be hydrolyzed to the acid melting at 213.8° — 214.9° C. (corrected).

Anthraquinone-1,5-disodium disulfonate was purified through the monomethylaniline salt which had a constant melting point of 248.5° — 250.0° C. (corrected).

Anthraquinone-2,7-disodium disulfonate was prepared from the crude compound by recrystallization from water.

Anthraquinone-beta-sodium sulfonate, anthraquinone-1,8-disulfonic acid, and anthraquinone-2,6-disodium disulfonate were prepared as described in (*2*). (Anthraquinone-alpha-sodium sulfonate, anthraquinone-1,5-disodium disulfonate, anthraquinone-beta-sodium sulfonate, anthraquinone-1,8-disulfonic acid, and anthraquinone-2,6-disodium disulfonate were prepared by W. J. Mader.)

PREPARATION OF SAMPLE SOLUTION. A 0.5-gram sample is weighed out into a 15 × 2.5 cm. (6 × 1 inch) test tube and the beta constituent is extracted with 10 ml. of boiling water, solution of the beta being aided by means of a stirring rod. After cooling the mixture to room temperature, the excess alpha is filtered off; the filtrate containing the more soluble beta is used in subsequent tests. It may be necessary to refilter one or more times in order to obtain a clear filtrate.

METHOD OF PRECIPITATION. *Concentrations of Beta 0.5 Per Cent and Higher.* Although two types of cells were tried, a brass

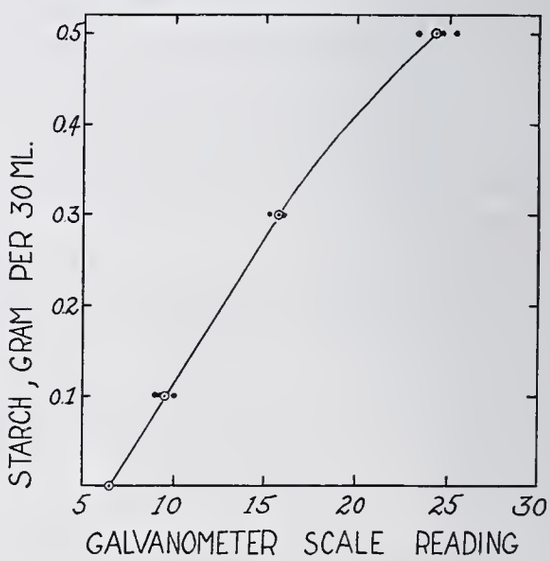

FIGURE 2. POTATO STARCH READINGS
⊙ Mean values
● Individual values

FIGURE 3. ANTHRAQUINONE-BETA-SULFONATE

Left. 2.5 per cent
Right. 1 per cent

ring and a Howard mold-counting cell, it was found unnecessary to use any of these for this work. The estimation may be carried out on an ordinary microscope slide. A drop of the filtrate is added to the slide by means of a 0.1-ml. pipet. One drop of 10 per cent barium chloride solution is then added directly to the first drop from a 10-ml. buret without stirring. After standing for 5 minutes, the drop is stirred with a small rod, scratching the slide and taking care not to spread the drop to a size greater than that of the 1.88-cm. (0.75-inch) cover glass. After an additional 5 minutes, the stirring is repeated and the drop is covered with a 1.88-cm. (0.75-inch) cover glass. Anthraquinone-alpha-barium sulfonate precipitates immediately, whereas the beta-isomer comes out only on standing. Readings are taken 30 minutes after addition of the barium chloride solution.

Under the conditions prevailing in this precipitation, a sample containing more than 2 per cent beta gave erratic and very often low results. A high beta content often tends to give many fine crystals and clusters of the barium salt instead of the usual fewer large crystals. Figure 3 (left) shows the type of field obtained with a high concentration of beta (2.5 per cent); Figure 3 (right) shows a field within the proper concentration range of beta (1.0 per cent). The greater variation in crystal size is also the explanation for the lower precision in the case of samples with a higher beta content. Thus, any sample containing more than 2 per cent beta must be run by using only a fractional part of the full sample. A sample showing a field filled with beta-barium salt and still giving a low light value, as well as any sample which is doubtful, should be repeated using a fractional sample. After observing a few slides representing decreasing sample weights with the difference in weight being made up with pure alpha, it becomes simple to pick out samples which are giving low results because of the physical condition of the barium beta salt. This reduction in the size of the sample is continued until a maximum percentage of beta is obtained; further dilution gives a constant percentage of beta within the lessened precision caused by multiplication of the error.

Concentration of Beta below 0.75 Per Cent. In order to obtain crystals for concentrations below 0.75 per cent beta, the slide must be allowed to come almost to dryness without a cover glass. The slide is stirred every 5 minutes as long as the rod leaves an even field. The drop is spread to a diameter of about 1.88 cm. (0.75 inch) and the readings must be made just before the preparation dries, which usually requires about 20 minutes. If the reading is made too late, this is obvious because the barium chloride crystallizes and the galvanometer needle goes off the scale.

ADJUSTING APPARATUS PRIOR TO MAKING READINGS. The lamp amperage is set at about 4.3 amperes and the filament is focused on the center of the mirror by means of the condenser lens and the lamp adjustment. The standard potato starch field is then set at the cross hairs. With the tube length set at 170 mm. and the Nicol prisms crossed, the maximum value is obtained for the field by manipulation of the mirror. The final adjustment is made by altering the lamp amperage to give a deflection of 28.5 cm. on the galvanometer scale from the zero position, which in all of this work was set at 5.5 cm. The 5.5-cm. position must be checked from time to time, for there is a gradual drift upward due to the heating action of the beam of light. Because of the difficulty, at times, in making the 28.5-cm. adjustment precisely, no attempt was made to adjust it any closer than between 28.5 and 29.0 cm.

METHOD OF TAKING READINGS. Three slides for each sample and standard were prepared and nine readings were taken on each, three fields across the top, three fields across the center, and three fields across the bottom. The optical system should be checked against the standard starch field before taking readings on each slide and the lamp should be on at least for 30 minutes before the readings are made in order to allow the filament to come to equilibrium. It is not necessary to return the galvanometer to zero after each slide is read. Occasionally the galvanometer suddenly jumps to give an unusually high reading, or it jumps off the scale because some fibrous material is included in the field. Such fields must be examined and discarded if the high reading is caused by the presence of foreign matter. Before readings were made for each slide, the particular field was refocused in order to take care of possible thickness differences, although it was found that the effect of neglecting this precaution was negligible.

STANDARDIZATION CURVES. Table I gives the mean galvanometer readings for a number of determinations with various percentages of pure beta mixed with pure alpha. Each mean reading is accompanied by the number of values from which it was obtained. Each individual value is itself the mean of nine readings on one microscope slide preparation.

In the case of values obtained with the high concentration method, a straight line was constructed by the method of least squares. Figure 4 gives the straight line, including the individual values from which the mean values were calculated.

TABLE I. STANDARD MIXTURES OF ALPHA AND BETA SALTS

% Beta	Mean Galvanometer Reading	No. of Readings	Standard Deviation of Single Reading (± % Beta)[a]
	Method Used for 0.5% and Higher		
0.50	23.1	17	0.12
0.63	23.4	6	0.22
0.75	25.8	8	0.17
0.88	26.7	12	0.15
1.00	29.4	16	0.24
1.25	30.6	10	0.26
1.50	35.0	8	0.27
1.75	37.1	10	0.33
2.00	38.1	17	0.37
2.25	37.3	9	0.50
2.50	45.3	8	.38
	Method Used for Less Than 0.75%		
0.25	20.0	12	0.05
0.50	25.8	9	0.08
0.75	30.4	10	0.05

[a] Standard deviation for mean would be that for a single reading divided by square root of number of readings.

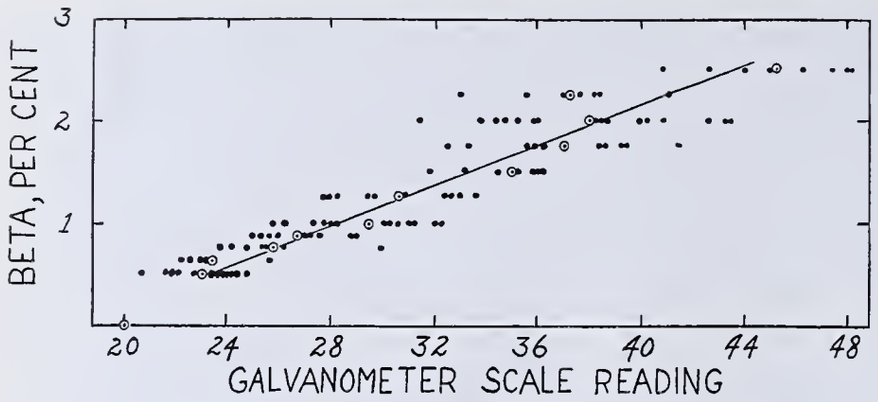

FIGURE 4. CALIBRATION CURVE
Per cent beta between 0.5 and 2.0
⊙ Mean values
● Individual values

Between beta concentrations of 0.5 to 2.0 per cent, the beta content can be calculated by the following equation which is derived from the curve:

$$\% \text{ beta} = \frac{\text{galvanometer scale divisions} - 18.2}{10}$$

In the case of the low concentration method, a straight line was drawn through the mean values as shown in Figure 5. The beta content for this range of concentration can be calculated by the following equation which is derived from the curve:

$$\% \text{ beta} = \frac{\text{galvanometer scale divisions} - 15.2}{20.6}$$

ACCURACY. From the deviations of the individual values from the curves, the standard deviation of a single value has been calculated. This refers to the accuracy, since the deviations are from known values. The precision would frequently be better than the accuracy, but it is not reported because it is of minor importance in this connection. The standard deviation was calculated separately for each concentration and is given in Table I. There is an upward trend of the standard deviation with higher beta concentrations, as would be expected.

In Table II are given results for some commercial samples analyzed. The percentage of beta found for commercial samples is often lower than the percentage of beta added, undoubtedly because of traces of impurities. The precision for commercial samples is of the same order of magnitude as that of pure mixtures.

CORRECTION FOR BACKGROUND. The 100 per cent pure alpha samples gave galvanometer readings of 20.1 ± 0.8 divisions (standard deviation). Some commercial batches, when precipitated and the readings taken immediately, gave blank values which were less than 19.3 (20.1 − 0.8). For this reason, a correction must be applied whenever the blank value differs from 20.1 by more than the standard deviation of 0.8 division. This correction takes care of differences in background due to impurities. The blank values are obtained by taking readings before the beta-barium salt starts to come out. It is, in reality, the light value of the field containing everything but the beta. Samples 1 and 3 of Table II were corrected for background. Samples 2 and 4 did not require corrections.

FACTORS AFFECTING READINGS. Stirring of the preparations was found essential in order to obtain uniform slides, while seeding by scratching gave larger crystals of the beta and eliminated erratic slides.

After standing for 30 minutes, the high concentration slides were always found to have reached equilibrium. Shorter times were likely to cause lower values. In the case of the low concentration method where the preparation was left uncovered, time is an important factor, as shown by the following readings for a 0.75 per cent beta sample: At 10, 15, 20, and 25 minutes after the precipitation the readings obtained were 22.4, 23.4, 24.3, and 25.0 cm., respectively. The slide started to go to dryness at the beginning of the 25-minute reading and all subsequent readings were much too high, giving deflections of the galvanometer which were off the scale. For this reason, the readings must be made just before going to dryness.

Impurities have a marked effect on readings, especially some of the disulfonic acid derivatives of anthraquinone. Sodium chloride has no serious effect. Table III shows the effects of sodium chloride and of some of the anthraquinonedisulfonic acids which were added to known mixtures of pure alpha and beta in the dry state.

Estimation of Anthraquinone-1,8-Disulfonic Acid as Barium Salt

The primary purpose of this work was to see whether the counting method (*2*) previously described (involving the

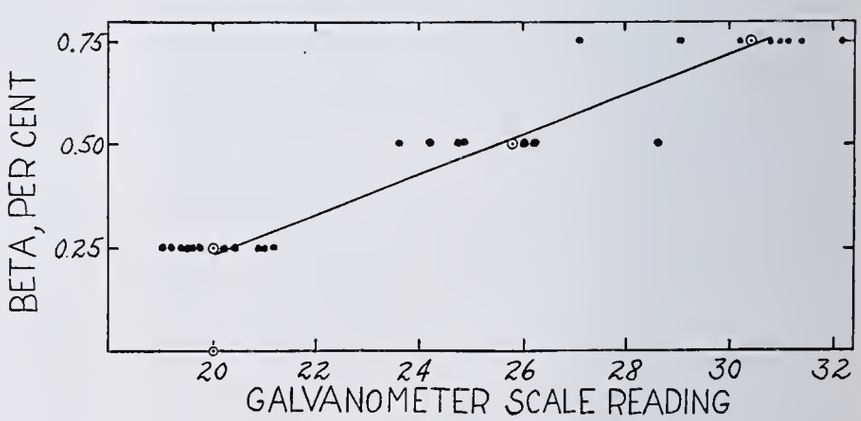

FIGURE 5. CALIBRATION CURVE
Per cent beta below 0.75
⊙ Mean values
● Individual values

TABLE II. ANALYSES OF COMMERCIAL SAMPLES OF ANTHRAQUINONE-ALPHA-SODIUM SULFONATE

Sample No.	Beta Added to Sample %	Beta Found %
1	0	0.0 (corrected for background)
	0.5	0.5 (corrected for background)
	0.7	0.5 (corrected for background)
	1.0	0.8 (corrected for background)
	1.5	0.9 (corrected for background)
2	0	0.0
	0.5	0.3
	0.7	0.7
	1.0	0.8
	1.5	1.2
3	0	0.8 (corrected for background)
	1.0	1.6 (corrected for background)
4	0	0.0
	1.0	0.7

Table III. Effects of Impurities on Values for Beta Content

Beta Present	Impurities Added					Beta Found
	NaCl	1,5-	1,8-	2,6-	2,7-	
%	%	%	%	%	%	%
1.5	2.0	...	...	...	...	1.4
1.0	2.0	...	...	...	...	0.9
0.5	2.0	...	...	...	...	0.7
2.0	...	1.0	...	...	...	1.1
2.0	...	0.5	...	...	...	0.9
1.0	...	1.0	...	...	...	0.5
1.0	...	0.5	...	...	...	0.5
0.5	...	1.0	...	...	...	0.0
0.5	...	0.5	...	...	...	0.0
2.0	...	...	1.0	...	...	0.1
2.0	...	...	0.5	...	...	0.2
2.0	...	...	0.2	...	...	0.7
1.0	...	...	1.0	...	...	0.0
1.0	...	...	0.5	...	...	0.0
1.0	...	...	0.2	...	...	0.3
0.5	...	...	1.0	...	...	0.0
0.5	...	...	0.5	...	...	0.0
0.5	...	...	0.2	...	...	0.0
1.0	...	...	...	1.0	...	0.3
1.0	...	...	...	0.5	...	0.2
1.0	...	...	...	0.3	...	0.4
1.0	...	...	...	0.1	...	0.3
0.5	...	...	...	0.5	...	0.0
1.0	...	...	...	...	0.5	0.2
1.0	...	...	...	...	0.3	0.3
1.0	...	...	...	...	0.1	0.2

preliminary precipitation of the barium salt of the 1,8- acid in the form of small needles against an amorphous background of barium sulfate) could be replaced by a photoelectrometric estimation of the precipitated needles. The method of precipitation of the crystals in preparation for the photoelectrometric estimation, the method of sampling of the precipitated crystals, and the microscopic cell which was used were the same as outlined in the previous paper. Instead of a calibration curve based on counting crystals, one was prepared which was based on photoelectrometric measurements in accordance with the technique described in the section on estimation of anthraquinone-beta-sodium sulfonate in the presence of the alpha-isomer. Figure 6 gives the curve obtained for known mixtures containing 0, 2, 5, and 10 per cent, respectively, of 1,8- acid.

The 1,8-anthraquinone-disulfonic acid content can be calculated by the following equation which is derived from the curve:

$$\% \text{ 1,8-} = \frac{\text{galvanometer scale divisions} - 17.6}{0.26}$$

Attempts were made to increase the sensitivity of the method by increasing the number of needles per field for a given concentration by a decrease in the dilution of the sample, but without success because of the formation of clusters of crystals (most likely 1,5-barium salt). Higher concentrations of barium chloride caused the same effect. The barium chloride was then replaced as a precipitant by manganous acetate. The accuracy and precision of this method, together with the accuracy of the manganese method, are discussed in the next section.

Effect of Impurities. It was assumed that the study of the effect of impurities which was made previously (*2*) for the counting method would apply here. The possibility that the calibration curve which was made up on the basis of the free acids might not apply for the sodium salts was investigated and it was found that substitution of sodium salts for the free acids did not influence the crystal count.

Estimation of Anthraquinone-1,8-Disulfonic Acid as Manganese Salt

Chemicals. The manganous acetate, sodium chloride, and sodium sulfate were C. P. grade. The remaining chemicals have been described under the estimation of anthraquinone-beta-sulfonate.

Reagent Solutions. The anthraquinone-1,8-disulfonic acid, anthraquinone-1,5-disulfonic acid, anthraquinone-2,6-disodium sulfonate, anthraquinone-2,7-disodium sulfonate, sodium chloride, and sodium sulfate were dissolved to make solutions containing 1 gram in 100 ml. of solution. Anthraquinone-alpha-sodium sulfate and anthraquinone-beta-sodium sulfonate were made up to contain 0.5 gram per 100 ml. of solution. The sulfuric acid was 0.1 *N;* 0.4 ml. equals 2 mg. of sulfuric acid.

Standard Solutions. Mixtures of the reagent solutions were made up so that each solution contained 10 mg., distributed between the sulfuric acid and the various sulfonic acids. All solutions had 0.4 ml. of 0.1 *N* sulfuric acid plus 8 mg. of total sulfonic acids. The various solutions of known sulfonic acid content were prepared by mixing appropriate volumes of the solutions named.

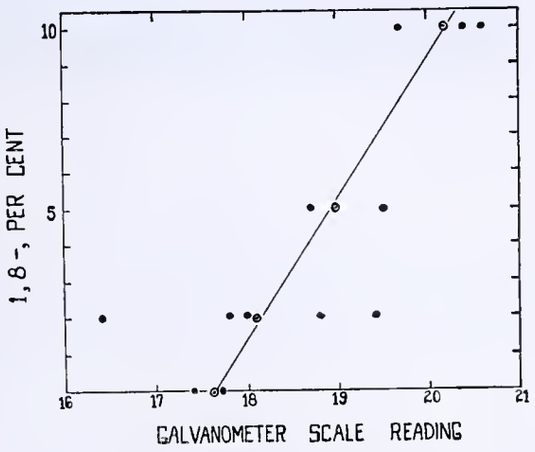

Figure 6. Estimation of Anthraquinone-1,8-Disulfonic Acid by Barium Chloride

⊙ Mean values
● Individual values

Preparation of Sample Solution. Solutions of actual samples were made up by dissolving sufficient sample in water to make a solution containing the equivalent of 10 mg. of sulfonic acids and sulfuric acid in from 1.36 to 1.37 ml.

Method of Precipitation. The estimation was carried out on a microscope slide. Three precipitations were made on each slide. A drop of the sample solution was placed on the slide by means of a 0.1-ml. pipet in a 19-mm. ring of vaseline formed with a piece of 19-mm. tubing. One drop of manganous acetate solution (10 grams per 100 ml. of solution) was then added by means of a10-ml. buret without stirring. Each preparation was stirred after standing for 5 minutes under the warmth of an electric bulb, and every 5 minutes thereafter as long as the rod left an even field. Readings can be made immediately after drying and up to 24 hours later.

Samples containing less than 4 per cent of 1,8- do not form crystals and it is necessary to add 1,8- to the sample to bring the 1,8- content above 4 per cent. The values obtained with samples containing more than 10 per cent of 1,8- are erratic, so that it is necessary with such samples to mix them with known amounts of 1,5-.

Figure 7 shows samples containing 5 and 10 per cent 1,8-, respectively.

Adjusting Apparatus Prior to Making Readings. The adjustment of the apparatus was the same as for the determination of anthraquinone-beta-sodium sulfonate, except that the zero point was adjusted to the actual zero on the scale for convenience and the standard starch field was made to give a deflection of 23.0 cm.

Method of Taking Readings. In this analysis, much higher readings were obtained than for the anthraquinone-beta-sodium sulfonate method, and in samples containing 9 per cent and more of 1,8-anthraquinonedisulfonic acid, it was necessary to alter the shunt resistance of the galvanometer. The resistance was increased approximately 10 times, thereby decreasing the deflection approximately 10 times. The reading was then recalculated to the basis of the original resistance. The reading obtained with the high resistance was corrected

FIGURE 7. ANTHRAQUINONE-1,8-DISULFONIC ACID
Left. 5 per cent
Right. 10 per cent

to give readings corresponding to those obtained with the low resistance by applying the proper factor.

STANDARDIZATION CURVES. Figure 8 gives the mean galvanometer readings for a number of determinations with various percentages of pure 1,8- mixed with pure 1,5- and sulfuric acid. Each individual value from which the mean values were determined is the average of three separate determinations of nine fields each from a single solution. The percentage of 1,8- may be calculated by the formula:

$$\% \text{ 1,8-} = \frac{\text{galvanometer scale reading} + 9.0}{6.8}$$

EFFECT OF IMPURITIES. With samples having concentrations of 1,8- of about 5 per cent in addition to 1,5- and sulfuric acid, the effect of 1 per cent of impurities such as the sodium salts of 2,6-, 2,7-, alpha-, and beta-anthraquinonesulfonic acids, sodium chloride, and sodium sulfate is very serious. In one series of experiments, the 2,6- and beta produced black fields containing no 1,8- crystals; the 2,7- and the sodium sulfate showed a few 1,8- crystals in addition to other anisotropic crystals; and alpha and sodium chloride showed many fine particles which were definitely not 1,8- crystals. Samples containing 0 per cent 1,8- with 1,5- and sulfuric acid and 1 per cent of impurities showed dendrites and crystallized clusters which would not be confused with 1,8-, but nevertheless interfered with the analysis. In mixtures containing 10 per cent of 1,8- acid, no interference was noted except in the case of sodium sulfate. Table IV shows the effects on the values of the impurities studied.

The effect of impurities upon this method in some cases is due to the crystallization of chemicals other than the 1,8-manganous salt when these impurities are present in too high a ratio to the 1,8-. In other cases, impurities have a tendency to prevent the crystallization in the anisotropic form of the 1,8-manganous salt. In order to be certain that impurities are not lowering the values obtained for 1,8- content, successive known quantities of 1,8- should be added, and the 1,8- found by the analysis should check the amount added plus the amount found originally. A doubtful sample may be checked by increasing the ratio of 1,8- to impurity (by adding pure 1,8-) for, as shown in Table IV, no serious interference takes place when the ratio of impurity to 1,8- is no more than 1 to 10 except in the case of sodium sulfate. A sample showing dendrites or other crystalline forms not characteristic of 1,8- must likewise be rerun with a known quantity of 1,8- added to overcome the influence of impurities present.

TABLE IV. EFFECTS OF IMPURITIES

(Manganous acetate method for anthraquinone-1,8-disulfonic acid)

1,8- Present	Impurities Added						1,8- Found
	2,6-	2,7-	β-	α-	NaCl	Na_2SO_4	
%	%	%	%	%	%	%	%
10	1.0	...	...	...	...	...	10.2
10	...	1.0	...	...	...	...	10.7
10	...	...	1.0	...	...	...	9.9
10	...	...	...	1.0	...	...	9.2
10	...	...	...	...	1.0	...	10.2
10	...	...	...	...	...	1.0	11.8

TABLE V. ACCURACY OF METHODS FOR ANTHRAQUINONE-1,8-DISULFONIC ACID

1,8- Present	Standard Deviation of Single Reading, 1,8-	
	Barium method	Manganese method
%	± %	± %
0	0.7	...
2	4.8	...
4	...	0.3
5	2.1	0.6
7	...	0.5
9	...	1.9
10	2.8	1.5

Comparison of Barium and Manganese Methods

Table V gives a comparison of the accuracy of the values read from the curves by the barium and the manganese methods. The manganese method is more accurate than the barium method. Because of the good agreement of the mean values with the straight line drawn through them, the precision of this method is of about the same order as the accuracy. The effect of the errors involved in the adjustment of the apparatus on the values by the barium method is much greater than for the manganese method.

The barium method has an advantage that concentrations of 1,8- below 4 per cent can be analyzed. Although the concentration range for analysis is limited, the manganese method is much more rapid because it requires only a short time for the preparation of the sample. Because of this factor, it would be possible to run a number of determinations rapidly and thus obtain an accuracy for the mean value which would be equal to the standard deviation divided by the square root of the number of values.

Effect upon Precision of Variations in Adjusting the Apparatus

A standard potato starch field was marked off and readings were taken by removing the slide after each reading in order

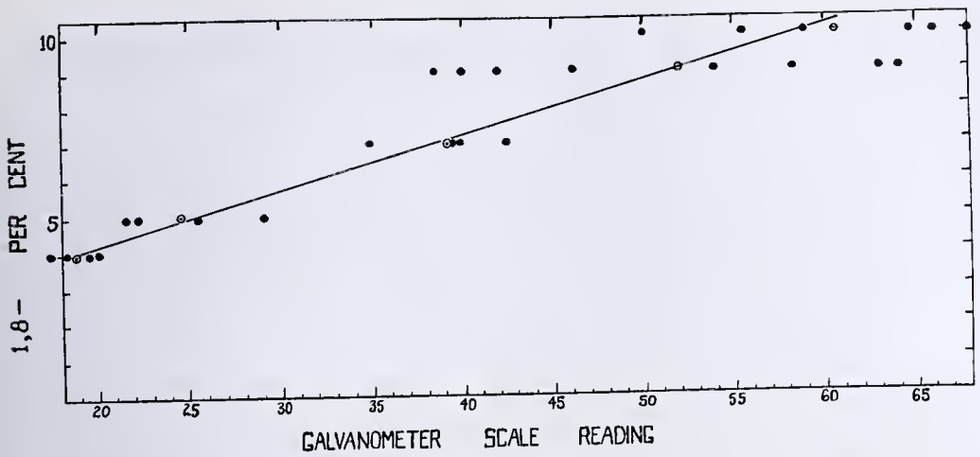

FIGURE 8. ESTIMATION OF ANTHRAQUINONE-1,8-DISULFONIC ACID BY MANGANOUS ACETATE

⊙ Mean values. ● Individual values

to check the exactness with which the field could be found. The mean of nine readings was 27.7, and the standard deviation 0.80.

When the mirror was reset but the slide was not moved after each observation, in order to test this operation, the average of nine readings was 27.7 and the standard deviation 0.35.

Next, a series was run by shutting off the lamp but not disturbing anything else. Out of nine readings, it was found necessary to readjust the amperage to 4.6 four times. The average of nine readings was 26.5, and the standard deviation 0.35.

These errors, which represent those encountered in the analysis of preformed particles, give a net standard deviation for a single reading of $\sqrt{0.80^2 + 0.35^2 + 0.35^2}$, or 0.94 divisions. In terms of anthraquinone-beta-sodium sulfonate this corresponds to 0.1 and 0.05 per cent, respectively, for the high and low concentration methods of analysis. In terms of anthraquinone-1,8-disulfonic acid by the barium method this corresponds to 3.5 per cent and by the manganese method to 0.1 per cent. The standard deviation for precipitated particles by the photoelectrometric method varies with the particular method in question, and for a definite method it also varies with the concentration of the substance to be determined. As the concentration increases, there is likely to be more variation in the size and number of crystals with a consequent variation in the precision.

Acknowledgments

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A Fuzz Detector for Viewing Glass Weighing Vessels in Organic Quantitative Microanalysis

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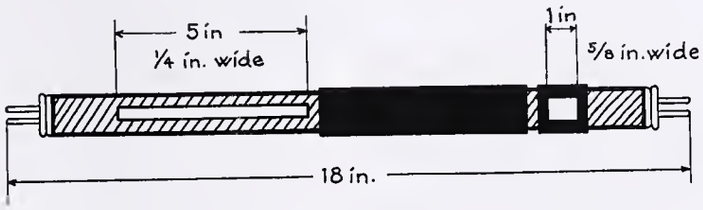
THE fuzz detector is a viewing light devised for detecting foreign material on the surface of glass vessels. It has been found very useful in the inspection of micro absorption tubes and other micro glassware for cleanliness prior to weighing. As shown in the accompanying figure, the detector consists of a standard 18-inch, 15-watt fluorescent lamp (white or daylight type) covered with several coats of black enamel, except for two openings. The opening used for the viewing of carbon and hydrogen absorption tubes is 5 inches long and 0.25 inch wide and the other opening, used for shorter objects, is 1 inch long and 0.625 inch wide. A rotating tube made of heavy black paper placed around the lamp permits either opening to be covered while the other one is in use.

For the detection of fuzz on absorption tubes, filter tubes, etc., the tube are held up in front of the lamp about 0.5 inch from the narrow opening, so that the operator cannot see the light source but only the light coming over the surface of the tube. When the tubes are rotated slowly, any lint or foreign material on the glass surface appears sharply outlined against the black background of the painted portion of the lamp. The surface of the glass is lightly stroked with chamois while still under observation and the successful removal of lint and foreign material can be readily observed. Foreign material in the tip ends of absorption tubes is easily removed by using a steel wire with a knurled end.

The smaller opening is very useful for viewing glass vessels such as weighing bottles, Rast molecular weight tubes, chemical samples, etc. In the Rast molecular weight determination the tube can be carefully checked for any foreign material before the first weighing and again after each addition of sample and of camphor to ensure that none of the material remains in the upper part of the tube.



Semimicrochemical Assay for Diethylstilbestrol

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SINCE the discovery of diethylstilbestrol (alpha, alpha'-diethyl-4,4'-stilbenediol) by Dodds *et al.* (*2*) in England, the use of this potent estrogen has spread throughout the world.

The bioassay of diethylstilbestrol by the well-known Allen and Doisy method is both time-consuming and uneconomical; therefore a chemical procedure is needed which will serve as an analytical tool. [Since this paper was prepared a colorimetric method for the assay of diethylstilbestrol has been described by Dechne, (*1*).] Sprung (*4*) and Francis and Hill (*3*) describe a bromometric titration method for phenols, using standard bromide-bromate solution which may be applied to this drug. Bromination of known quantities of diethylstilbestrol (Table I) has established that the method is suitable for determining diethylstilbestrol in the amounts normally found in pharmaceutical preparations (1 to 40 mg.)

Table I. Bromination of Known Amounts of Diethylstilbestrol

(Dried at 100° C.)

Weight of Sample	Diethylstilbestrol Found	Recovery
Mg.	*Mg.*	%
1	0.960	96.0
1	0.982	98.2
2	2.05	102.5
2	1.94	97.0
5	4.98	99.6
5	4.91	98.2
5	5.00	100.0
10	9.92	99.2
10	9.81	98.1
10	10.18	101.2
40	39.81	99.55
40	40.06	100.15
40	40.06	100.15
40	40.37	100.93

Method

Analysis of Tablets. Weigh not less than 20 tablets; reduce them to a fine powder, and place an accurately weighed aliquot equivalent to about 10 mg. of diethylstilbestrol in a micro-Soxhlet extractor. Extract with ether for 1 hour, transfer the extract to a 125-cc. glass-stoppered iodination flask, and evaporate to dryness on a water bath. Dissolve the residue in 5 cc. of warm aqueous 2.5 per cent sodium hydroxide solution, cool, and add 10 cc. of standard U. S. P. XI bromide-bromate solution (0.1 *N* bromine solution) by means of a pipet.

Quickly add 5 cc. of hydrochloric acid, insert the stopper, and place 5 cc. of 10 per cent potassium iodide solution around the stopper. Set aside in a dark place for 30 minutes at 25° to 30° C. Remove the stopper just sufficiently to introduce the potassium iodide solution and shake the flask thoroughly, taking care that no bromine vapor escapes. Carefully rinse the stopper and the neck of the flask with a little distilled water and then titrate the liberated iodine with 0.05 *N* sodium thiosulfate solution, using starch indicator at the end of the titration.

Each cubic centimeter of bromide-bromate solution is equivalent to 2.233 mg. of diethylstilbestrol.

The presence of material capable of bromination will seriously affect the accuracy of the assay—for example, 60 mg. of stearic acid U. S. P. may contain sufficient oleic acid as impurity to introduce an error of 8 per cent in a determination of 10 mg. of diethylstilbestrol. Consequently, a blank determination on the filler and lubricant material in tablets should be made.

Oil Solutions of Diethylstilbestrol. Accurately measure a sample containing 5 mg. of diethylstilbestrol into a separatory funnel, add 30 cc. of light petroleum ether, and extract 4 times with 2.5 per cent sodium hydroxide solution, using two 10-cc. and two 5-cc. portions. Pass the extractions through a small funnel containing a plug of cotton into a 125-cc. glass-stoppered iodination flask. Add 5 cc. of 0.1 *N* bromide-bromate solution and proceed as directed for the tablets, beginning with "Quickly add 5 cc. of hydrochloric acid".

Glycerogelatin Suppositories of Diethylstilbestrol. Dissolve in hot water a sufficient quantity of the suppositories to yield 5 mg. of diethylstilbestrol, cool, and acidulate with dilute sulfuric acid. Extract 4 times with ether, using 20-cc., 15-cc., 15-cc., and 10-cc. portions, and pass through a funnel containing a plug of cotton into a suitable vessel. Evaporate the extract nearly to dryness and pass steam over the residue for 1 hour to volatilize and remove any phenolic preservative present in the gelatin. Evaporate most of the water and dissolve the residue in 2.5 per cent sodium hydroxide. Transfer to an iodination flask, add 5 cc. of 0.1 *N* bromide-bromate solution, and proceed as directed for the tablets, beginning with "Quickly add 5 cc. of hydrochloric acid".

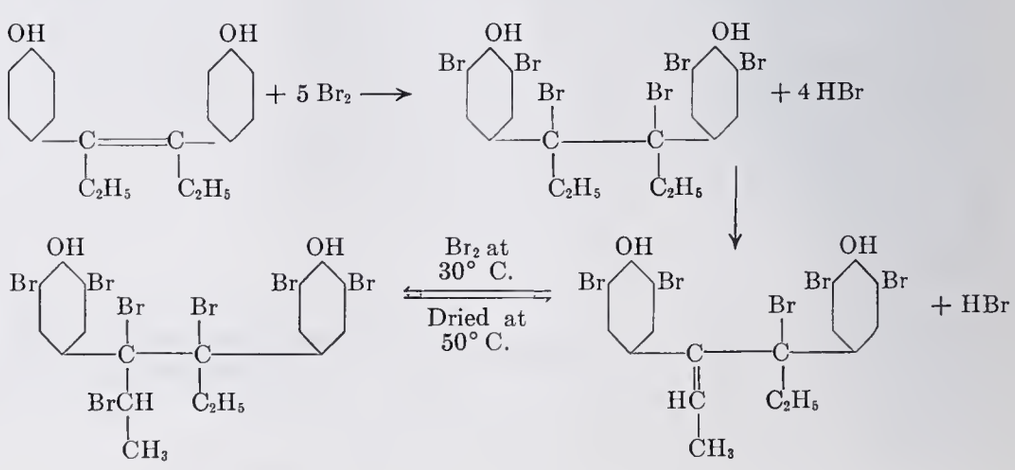
Table II. Temperature-Time Factor in Bromination of Diethylstilbestrol

Temperature	Time	Bromine Used
° *C.*	*Min.*	*Atoms*
5–10	60	9.9
5–10	180	10.0
5–10	380	10.2
5–10	960	11.6
15	15	9.5
15	30	11.0
20	15	10.0
20	30	11.2
25	15	11.0
25	30	12.0
30	15	12.0
30	30	12.0
30	360	12.0
60	60	12.0

From the brominations shown in Table II it is evident that diethylstilbestrol in a cold solution first removes ten atoms of bromine from solution. The removal of two atoms more takes place slowly in the cold, rapidly at higher temperatures. The removal of ten atoms can be made consistent by controlling the time and temperature, but since twelve atoms will eventually be removed if the reaction goes to completion it seems more logical to brominate for 30 minutes at above 25° C.

Discussion

Under the conditions described in the analytical procedure, twelve atoms of bromine enter into the reaction, accompanied by the release of 5 moles of hydrogen bromide for each mole



of diethylstilbestrol. Presumably the bromination product contains seven atoms of bromine. Studies made with 4,4′-dihydroxystilbene indicate that four of these are located in the 3,5- and 3′,5′-positions. It would be expected that simultaneously two atoms of bromine would add at the double bond. The splitting out of one of these bromine atoms as hydrogen bromide would provide a vinylene linkage into which two more bromine atoms could enter. The diagram appears to explain the data in the most satisfactory manner.

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Separation of Copper, Lead, and Zinc with Salicylaldoxime

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THE separation of copper from other metals which form complexes with salicylaldoxime is possible because copper salicylaldoximate may be precipitated in weakly acidic solutions, whereas the salicylaldoximates of most other metals are precipitated only from neutral or slightly basic solutions (*1–6*, *10*). Lead has been separated from silver, zinc, and cadmium by precipitation in strongly ammoniacal solution (*7*). The effect of hydrogen-ion concentration upon the precipitation of zinc with salicylaldoxime has been reported briefly (*5*).

The purpose of the present investigation was to make a more detailed study of the effect of pH on the precipitation of zinc salicylaldoximate, and to determine the best conditions for a separation of copper, lead, and zinc based upon pH control and ammonia-complex formation.

Precipitation of Zinc Salicylaldoximate

The fact that zinc forms an insoluble complex with salicylaldoxime was first reported by Ephraim (*4*). Pearson (*9*) studied the properties of the precipitate and concluded that it was not suitable for the quantitative determination of zinc because the pH range for complete precipitation was too narrow, the precipitate was appreciably soluble in various neutral salt solutions, and the compound slowly decomposed above 80° C. The compound precipitated by Pearson contained 19.45 per cent zinc by analysis and evidently was $Zn(C_7H_6O_2N)_2$, which contains 19.36 per cent zinc. Flagg and Furman (*5*) found that if zinc salicylaldoximate, together with the solution from which it has been freshly precipitated, is warmed to 90° C. for 10 minutes, a compound corresponding to $Zn(C_7H_5O_2N)$ results. This compound, containing 32.61 per cent zinc, can be dried at 110° C. without decomposition. Since the compound obtained by Flagg and Furman is obviously more suitable for quantitative purposes, the work described below is based upon precipitation of the compound $Zn(C_7H_5O_2N)$.

Reagents and Apparatus. The salicylaldoxime used was obtained from the Eastman Kodak Company. A 1 per cent solution was prepared by dissolving 1 gram of the reagent in 5 ml. of alcohol and slowly pouring the alcohol solution into 95 ml. of water warmed to 80° C. The reagent solution was cooled and filtered before use.

The zinc solution was prepared from reagent grade zinc nitrate. It was standardized by precipitation and weighing as $ZnNH_4PO_4$, and by precipitation as $ZnNH_4PO_4$ and ignition to ZnP_2O_7.

A glass electrode pH meter as described by Mellon (*8*) was used. It was calibrated with Clark and Lubs buffer solutions.

The reagents used in standardization of the zinc solution, those used in preparation of the buffers, and the ammonia used for adjustment of pH were all of reagent grade.

Calibrated weights and volumetric ware were used. Redistilled water was employed for the preparation of all solutions, for dilutions, and for washing the precipitates.

Precipitation and pH Measurement. In each case, 25 ml. of a 1 per cent salicylaldoxime solution were added dropwise to 25 ml. of zinc solution containing 0.0999 gram of zinc and ammonia in an amount estimated to give the desired pH. Water was added to make 100 ml. The mixture was mechanically stirred during addition of the reagent. After all the reagent had been added, solution and precipitate were heated to about 90° C. for 10 minutes, cooled to room temperature, filtered with suction on No. 4 Jena glass crucibles, washed with water, and dried at 110° C. for 1 hour. Ferric chloride solution was used to test for complete washing of the precipitate, thorough washing

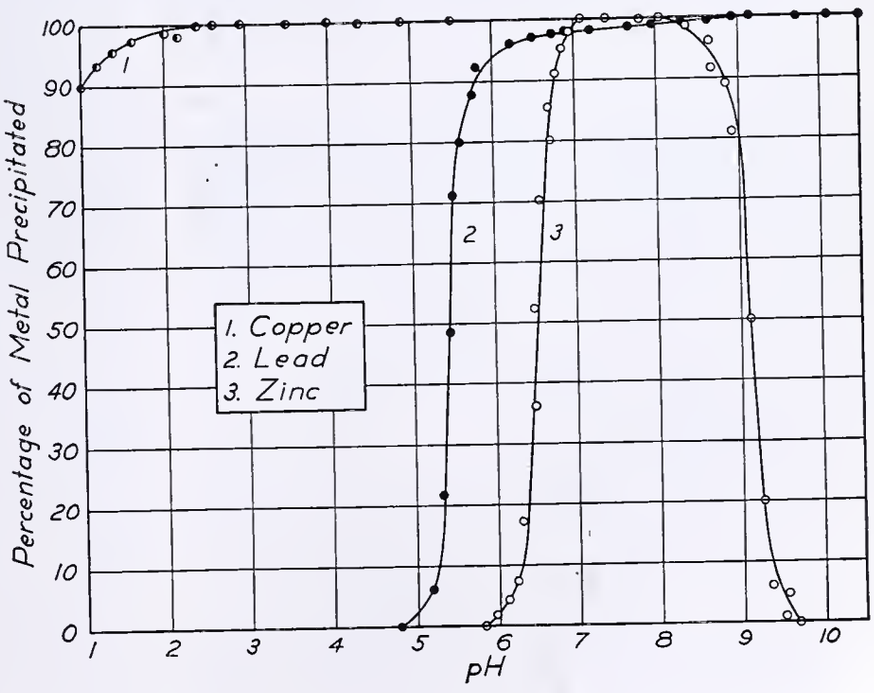

Figure 1. Precipitation of Copper, Lead, and Zinc

being indicated by the absence of coloration in a final portion of filtrate. The pH measurement was made upon the filtrate before the addition of any wash water.

pH Range for Precipitation. The theoretical factor 0.3261 for zinc in $Zn(C_7H_5O_2N)$ was used in calculating the results which are presented in Figure 1, curve 3. As shown by the curve, the precipitation of zinc salicylaldoximate is complete only in the pH range 7.1 to 8.1. It is completely soluble below pH 5.8 and above pH 9.7 if the pH is regulated with ammonia.

Separation of Copper and Lead

Curves 1 and 2, Figure 1, reproduced from papers by Biefeld and Howe (*2*) and Ligett and Biefeld (*7*), respectively, show that the separation of copper from lead by precipitation with salicylaldoxime may be effected easily by control of the pH. The adjustment of the pH is usually made with acetic acid. Hydrogen ion is formed by the reaction between the metal ion and the reagent, and if a considerable amount of copper is present the hydrogen-ion concentration may be so increased that precipitation of copper will be incomplete (*2*). The simplest procedure is to buffer the solution with acetate ion. However, this is not satisfactory if it is desired to determine lead in the filtrate by precipitation with salicylaldoxime because the lead complex is appreciably soluble in acetate solutions (*5*).

It was found that the correct pH could be readily attained by the use of modified methyl orange as indicator. After the addition of reagent to the solution of copper and lead, one or two drops of indicator were added. Then the solution was neutralized with ammonia, finishing with 0.5 *M* ammonia added dropwise with stirring. After standing for a few minutes, the supernatant liquid again shows the acid color of the indicator. This is due to the fact that the precipitation reaction does not immediately reach equilibrium after neutralization and a small additional amount of hydrogen ion is formed as the reaction goes to completion. It was unnecessary to bring the solution back to the neutral point again.

Separation of Lead and Zinc

Curve 2, Figure 1, is reproduced from a previous paper of the authors (*7*). Together with curve 3, it indicates why a separation of lead from zinc is possible in highly ammoniacal solution, although considerably more ammonia is required to prevent the precipitation of zinc with lead than would seem to be necessary.

Attempts were made to precipitate zinc from the filtrate after the separation of lead from zinc in highly ammoniacal solution. Neutralization of the large excess of ammonia present and the addition of salicylaldoxime did not result in the precipitation of zinc salicylaldoximate. Evidently the high concentration of ammonium salts present after neutralization of the ammonia acts to prevent the precipitation.

Removal of the excess ammonia in the filtrate by boiling the solutions was attempted. Prolonged boiling resulted in precipitation, but the precipitate formed was light brown in color and gave results only approximating the amount of zinc known to be present.

In view of these negative results, it was concluded that salicylaldoxime was not suitable for the determination of zinc in the filtrate after the separation of lead from strongly ammoniacal solution. However, the fact that the high concentration of ammonium salts was so effective in preventing the precipitation of zinc salicylaldoximate suggested an improvement upon the previously presented method (*7*) of separating lead and zinc. It seemed that a high concentration of ammonium salts might make it possible to use a lower concentration of ammonia when attempting to separate lead and zinc with salicylaldoxime. This would be desirable, because if too much ammonia is present in this determination, an appreciable amount of the lead is not precipitated, and if too little ammonia is present, zinc salicylaldoximate coprecipitates with the lead complex.

The purpose of the experiments outlined below was to determine whether the permissible range of ammonia concentration in the separation of lead from zinc might be widened by the use of a high concentration of salts.

Reagents and Apparatus. Lead and zinc solutions were prepared from the corresponding reagent grade nitrates. The lead solution was standardized by precipitation as the sulfate. The same care with reagents, volumetric ware, and weights was taken as in the precipitation of zinc salicylaldoximate, with the added precaution that the ammonium nitrate used was tested for insoluble matter.

Procedure for Separation. A 12-ml. portion of 1 per cent salicylaldoxime was added to a solution containing 0.0398 gram of lead and 0.0400 gram of zinc. This is an excess of reagent over that required for the precipitation of both metals. Then 5 grams of ammonium nitrate were added, followed by varying amounts of concentrated ammonia solution and water to make 65 ml. The precipitate was allowed to stand in contact with the solution, with occasional stirring, for 1 hour. It was filtered, washed with 20 per cent alcohol, and dried at 110° C. for 1 hour. The amount of lead was calculated from the weight of precipitate, using the factor 0.6053 for lead in $Pb(C_7H_5O_2N)$. The results are given in Table I.

Table I. Separation of Lead and Zinc

(0.0398 gram of lead present)

Concd. NH_3 Added	Precipitate Found	Lead Found	Error
Ml.	*Gram*	*Gram*	*Mg.*
3.0	0.0659	0.0399	+0.1
3.5	0.0661	0.0400	+0.2
4.0	0.0656	0.0397	−0.1
5.0	0.0656	0.0397	−0.1
5.0	0.0655	0.0397	−0.1
7.0	0.0658	0.0398	0.0
9.0	0.0655	0.0397	−0.1
10.0	0.0657	0.0398	0.0

Discussion of Results. The data of Table I demonstrate that a wide range of ammonia concentrations will suffice to keep the zinc from precipitating and still permit complete precipitation of lead salicylaldoximate, provided a large amount of ammonium nitrate is present. In the absence of ammonium nitrate, however, concentrations of ammonia as low as those given are not adequate, and the permissible range of ammonia concentration is much less.

The precipitate obtained in the presence of high concentrations of ammonium nitrate is very satisfactory with respect to ease of handling. Freshly precipitated, it is gelatinous, but during a period of about 30 minutes it gradually changes to a form which is easily filtered. The progressive change is readily apparent when three or four separate solutions are precipitated at intervals of a few minutes.

Separation of Copper, Lead, and Zinc

The procedure described is based upon the facts outlined above, and is intended to be applicable to solutions containing varying proportions of the three metals.

Solutions. The copper solution was prepared from reagent grade copper nitrate, and was standardized by electrodeposition and by precipitation with salicylaldoxime. The lead and zinc solutions and the reagent solution were prepared as described above.

Procedure for Analysis. Portions of the copper, lead, and zinc solutions were taken to give solutions with varying quantities of each metal. A 30-ml. portion of 1 per cent salicylaldoxime was added. The solutions were then made neutral to modified methyl orange by the dropwise addition of 0.5 *M* ammonia solution. The resulting precipitate and solution were allowed

Table II. Determination of Copper, Lead, and Zinc

Copper				Lead				Zinc			
Metal present	Precipitate found	Metal found	Error	Metal present	Precipitate found	Metal found	Error	Metal present	Precipitate found	Metal found	Error
Gram	*Gram*	*Gram*	*Mg.*	*Gram*	*Gram*	*Gram*	*Mg.*	*Gram*	*Gram*	*Gram*	*Mg.*
0.0089	0.0471	0.0089	0.0	0.0597	0.0987	0.0597	0.0	0.0694	0.1896	0.0695	+0.1
0.0089	0.0469	0.0089	0.0	0.0597	0.0986	0.0597	0.0	0.0694	0.1893	0.0694	0.0
0.0312	0.1649	0.0312	0.0	0.0199	0.0329	0.0199	0.0	0.0926	0.2526	0.0926	0.0
0.0312	0.1645	0.0311	−0.1	0.0199	0.0330	0.0200	+0.1	0.0926	0.2527	0.0926	0.0
0.0223	0.1177	0.0223	0.0	0.0796	0.1311	0.0794	−0.2	0.0231	0.0633	0.0232	+0.1
0.0223	0.1178	0.0223	0.0	0.0796	0.1313	0.0795	−0.1	0.0231	0.0631	0.0231	0.0
0.0446	0.2354	0.0446	0.0	0.0398	0.0656	0.0397	−0.1	0.0463	0.1259	0.0461	−0.2
0.0446	0.2356	0.0446	0.0	0.0398	0.0656	0.0397	−0.1	0.0463	0.1258	0.0461	−0 2

to stand 0.5 hour, filtered on a Gooch crucible, washed with 20 per cent alcohol, dried at 110° C., and weighed as $Cu(C_7H_6O_2N)_2$. The factor for copper is 0.1893.

The filtrate was transferred to a beaker and 0.7 gram of ammonium nitrate and 1.0 ml. of concentrated ammonia were added for each 10 ml. of solution. This resulted in precipitation of lead salicylaldoximate. After standing 1 hour, the precipitate was filtered on a Gooch crucible, washed with 20 per cent alcohol, dried at 110° C., and weighed as $Pb(C_7H_5O_2N)$. The factor for lead is 0.6053.

Zinc was determined in the filtrate by precipitation and weighing as $ZnNH_4PO_4$. The results are given in Table II.

Summary

Zinc salicylaldoximate begins to precipitate at pH 5.8, is completely precipitated in the range 7.1 to 8.1, and is again soluble above pH 9.7 if the pH is regulated with ammonia.

Copper, lead, and zinc in the same solution may be separated with salicylaldoxime by precipitating the copper in weakly acidic solution, filtering it off, and then making the filtrate strongly ammoniacal to cause precipitation of the lead complex. The correct pH for precipitation of the copper complex is easily obtained by the use of modified methyl orange. The separation of lead from zinc in highly ammoniacal solution is improved by the addition of a large amount of ammonium nitrate. In the presence of a high concentration of ammonium nitrate, a wide range of ammonia concentration may be used to prevent coprecipitation of zinc salicylaldoximate while still permitting complete precipitation of the lead. Zinc cannot be quantitatively precipitated as the salicylaldoximate in the strongly ammoniacal filtrate from the lead separation.

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Color Reactions of Organic Nitrogen Compounds with Selenious Acid-Sulfuric Acid Solution

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SELENIOUS and selenic acids were proposed as reagents for the identification of the opium alkaloids by Brandt (*1*), Lafon (*3*), and Ferreira da Silva (*2*). Mecke (*5*) observed the colors produced on the addition of a 0.5 per cent solution of selenious acid in concentrated sulfuric acid to the opium alkaloids, and recommended this solution as a reagent for the identification of minute quantities of these compounds. Levine (*4*) observed the color reactions of phenols and phenolic ethers with selenious acid–sulfuric acid reagent. He stated that the color production is a specific test for phenolic hydroxyl groups. The production of colored compounds he attributed to the oxidizing action of selenious acid, and he stated that a red or red-brown precipitate accompanying or following the primary color reaction is due to the reduction of selenious acid with the liberation of elemental selenium. Levine concluded that a positive reaction cannot be accepted as indicative of the presence of the opium alkaloids unless other phenolic compounds are absent.

The present work reports the color reactions of some organic nitrogen compounds with selenious acid–sulfuric acid solution.

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Experimental

One milligram of the compound was placed on a spot plate and a drop of a 0.5 per cent solution of selenious acid in concentrated sulfuric acid was added. Chemicals used were Eastman grade supplied by Eastman Kodak Co., Rochester, N. Y., and C. P. quality of the Pfanstiehl Chemical Co., Waukegan, Ill. To determine if the reaction is characteristic of the selenious acid reagent, a similar test was carried out simultaneously with sulfuric acid.

The color changes which took place during a 3-hour period are recorded in Table I.

Some compounds produced the same color in the sulfuric acid as in the selenious acid–sulfuric acid solution: benzidine, *o*-bromoaniline, *m*-bromoaniline, *p,p'*-diaminodiphenylmethane, dibenzylamine, diphenylnitrosoamine, diphenylthiocarbazone, methyl-*o*-toluidine, methyl-*m*-toluidine, methyl-*p*-toluidine, *p*-nitroaniline, *p*-nitrosodiphenylamine, *m*-phenylenediamine hydrochloride, and *p*-phenylenediamine.

The following compounds gave either a faint pink or no color at all: allylthiourea, *o*-aminobenzoic acid, *m*-aminobenzoic acid, *p*-aminobenzoic acid, *dl*-α-amino-α-methylbutyric acid, *d*-arginine monohydrochloride, asparagine, barbituric acid, *o*-chloroaniline, *p*-chloroaniline, creatinine, *l*-

Table I. Color Changes

Compound	H_2SO_4	H_2SeO_3–H_2SO_4	Compound	H_2SO_4	H_2SeO_3–H_2SO_4
o-Aminodiphenyl	Pink	Pale green	4,4-Diphenylsemicarbazide	Greenish brown to brown	Intense blue to blue-green
p-Aminodiphenyl	Colorless	Pink to dirty gray	Diphenylthiocarbazide	Pink to blue-green to red-orange	Purple to brown
4-Aminodiphenylamine HCl	Purple to pale green	Brown to red-brown to purple	*s*-Di-*o*-tolylthiourea	Colorless	Greenish yellow to yellow
Aniline	Yellow	Yellow to pale blue	*s*-Di-*p*-tolylthiourea	Colorless	Greenish yellow to green to red-orange
Benzeneazodiphenylamine	Green to pink-purple to purple	Green to blue-black to purple	*s*-Di-*o*-tolylurea	Colorless	Colorless to pale yellow
p-Bromoaniline	Colorless	Pink	*s*-Di-*m*-tolylurea	Colorless	Pale blue to faint yellowish green
Carbanilide	Colorless	Yellow	*s*-Di-*p*-tolylurea	Colorless	Faint blue to pale yellow
m-Chloroaniline	Pink	Pink to brown	Formyl diphenylamine	Colorless	Blue to gray
Cholesterol	Pink	Pink to brown	*l*-Leucine	Colorless	Purple to pink
Cysteine HCl	Colorless	Yellow to pink	Methyldiphenylamine	Colorless	Pinkish blue to purple to rose-brown
2,4-Diaminodiphenylamine	Rose-red to dark red to brown to gray-green	Rose-purple	Methylthiocarbanilide	Colorless	Yellow-green to orange to purple
Dibenzylaniline	Colorless	Rose-pink to pale purple	1-Naphthylamine	Colorless	Greenish brown to reddish brown
s-Dimethylcarbanilide	Colorless	Pale pink to light brown	2-Naphthylamine	Colorless	Greenish yellow to yellowish brown
Di-2-naphthylamine	Yellow	Brown	4-Nitrodiphenylamine	Purple to blue to blue-gray	Purple
Di-*p*-phenetylurea	Colorless	Green to olive-green to yellow to tan	*p*-Nitrophenylhydrazine	Yellow to yellow-green	Yellow to brown
Diphenylamine	Colorless	Intense blue to dirty green to brown	Phenylthiourea	Colorless	Pale yellow
Diphenylbenzidine	Colorless	Blue	Thiocarbanilide	Faint pink	Greenish yellow to yellow-orange
Diphenylcarbamine Cl	Colorless	Green to blue-green to intense blue	Tolidine	Faint pink	Yellow
s-Diphenylcarbazide	Colorless	Purple to blue to brown to greenish brown	*o*-Toluidine HCl	Colorless	Yellow to brown to blue-green to blue to reddish brown
s-Diphenylcarbazone	Rose-pink to red-orange	Dark blue to purple to brown	*p*-Toluidine HCl	Colorless	Greenish blue to brown
s-Diphenylethylenediamine	Colorless	Faint pink to pale yellow to brown	Triphenylguanidine	Colorless	Pale blue to pale yellow
4,5-Diphenylglyoxalone	Salmon-pink to colorless	Pink to orange-brown to brown	Tryptophan	Faint yellow	Yellowish brown
Diphenylpiperazine	Colorless	Yellow to yellowish green to brown			
1,4-Diphenylsemicarbazide	Pale pink	Intense bluish purple to dirty gray-green to brown			

cystine, diethylaniline, *s*-diethylcarbanilide, diiodotyrosine, *p*-dimethylaminobenzaldehyde, dimethylaniline, diphenylguanidine, di-*o*-tolylguanidine, ethylamine hydrobromide, ethylenediamine, glycine, guanidine hydrochloride, hexamethyleneamine, hydroxylamine hydrochloride, *dl*-isoleucine, methylaniline, morpholine, *o*-nitroaniline, *m*-nitroaniline, *l*-oxyproline, phenobarbital, β-phenylalanine, phenylhydrazine, phenylurea, α-picoline, *l*-proline, isopropanolamine, pyridine, quinoline, *dl*-serine, sulfanilic acid, thiourea, tribenzylamine, triethanolamine, triisopropanolamine, urea, and *dl*-valine.

In general, compounds containing two or three aromatic nuclei gave the most intense reactions. Colors could be easily distinguished from those produced by the sulfuric acid alone. Compounds containing one aromatic nucleus produced pale colors or none at all. Freshly distilled aniline produced a pale blue color after 30 minutes' contact with the reagent. Aniline which remained for 3 hours in air after distillation gave an intense blue color immediately upon the addition of the reagent. Few aliphatic amines showed any coloration except a pink or brown, which was probably due to the precipitation of selenium.

The minimum amounts necessary to produce the characteristic colors were determined for those compounds which gave intense colors.

Solutions in ethanol, benzene, toluene, or chloroform were prepared. Amounts of the solutions containing from 100 micrograms to 0.01 microgram of the compound were placed in porcelain evaporating dishes and the solvent was removed on a steam bath. A few drops of selenious acid–sulfuric acid reagent were added to each one and the dishes were rotated so that the reagent flowed over the bottoms of the dishes.

The smallest quantities of the compounds which gave recognizable colors are recorded in Table II. Five compounds were sensitive in amounts as small as 0.1 microgram. The sensitivity of the test was only one one-hundredth as great when the selenious acid–sulfuric acid solution was added to the ethanol or chloroform solutions of the compounds on a spot plate. It was possible to detect as small amounts of the compounds when the reagent was added to the benzene or toluene solutions as when the solvent had been removed.

Table II. Minimum Production of Color

Compound	Limit in Micrograms
4-Aminodiphenylamine HCl	0.5
Benzeneazodiphenylamine	0.1
2,4-Diaminodiphenylamine	1.0
Di-2-Naphthylamine	0.1
Diphenylamine	10.0
Diphenylbenzidine	1.0
Diphenylcarbamine chloride	7.0
s-Diphenylcarbazide	1.0
4,5-Diphenylglyoxalone	0.5
Diphenylpiperazine	10.0
1,4-Diphenylsemicarbazide	0.1
4,4-Diphenylsemicarbazide	50.0
Diphenylthiocarbazide	0.1
Formyl diphenylamine	10.0
Methyldiphenylamine	10.0
Methylthiocarbanilide	30.0
4-Nitrodiphenylamine	0.1
Thiocarbanilide	1.0
Tolidine	2.0

Conclusions

Color production with selenious acid–sulfuric acid solution is not a specific reaction of phenolic compounds. Many nitrogenous compounds—especially those containing two or three aromatic nuclei—give intense color reactions with this reagent.

The colors produced with selenious acid–sulfuric acid reagent provide a sensitive method for detecting and distinguishing between certain nitrogen compounds. The colors produced by 1-naphthylamine, 2-naphthylamine, and di-2-naphthylamine can be readily distinguished. Diphenylguanidine shows no color. Triphenylguanidine yields a pale but definite blue which changes to yellow. Neither becomes colored when treated with sulfuric acid alone.

The test with selenious acid–sulfuric acid reagent for the presence of the opium alkaloids cannot be considered conclusive unless interfering phenols and nitrogen compounds are known to be absent.

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A Modified Micropipet

For Density Determinations in Heavy Water Analysis

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THE instrument here described is a modification of the miropipet of Keston, Rittenberg, and Schoenheimer (*3*) from which drops of water of highly uniform size are expelled and measured for density by the falling-drop method of Barbour and Hamilton (*1*), as adapted by Vogt and Hamilton (*4*) and Fenber-Eriksen, Krogh, and Ussing (*2*).

Figure 1 shows the construction of the instrument. All parts are of stainless steel or cold-rolled steel unless otherwise stated.

The screw, *C*, having a pitch of 9.3 mm. (0.037 inch), to which is attached the Bakelite knob, *N*, and the stop-arm, *I*, is threaded into the upper cap, *E*, and presses upon the head of the piston, *H*, the shank of which is seated in the hole in the platform, *F*. The shank of *H* has a diameter of 2.90 mm. (0.114 inch) and the hole is 2.95 mm. (0.116 inch) in diameter (No. 32 drill). The pressure of *C* is opposed by a thick compression gasket, *M*, of pure live gum rubber which fits snugly on *H* and thereby also serves as a seal to the clearance space between *H* and *F*. The extension at the bottom of *F* is turned as shown to correspond to the inside and outside diameters of a short length of glass tubing, *R*. These diameters in the authors' instrument are, respectively, 6.0 mm. (0.237 inch) and 8.3 mm. (0.327 inch). *R* is sealed to the capillary glass tubing, *S*, which has the same outside diameter, and an inside diameter of not more than 2 mm. In order to preclude air pockets, the glass and glass-metal joints must be smooth, and the bottom of the hole in *F* must be smoothly countersunk as indicated. The end of *H* is turned conical for the same reason. *J* is a piece of rubber pressure tubing or a rubber stopper with a snug-fitting hole, and *K* and *L* are rubber gaskets having the diameters, respectively, of the inside of the housing cylinder, *A*, and of the thread undercut at the bottom of the screw cap, *B*. These gaskets are cemented together with rubber cement and are provided with tight-fitting holes accurately centered for *S*. *B* is threaded to *A* and is knurled on the outside. The hole in *B* through which *S* passes is 9.52 mm. (0.375 inch) in diameter.

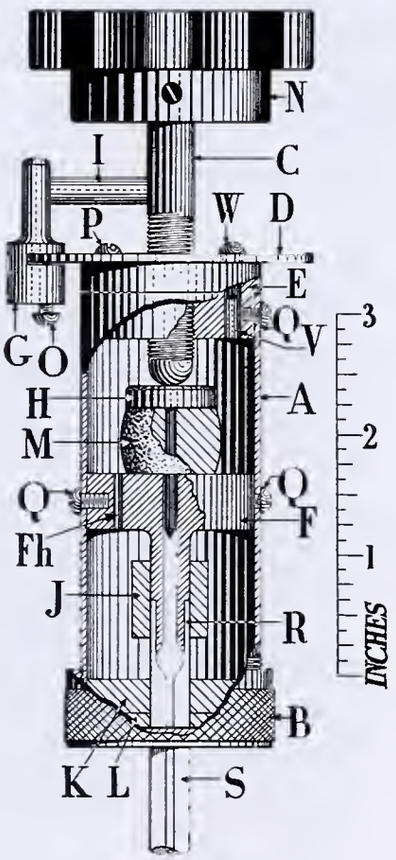

FIGURE 1. MICROPIPET CONSTRUCTION

The top plate, *D*, carries one or two movable stops, *G*, which in use are held rigidly in place with the setscrews, *O*. *D* and *E* may be integral or firmly attached together with the screws, *P*. *F* and *E* are held securely in their respective places within *A* by means of the screws, *Q*. The upper cap is provided with a filler hole, *V*, which is protected from dust with the screw, *W*, from which the threads have been turned off.

The remainder of the pipet (Figure 2) consists of the stopcock, *T*, the reservoir, *U*, and the connecting rubber tubing. The end of the capillary tubing, *S*, is drawn out to a smaller diameter. *U* when not in use is hung on a hook which is carried on the bracket, *X*. This bracket also carries an adjustable clamp, *Y*, to which *S* is attached and is in turn carried on any smoothly operating rack-and-pinion device, *Z*, with a vertical travel of about 10 cm. (4 inches), and which is rigid enough to carry the weight of the pipet when it is filled with mercury. (The authors use the modified head and spindle of a No. 620 Delta drill press, in which the two ball-bearing assemblies are replaced by solid bearings each carrying a setscrew which engages the keyway in the upper end of the spindle, thus preventing rotation of the spindle but permitting the normal vertical motion.)

In assembling and setting up the instrument for use, the following procedure is suggested:

All parts should be carefully cleaned before assembly. *F* (Figure 1) is first screwed in place in *A*. The glass system is passed through *B* and rubber gaskets *L* and *K*, inserted in *J*, and then cemented to the platform extension with de Khotinsky or other suitable cement. *J* is slid over the joint and *B* is brought up and screwed tight. Mercury is then poured into *U* (Figure 2), stopcock *T* is opened, and the mercury is allowed to flow into both branches of the capillary. When the mercury has risen so as slightly to overflow the hole in *F*, the stopcock is closed, *H* carrying the gum rubber compression gasket is inserted, *E* carrying plate *D* is fastened in place with screws *Q*, and the main screw with knob *N* is screwed in and brought down, so as to press firmly but not too heavily upon the head of the piston. A little experimenting will determine the proper pressure.

Petroleum jelly should be applied sparingly to the thread and to the rounded end of *C* before assembling. Additional mercury is introduced through *V* until it can just be seen at the bottom of the hole, and *W* is then inserted. Since *H* and *M* are in place, this mercury runs down through holes *Fh* to fill the lower compartment, but has no connection with that in the capillary; its function is to prevent leaks at the two joints. Stops *G* are put in place on *D*, only one stop being used when maximum drop size is required.

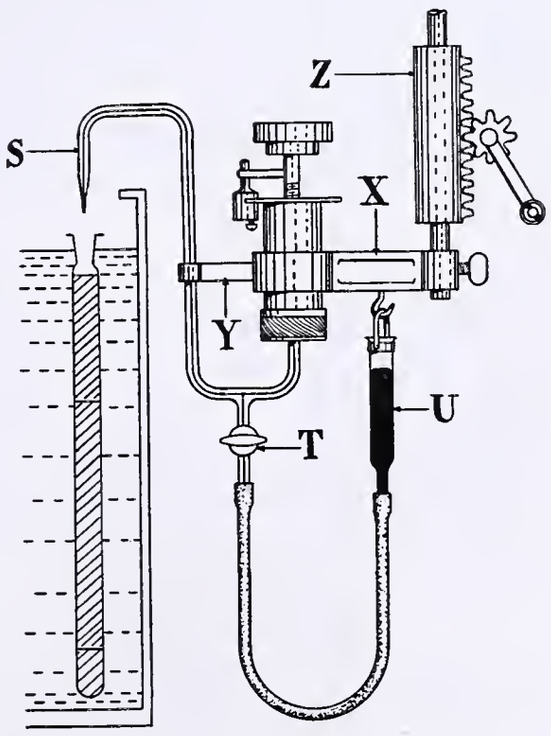

FIGURE 2. DIAGRAM OF PIPET

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In operation, C is first backed off around to the stop, U is held at a high level, and with the drawn-out end of S dipping into the vessel containing the sample to be measured, T is opened, allowing a drop or two of mercury to run out. The reservoir is then brought to a lower level, thus drawing some of the sample into the end of the pipet, the stopcock is closed, and the sample vessel is removed. The end of the pipet is cleaned with hard filter paper and then lowered with the rack and pinion, so that the end of the capillary dips into the cylinder containing immiscible fluid having a density slightly less than that of water (*3*) and maintained at constant temperature in a water bath (Figure 2). N is then turned down against the stop, thus expelling a drop of the sample, the volume of which is determined by the formula:

$$V = \pi r^2 P \frac{A}{360}$$

in which V is the volume, r the radius of piston shank H, P the pitch of screw C, and A the angle in degrees through which C has been turned. The pipet is then raised and as the end of S passes through the surface of the fluid in the cylinder the drop is detached and its rate of fall between two marks is determined with a stop watch.

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Photometric Determination of Copper and Iron in Distilled Liquors

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COPPER and iron are so frequently estimated in biological experimentation that a number of methods and reagents for their determination have been described. In view of the low concentrations of copper and iron found in alcoholic liquors, the colorimetric procedures only were considered.

The merits of sodium diethyldithiocarbamate as a color reagent for copper have been established by Conn and co-workers (*3*), Coulson (*4*), and Parker and Griffin (*11*). Drabkin (*5*), using a spectrophotometer in his critical study of the color produced by the diethyldithiocarbamates of copper, nickel, cobalt, and bismuth, reported fair agreement with Beer's law for copper concentrations in the range 0.02 to 0.1 mg. in 10 ml. of isoamyl acetate as extraction solvent. Iron in biological materials has been determined colorimetrically with 2,2′-bipyridine as the color reagent by Hill (*9*), Jackson (*10*), and others. Not recommended in the report of Harrison (*8*) is the application of the reagent to the estimation of iron in beer described by Gray and Stone (*6*). However, the success of Parker and Griffin (*11*) with the above reagents in the determination of iron and copper in the same sample of plant extract prompted the application of their method to distilled liquors.

Both copper and iron form yellow carbamate complexes soluble in isoamyl alcohol. Parker and Griffin (*11*) converted the iron into non-ionized ferrous bipyridine, which is insoluble in isoamyl alcohol, thus permitting the quantitative separation of the copper. As recommended by Campbell and Dauphinee (*2*), the iron was first reduced to the ferrous form by means of *p*-hydroxyphenylglycine.

Preliminary tests indicated no advantage in the use of isoamyl acetate or bromobenzene in the extraction of the copper carbamate under the experimental conditions involved. Hence, all extractions were made with isoamyl alcohol. In the scheme of separation of cations as applied to the ash of distilled liquors, the copper and iron are precipitated with lead and tin from ammoniacal solution by hydrogen sulfide. In this manner possible interference of calcium is removed; no other interfering metal is present in the copper-iron fraction. The use of a small excess of the carbamate reagent serves to overcome any effects of calcium when the method is applied directly to freshly distilled whisky.

Reagents

Water redistilled in Pyrex glass apparatus was used for all solutions and dilutions. Reagent grade chemicals must be used wherever obtainable.

2,2′-Bipyridine. Dissolve 0.2 gram of 2,2′-bipyridine (also marketed as α,α'-dipyridyl) in 100 ml. of 10 per cent acetic acid. In a few hours the solution turns pink, but this coloration does not interfere with its use.

Sodium Diethyldithiocarbamate. Dissolve 1 gram of the salt in 100 ml. of water.

p-Hydroxyphenylglycine. 0.1 gram dissolved in 100 ml. of 0.4 N sulfuric acid. This solution must be freshly prepared the day it is used.

Ammonium Acetate. Prepare a normal solution by dissolving 77 grams of ammonium acetate per liter of solution. Adjust to pH 6.

Standard Copper Solution. Weigh accurately 0.2000 gram of C. P. copper wire and dissolve in 15 ml. of nitric acid (1 + 4), warming to complete solution. Expel fumes by boiling and, when cool, dilute to 200 ml. An intermediate standard containing 0.1 mg. per ml. is prepared by a tenfold dilution of the first solution. The working standard containing 1 microgram per ml. (1 p. p. m.), prepared just before use, is made by diluting 10 ml. of the intermediate standard to 1 liter.

Standard Iron Solution. Weigh 0.2000 gram of pure iron wire or electrolytic iron accurately, dissolve in about 10 ml. of 10 per cent sulfuric acid, and dilute to 200 ml. Further dilutions are made as described for copper.

Lead Nitrate Solution. Dissolve 1.6 grams of reagent grade lead nitrate in water to make 1 liter of solution.

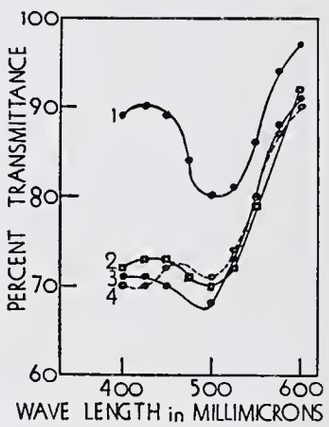

Figure 1. Influence of Reagents on Transmittance Curve of Iron Bipyridine

1. 1 p. p. m. of iron
2. Iron extracted with isoamyl alcohol
3. Iron plus carbamate plus isoamyl alcohol extraction
4. Iron plus 1 p. p. m. of copper plus all reagents

Effect of pH on Intensity and Stability of Color

Color development, characteristics, and stability were investigated using a Coleman Universal spectrophotometer, single monochromator type, with band width of 35 mμ.

To establish the influence of pH on the color 1 ml. of the intermediate standard (containing 0.1 mg.) of iron was diluted to about 50 ml. with water, and 1 ml. of the *p*-hydroxyphenylglycine solution and 2 ml. of 2,2′-bipyridine reagent were added. The solution was adjusted to the desired pH, as measured with a glass electrode, by the addition of ammonium hydroxide and transferred to a 100-ml. volumetric flask. The solution was diluted

to the mark and mixed thoroughly. A blank was prepared from all reagents used with exclusion of the standard iron solution. The pink colored solution was read in the spectrophotometer against the blank.

Table I shows that the color is not so stable in the solutions at higher pH. Transmittances greater than 90 per cent do not accurately represent color intensity; hence, these values are approximations. The effect of reaction on the copper carbamate color was followed by adjusting to the desired pH 1 ml. of the intermediate standard solution (100 p. p. m.) previously diluted and treated with 1 ml. of the carbamate reagent, diluting to 100 ml., and extracting a 25-ml. portion with 10 ml. of isoamyl alcohol. The data of Table II reveal no significant differences in stability within the range pH 5 to 12.

Table I. Transmittance of Aqueous Solutions of Ferrous Bipyridine at Wave Length 500 mμ

Time after Mixing	pH 5	pH 6	pH 7	pH 8	pH 9
	Transmittance				
Min.	%	%	%	%	%
10	81	80	84	83	82
60	81	79	88	90	96[a]
120	81	79	90	94[a]	98[a]

[a] Faint pink coloration visible.

Table II. Transmittance of Isoamyl Alcohol Solutions of Copper Diethyldithiocarbamate at Wave Length 450 mμ

Time after Mixing	pH 5	pH 6	pH 7	pH 8	pH 9	pH 12
	Transmittance					
Min.	%	%	%	%	%	%
20	33	35	39	35	33	31
120	33	35	39	35	32[a]	31[a]
240	34	34	39	35	33	32

[a] Slight turbidity, removed by centrifuging.

Effect of Reagents on Color Development

Inasmuch as the complexes for iron and copper were developed simultaneously in the same solution, it was necessary to know what influence the reagents exerted on the color development of these complexes. From the data previously obtained it was decided to buffer the solution at pH 6. Two reference spectral transmittance curves were prepared from standard solutions of copper and iron containing 1 microgram per ml. (curve 1, Figure 1, and curve 1, Figure 2). Extraction with isoamyl alcohol of the aqueous solution containing the colored iron complex decreased the transmittance at 500 mμ 10 per cent (curve 2, Figure 1). Addition of the carbamate reagent, with or without the presence of copper, had very little influence on the transmittance of the extracted aqueous solution at 500 mμ (curves 3 and 4, Figure 1). Curve 2 in Figure 2 reveals that the 2,2′-bipyridine does not influence the transmittance of the isoamyl alcohol solution of the copper complex; but from curve 4, same figure, it is evident that the presence of iron in an amount equal to or more than that of the copper interferes with full color development. The addition of a fivefold excess of carbamate reagent relieves the competition of the copper and iron, curve 5, without in any way influencing the transmittance of the reference solution as shown in curve 3. It is apparent that it is essential to have all reagents used in the actual determination present in the preparation of the standard curves of reference.

Preparation of Standard Reference Curves

The standard reference curves for copper and iron were prepared, utilizing the information obtained in the preliminary tests.

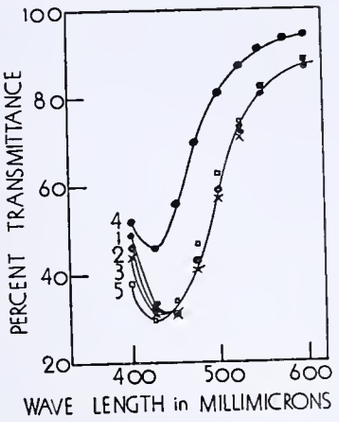

Figure 2. Influence of Reagents on Transmittance Curve of Copper Carbamate in Isoamyl Alcohol

1. ● 1 p. p. m. of copper plus carbamate
2. ○ 1 p. p. m. of copper plus all reagents
3. × 1 p. p. m. of copper plus excess carbamate
4. ● 1 p. p. m. of copper plus 1 p. p. m. of iron plus all reagents
5. □ 1 p. p. m. of copper plus 1 p. p. m. of iron plus excess carbamate

Copper standards varying in content from 2 to 100 micrograms in 20 ml. of solution (corresponding to 0.1 to 5 p. p. m.) were treated in the order given with 1 ml. of *p*-hydroxyphenylglycine solution, 2 ml. of 2,2′-bipyridine solution, 4 drops of concentrated ammonium hydroxide, 5 ml. of *N* ammonium acetate solution, and 1 ml. of carbamate reagent. Each standard was then extracted in a separatory funnel with 15 ml. of isoamyl alcohol. This volume of isoamyl alcohol was found sufficient to remove in one extraction all the carbamate complex formed by 100 micrograms of copper. The yellow alcohol layer was cleared by a short centrifugation at about 2000 r. p. m. and read in the spectrophotometer against a blank solution incorporating all reagents and steps except for the copper solution. The standards for iron varied from 2 to 100 micrograms in 20 ml. of solution and were treated in the same manner as the copper standards. Readings were made on the aqueous layer following a short centrifuging. Above concentrations of 60 micrograms in 20 ml. the bipyridine solution became the limiting factor for color development and it was necessary to increase the strength of the color reagent to accommodate all of the iron present.

The transmittance-concentration curves are given in Figure 3 for wave length 500 mμ for iron and wave length 450 mμ for copper. From the relationship between optical density, D, and transmittance, T, expressed and defined by Ashley (*1*) the equation $D_\lambda = \log \frac{1}{T} = K_\lambda cl$ may be derived. When c is expressed in moles per liter and l in centimeters, K_λ becomes the molecular extinction coefficient and may be calculated from the expression $K_\lambda = \frac{D_\lambda}{cl}$. Preference is given to this form of the Bouguer-Beer equation, as the value for D may be read directly from the spectrophotometer used. In Figure 3 the optical density is plotted against the concentra-

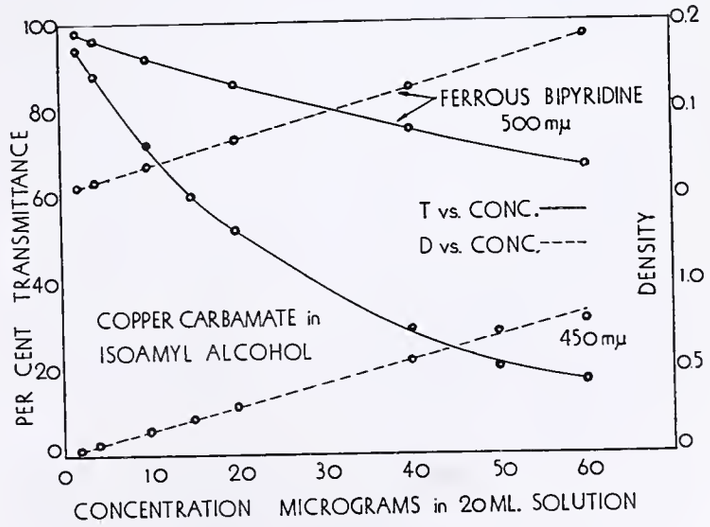

Figure 3. Transmittance Concentration Curve for Ferrous Bipyridine and Copper Diethyldithiocarbamate in Isoamyl Alcohol

tion for each of the metals. For the copper solutions Beer's law is obeyed in the range 2 to 60 micrograms per 20 ml. of solution (0.1 to 3 p. p. m.), whereas for the iron solutions the law holds less exactly over the range 20 to 100 micrograms per 20 ml. of solution (1 to 4 p. p. m.).

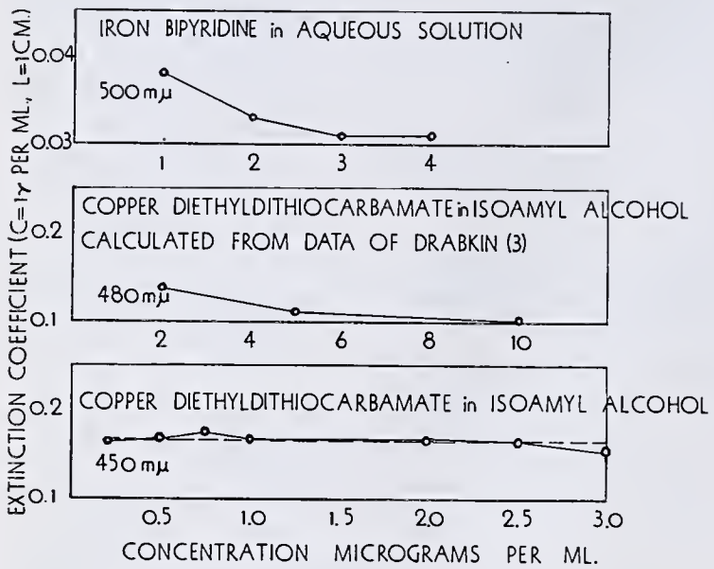

Figure 4. Extinction Coefficients for Copper and Iron Complexes

Under the conditions imposed and the concentrations used the procedure is much more satisfactory for copper than for iron. This is apparent from the values for the extinction coefficients shown in Figure 4 as calculated for a concentration of 1 microgram per ml. and cell depth of 1 cm. For comparison, the data of Drabkin (*5*) for wave length 480 mμ obtained by precise visual spectrophotometry have been converted to the same basis. For copper diethyldithiocarbamate in isoamyl alcohol there is apparently closer concordance with Beer's law at concentrations of 3 micrograms per ml. and less. Best agreement with Beer's law—that is, the optimum range for iron—is at concentrations of 40 to 80 micrograms per 20 ml.

Application to Distilled Liquors

Freshly distilled whisky, called "high wine", is clear, colorless, and ranges in alcohol content from 100 to 160 proof. When the reagents were added directly to this material two objections became manifest: (1) the isoamyl alcohol layer was diluted about 50 per cent, and (2) the aqueous layer became turbid. Both objections were overcome by diluting the original whisky (high wine) sample to 50 proof (25 per cent) or less. A disadvantage of this dilution is the reduction of the concentration of the copper and iron, particularly of the latter, to a point where poor color intensities may result. The added step of evaporating the original whisky sample to small volume on the steam bath to remove most of the alcohol, with subsequent dilution to any desired volume with redistilled water, repays the effort involved. Aged whiskies possessing a variety of shades of brown cannot be tested directly but must be ashed.

The sample, which may be as small as 100 ml., is transferred to a platinum dish and evaporated to dryness on the steam bath. The residue is ignited in a muffle furnace held at a barely perceptible redness. In about 30 minutes the residue assumes the form of a gray gossamer, which is taken up with 1 ml. of concentrated hydrochloric acid, warmed a few minutes on the steam bath, and transferred with redistilled water to a 25-ml. volumetric flask. Of the solution 20 ml. are transferred to a separatory funnel and treated with the reagents as described above for the reference standards. The yellow isoamyl alcohol solution obtained is read against the alcohol layer from a blank carried through at the same time, and the pink aqueous layer is read against the corresponding layer of the blank. Concentrations may be calculated from the equation $K_\lambda = \frac{D_\lambda}{cl}$ or read directly from the standard curve of reference.

As there are no interfering ions present in the ash of the freshly distilled or aged whisky, copper and iron may be determined directly on the solution thus obtained. When other metals such as calcium and magnesium are also to be determined, a preliminary separation is effected by precipitation of the copper and iron from ammoniacal solution with hydrogen sulfide. When this separation is to be carried out 5 mg. of lead are added as a gatherer before precipitation, as recommended by Greenleaf (*7*).

Copper and iron may be determined simultaneously in degassed but otherwise untreated beer by the direct method described above. Gray and Stone (*6*) utilized 2,2′-bipyridine in a direct procedure for beer, making visual comparison with permanent iron standards in a block comparator. The usual advantages are found in the use of the spectrophotometer.

Twenty milliliters of the degassed beer in a 50-ml. Erlenmeyer flask were treated with the reagents as above and placed in a water bath at 70° C. for 30 minutes. The solution was allowed to cool and transferred to a separatory funnel with 5 ml. of water. Copper carbamate was extracted with 15 ml. of isoamyl alcohol and clarified by centrifuging. The colors were read in the spectrophotometer against the alcohol and aqueous layers of beer not subjected to the reagents but diluted with water to the proper volume.

The analytical data for a few representative samples are given in Table III.

Table III. Copper and Iron Content of Distilled Liquors and Beer

Sample		Iron Added	Iron Found	Copper Added	Copper Found
		P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*
High wine	D[a]	None	0.2	None	0.62
	D[a]	1	1.08	1	1.67
	D[a]	2	2.10	2	2.70
	D[b]	None	0.2	None	0.60
	D[b]	1	1.05	1	1.57
Whisky	R[c]	None	0.5	None	1.76
	B[c]	None	0.2	None	0.82
Beer	L[d]	None	0.38	None	4.2
	L[d]	1	1.42	1	5.1

[a] Alcohol removed by evaporation.
[b] Alcohol concentration reduced by dilution.
[c] Ashed sample.
[d] Sample degassed only.

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Potentiometric Titrations

A Review with Bibliography

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THIS paper attempts to cite the chief aspects of published work in the field of potentiometric titrations and related simplified methods that depend upon changes in electrode potentials at the end points of titrations. The period included is that from the time of a preceding review (*162*) to the end of 1941. A partial review by the author based on this and other fields of electrotitration has appeared (*166*). More or less recent reviews of the field have been published by Lassieur (*329*), Fell (*135*), and Lederer (*333*). Mention should also be made of monographs or books in this field by Böttger (*47*), Hiltner (*229*), Kolthoff and Furman (*299*), and Müller (*337*). Useful data on oxidation-reduction potentials are to be found in Latimer's treatise (*330*).

The field will be reviewed under the general headings of Theory, Apparatus and Technique, and Applications.

Theory

THE ACID-ALKALI FIELD. A further detailed mathematical consideration of the appearance of inflections, the titration error, and the relative ultimate accuracy of indicator and e. m. f. methods has been given by Roller (*463*) and by Kilpi (*293, 294*). The latter has considered in detail the mathematical theory of the equivalence point, buffer action, the stepwise titration of dibasic acids, the theory of the titration error, and basic dissociation constants in glacial acetic acid (*293*). The conclusions of these recent studies are not widely different from those of Bjerrum (1911). In general, the appearance of an inflection is governed by the magnitude of Kc (where K is the ionization constant of the weak acid or base and c the concentration) relative to K_{solvent}. In aqueous solutions no inflection appears unless Kc is $> 27K_w$. For practical analytical purposes Kc should be greater than 10^{-10}. The classical potentiometric method is capable of about three times the sensitivity of titration to the color tint of a reference solution.

The calculation of approximate ionization constants from titration curves is described in detail by Hahn and Klockmann (*216, 219*).

The theory of the differential titration of a strong or a weak acid by the general procedure of MacInnes has been treated by Giraut-Erler (*185, 186*).

PRECIPITATION. The theory of second-class electrodes has been reviewed by Ringbom (*452; cf. 450, 451*), with especial reference to titrations with lead nitrate as reagent.

The chief novel trend in the precipitation field is the development of the principles of operation of so-called third-class electrodes as indicators for titrations. The electrode systems are of the type: metal$_1$/solid M_1A/solid M_2A, where M_1 and M_2 stand for different metals whose salts with A are slightly soluble. The electrode is desired to be an indicator for the ions of M_2, for which no convenient solid electrode is available. The electrodes that have been investigated have been chiefly those which might indicate the concentration of calcium ion—e. g., silver–silver oxalate–calcium oxalate. The theory has been developed by LeBlanc and Harnapp (*332*). The two solubility products are:

$$S_1 = (B_1^+)\,(A^-) \text{ and } S_2 = (B_2^+)\,(A^-)$$

where B_1^+ and B_2^+ are the cations and A^- is the common anion. The effect of a small increase in the concentration of B_2^+ from $(B_2^+)_0$ to $(B_2^+)_0 + c$ is considered in terms of c, S_1, S_2, and $(B_2^+)_0$. The expression that results for c is:

$$c = \frac{2S_1 + S_2}{2(S_1 + S_2)}\,(B_2^+)_0 + \sqrt{\frac{(2S_1 + S_2)^2}{4(S_1 + S_2)^2}\,(B_2^+)_0^2 - \frac{S_1}{S_1 + S_2}\,(B_2^+)_0 + \frac{S_2^2}{S_1 + S_2}}$$

The condition $c = 0$ is the most favorable one for a good indicator electrode because this means that no change in the cation content of the solution occurs through the formation or solution of the precipitate. The ideal condition is most closely approximated when $\frac{2S_1 + S_2}{2(S_1 + S_2)} = 0.5$. A summary of the electrodes of this class that have been studied is given under Applications.

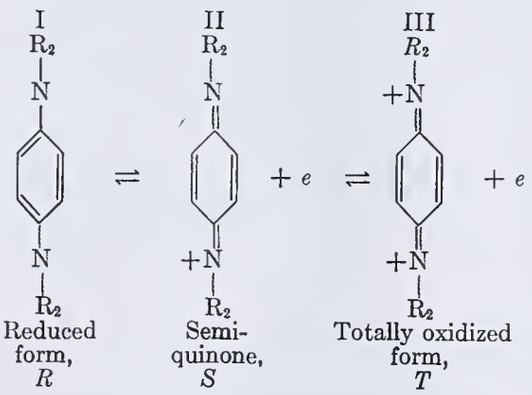
Polarometric Titrations. Although logically beyond the scope of this review, certain titrations based upon the dropping mercury electrode are closely analogous to titrations with polarized electrode systems. In the polarometric (amperometric) method it is often necessary to plot current *vs.* volume of reagent added with correction for the change in volume. The dropping mercury electrode may serve as an indicator for ions such as lead, calcium, barium, sodium, etc., for which in general no satisfactory direct indicator electrode can be prepared (*2, 298, 300–308, 328, 358, 411, 413*). This new technique is not limited to precipitations, but has been shown to be applicable also to oxidation-reduction titrations (*541, 553*). In certain cases the end point is marked by either an increase or decrease in current that is so abrupt that plotting of data is unnecessary (*306*).

OXIDATION-REDUCTION. Mohr (*379*) has pointed out again that the equivalence potential is not at the average between the two standard potentials of the higher and lower oxidation-reduction systems (cf. *299*, pages 45–6). As is well known, this point is at a weighted mean of the two standard potentials. In general, for the reaction $a\mathrm{Ox}_1 + b\mathrm{Red}_2 \rightleftharpoons a\mathrm{Red}_1 + b\mathrm{Ox}_2$, the equivalence potential is $E_E = \frac{bE_0' + aE_0''}{a+b}$, where E_0 is the standard potential for the higher and E_0'' for the lower of the oxidation-reduction systems.

Travers (*589*) has considered the question of making predictions from oxidation-reduction theory. Irreversible effects at electrodes and slow rate-determining steps often make predictions difficult.

The detailed mathematical theory of the course of e. m. f. during oxidation-reduction processes has been treated by Michaelis and associates (*369–371*) and by Geake (*180*). The polarographic technique has also proved to be significant in this field (*398*).

In the case of semiquinone formation, which was the chief point of interest in developing the theory, Michaelis formulates the transformations for substituted paraphenylene diamines, which are the types of compounds that were studied most exhaustively, as follows:



The respective concentrations of the three forms are symbolized by r, s, and t. The equilibrium constant is

$$K = \frac{s^2}{rt} \qquad (1)$$

The relation $r + t = 2s$ must also hold.

If the solutions are properly buffered during the oxidation studies, the two possible stages of the process are represented by

$$E = E_1 + \frac{RT}{F} \ln \frac{s}{r}$$

$$E = E_2 + \frac{RT}{F} \ln \frac{t}{s}$$

and for the complete process

$$E_{\text{total}} = E_m + \frac{RT}{2F} \ln \frac{t}{r}$$

where

$$Em = \frac{E_1 + E_2}{2}$$

Let the total concentration of substance be

$$a = r + s + t \qquad (2)$$

and let x be the molar concentration of oxidant added, starting with the pure reduced form of concentration r.

$$x = s + 2t \qquad (3)$$

Upon combining expressions 1, 2, and 3 with the electrode expression and letting x/a be represented by X, the following general equation is obtained:

$$E = E_0'' + \frac{RT}{2F} \ln \frac{X}{2 - X} + \frac{RT}{2F} \ln \frac{X - 1 \pm \sqrt{(X-1)^2 + 4X(2-X)/K}}{1 - X \pm \sqrt{(X-1)^2 + 4X(2-X)/K}} \qquad (4)$$

If the equilibrium constant of a particular semiquinone formation process is very small, Equation 4 reduces to the familiar equation for a process involving two electrons:

$$E = E_0'' + \frac{RT}{2F} \ln \frac{\frac{x}{r+s+t}}{2 - \left(\frac{x}{r+s+t}\right)}$$

whereas at large values of K the curve is composed of two branches, each of form corresponding to

$$E = E_0'' + \frac{RT}{F} \ln \frac{(\mathrm{Ox})}{(\mathrm{Red})}$$

In Figure 1 is given Michaelis' plot of values of E *vs.* values of X for various values of the constant.

Geake (*180*) has given a detailed mathematical analysis of the possibilities, including formation of slightly soluble intermediate products.

Müller (*399–402*) has shown that an appropriate modification of Equation 4 may be applied to a study of semiquinone formation by the polarographic technique. This is not so accurate as the potentiometric method, but permits the study of labile radicals, and may also be applied in the range of over-voltage where the platinum electrode is not suitable.

Computation of Inflections. When the plots of e. m. f. *vs.* milliliters of reagent are not sharp, or the corresponding observations do not give a clear indication of the end point, it may still be possible to estimate the end point with fair precision, provided the reagent has been added in small equal increments. Hahn (*217*), Hahn and Klockmann (*218*), Fenwick (*136*), and later Shibata (*496*) have dealt with this question.

An illustration will make clear the simple application of the change in the second difference quotients of e. m. f. with milliliters of reagent. Let V_1, V_2, V_3, and V_4 be volumes read at equal

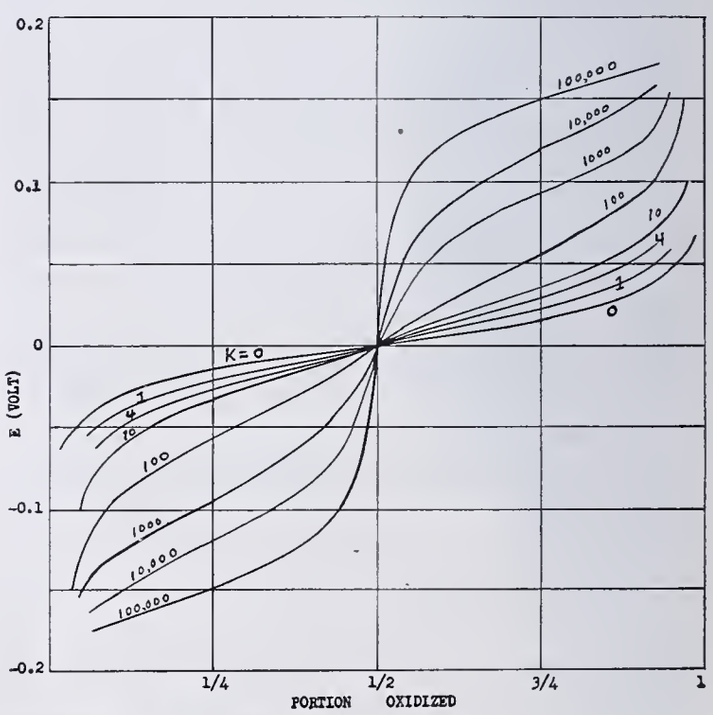


FIGURE 1. E *vs.* X, FRACTION OXIDIZED, FOR VARIOUS VALUES OF CONSTANT K OF SEMIQUINONE FORMATION

increments of reagent and the corresponding e. m. f. values be E_1, E_2, E_3, and E_4.

	E_1		E_2		E_3		E_4		E_5
E. m. f.	0.422		0.437		0.458		0.475		0.489
	V_1		V_2		V_3		V_4		V_5
Volume of reagent	30.20		30.40		30.60		30.80		31.00
$\Delta E/\Delta$ml.		7.5		10.5		8.5		7	
Δ^2E/Δml.^{2}			3.0		−2.0		−1.5		
			D_1		D_2				

The end point lies between 30.4 and 30.6, since the values of Δ^2E/Δml.^{2} change sign, and the end point is calculated to be at $30.4 + 0.2 \times \frac{3}{3+2} = 30.5_2$. In general, the volume to be added to the last buret reading before the change in sign of the $\Delta^2E/$ml.^{2} values is $\frac{D_1}{D_1 + D_2} \times$ volume increment, using the numerical values of D_1 and D_2 without regard to their signs.

The deviation between the theoretical and actual volumes in titrations with unsymmetrical curves has been discussed by Murgulescu and Drăgulescu (*404*), and by Hahn, Frommer, and Schulze (*214*). Hahn (*206*, *213*) has treated the effect of errors and interferences upon accuracy, and the subject of titration errors has been handled mathematically by Shakhkeldian (*493*). Hahn (*209*) and Hahn and Klockmann (*215*) describe the methods of deriving equilibrium constants from titration data.

Apparatus and Technique

Electrode Devices. An excellent type of salt bridge with a ground-glass stopper built in each end of the bridge (Figure 2) has been described by Irving and Smith (*252*). A bridge with sintered-glass ends is described by Laitinen (*318*). Gotō (*190*) has described the use of micro filter tubes, or tubes provided with either ground-glass joint, sintered glass, or a stopcock to construct reference electrodes free from diffusion effects. Some of the commercially available forms of electrodes that have been developed for pH measurements may be used almost indefinitely in titrations without serious diffusion errors. Lykken and Rolfson (*347*) have advocated the use of such electrodes.

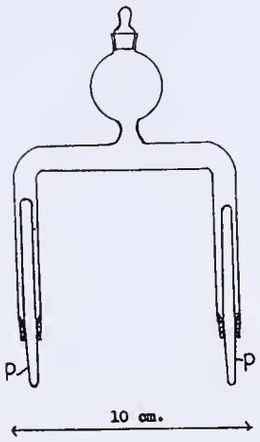


Figure 2. Salt Bridge

Tapered glass rods fit snugly through ground-glass connections.

Vance (*597*) has advocated the more general use of the silver–silver chloride electrode for reference in acid-alkali titrations. Capillary electrodes of platinum and silver have been proposed as indicator electrodes by Poupé (*434*).

Hiltner (*229*) attaches the upper ends of reference and indicator electrodes, with insulation to prevent short circuits, to a stout piece of wire bent to fit the rim of a beaker (Figure 3). Herzog (*228*) has proposed the use of flanged tubing, or tubes cut from the tops of test tubes, to hold electrodes which are mounted in rubber stoppers. The author has for many years mounted electrodes in holes drilled in Bakelite, the electrode mount being held in place by a ring of rubber tubing (*165*). Sholes (*499*) advocates protecting platinum electrode wires with glass to a point below the surface of the liquid that is titrated. The question of strain in glass and the failure of electrodes sealed through glass has been treated by Garrett *et al.* (*177*).

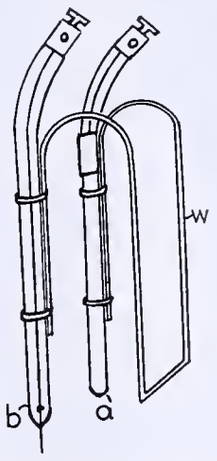


Figure 3. Simple Electrode Holder

Wire *w* serves as a support for electrodes *a* and *b* and will fit several sizes and types of beakers.

Atanasiu and Velculescu (*13*, *24*) have proposed the platinum-nickel electrode pair as a universal indicating system for neutralizations, oxidation-reduction titrations, and argentometry. In the last field the system appears to be unreliable, according to a few experiments by the author's students. Better combinations are available for the other fields.

Erbacher (*128*) has proposed the use of Ostwald decade boxes in a Wheatstone bridge arrangement for potentiometric titrations.

Simplified Devices for Determining End Points. The differential method has been further applied to the use of hydrogen electrodes in precision measurements by MacInnes and Cowperthwaite (*351*). Tungsten electrodes have been used by Abichandani and Jatkar (*1*). The method can be recommended in general for any type of problem where the use of a reference electrode presents difficulty. Orloff (*423*) uses the method, with platinum–lead peroxide electrodes, for controlling on an industrial scale the conversion of chromate to bichromate.

Guzmán and associates have used a thermocouple as a source of e. m. f. for polarizing electrodes (*203*) and have used the silver-platinum electrode system short-circuited through a sensitive galvanometer ($i = 7 \times 10^{-6}$ ampere per division) in the titration of the halides (*204*). The phenomena are analogous to the dead-stop method of Foulk and Bawden. The latter method has been extended by Clippinger and Foulk (*93*) to acid-alkali titrations with the addition of iodate and iodide or of hydrogen peroxide to serve as an electrometric indicator. In titrations with silver, cyanide acts as a depolarizer. In certain types of titration sodium nitrite is added to keep the anode depolarized.

Gay (*179*) has proposed the use of a small dry cell to polarize a tungsten-platinum electrode pair, in series with a 1500-ohm resistance, and a 0–1 milliammeter as indicating instrument. Under certain circumstances rather large currents can flow to or be drawn from the electrodes. The apparatus was found satisfactory for titrations with 0.1 *N* oxidants or reductants that show large breaks in potential, but was rather unsatisfactory for the titration of vanadyl solution with potassium permanganate (error 0.06 ml. in 25.00 ml.).

Multiple-electrode pairs have been proposed as a means of expanding the change in e. m. f. near the end point and thus making it possible to use cheaper instruments. Szebellédy and Jónás (*558*) placed the three duplicate electrode systems, connected in series, in the titration vessel and in two side tubes sealed to it. Upon shaking liquid into the side tubes three cells are created if electrical leakage across the wet glass surfaces is prevented by paraffin oil. Wolf (*621*) used four bimetallic electrode pairs connected in series and built as a stirrer. Upon stopping and lifting the stirrer or lowering the vessel, four cells are formed by cups, one below each electrode pair.

Stansby and Fitzgerald (*545*) have devised a semiautomatic arrangement with multiple electrodes for speeding the routine application of the method to large numbers of similar samples per day. A mounting for multiple electrodes in comparative

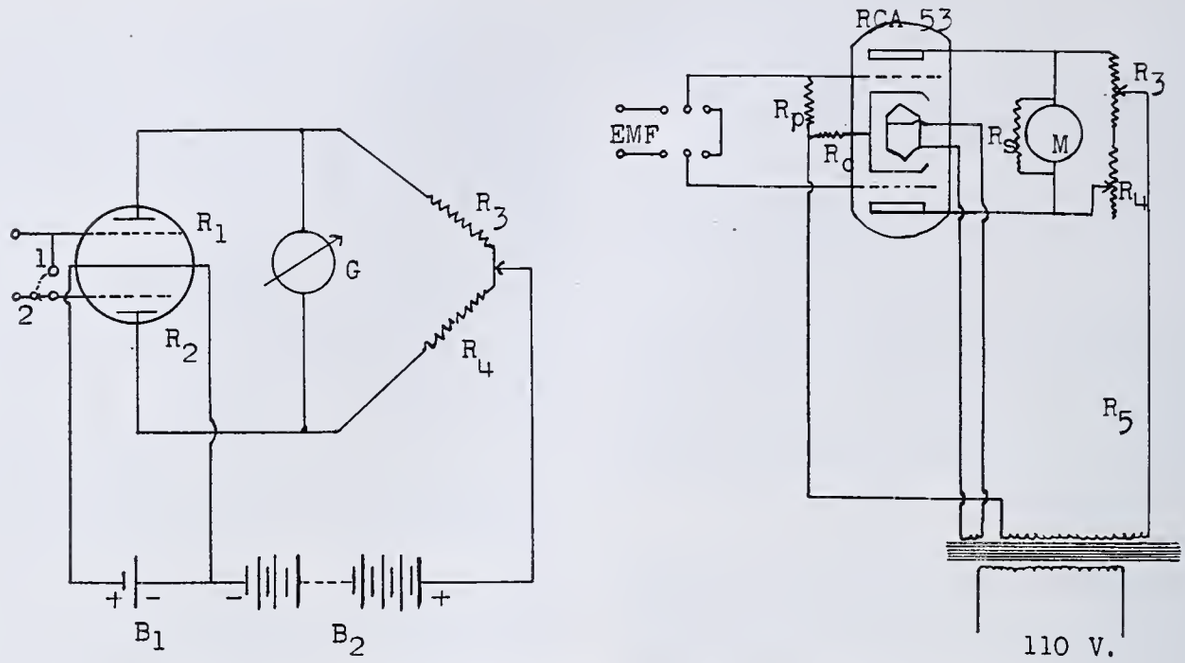


FIGURE 4. CIRCUITS FOR CONTINUOUS INDICATION IN POTENTIOMETRIC TITRATIONS

Left, Hiltner type (*230*)

R_p. From 50,000 to 200,000 ohms
R_c. 1000 ohms
R_s. 20 ohms
R_3. 2000 ohms

Right, Willard and Hager type (*615*)

R_4. 50 ohms
R_5. 480 (E_T − 220). E_T is transformer voltage
M. 0 to 150 microamperes

studies has been described in detail by Frediani and Warren (*150*). Tronstad (*594*) devised an automatic apparatus for estimating copper.

VACUUM-TUBE VOLTMETERS. As new types of radio tubes are developed, new or revised circuits are applied either in the field of pH measurement or for titration purposes. Circuits for measurement of pH with glass electrodes have reached a high degree of excellence. Detailed papers that have special merit are those of Cherry (*84*), Müller and Dürichen (*397*), Gilbert and Cobb (*184*), Stadie (*544*), Rosebury (*465*), and Morton (*384*); many other papers are important in this field. A review of this field is beyond the scope of this paper, and the reader is referred, for a critical discussion and review of the various types of circuits for use with the glass electrode, to the recent monograph by Dole (*112*).

The circuits that are finding most favor for continuous observation during titrations involve a Wheatstone bridge network in the output circuit. Some of the simpler circuits are shown in Figures 4 and 5. Circuits worthy of special mention are those of Baldinger (*31*), Compton and Haring (*94*), Ehrhardt (*126*), Garman and Droz (*175*), Goodwin (*189*), Hahn (*207*), Hiltner (*230*), Müller (*394, 395*), Müller and Dürichen (*396, 397*), Nottingham (*419*), Pollatschek (*433*), West and Robinson (*609, 610*), and Willard and Hager (*615*).

The use of the visual tuning tube for indication of a sudden change in potential at the end of a titration was first described by Smith and Sullivan (*515*). Subsequently, Serfass (*486*) described still simpler circuits that give the same general performance as that of Smith and Sullivan. Instruments based on this principle are commercially available.

The idea of using the same vacuum tube as both polarizing and measuring device has been developed by Kassner *et al.* (*275*) and by Masaki and Hirabashi (*365*). The use of radio tubes to construct automatically operated titration assemblies has been described by Kordatzki and Wulff (*310, 625*). Hahn (*211*) has criticized the work of Kordatzki and Wulff. A tube-operated circuit to close the stopcock of the buret was described by Shenk and Fenwick (*495*).

An ingenious circuit which gives a pulsation proportional to the change produced by each drop of reagent (pulse amplifier) has been developed by Baker and Müller (*30*). The maximum pulse per drop of reagent marks the end point. Another manner in which a vacuum tube voltmeter may be used to get differential readings is to reset the meter after each drop is added and a steady reading is found, as proposed by Clarke, Wooten, and Compton (*92*).

MICROMETHODS. The microchemical aspects of the potentiometric method have by no means been neglected. Ashcroft (*11*) has given an excellent review on the general subject of electrochemical methods in microchemistry, with bibliography. A similar review has been made by Ehrhardt (*125*). Chirkov (*85*) has studied microchemical applications of potentiometry, noting impulses in e. m. f. rather than plotting e. m. f. readings.

Suitable types of microburets are described by Mika (*375*) and by Lochte and Hoover (*340*). Krumholz (*313*) (Figure 6) and Raspopov and Finkelshtein (*447*) place the beaker on a platform that is rotated to effect stirring. The salt bridge (*313*) is one with sintered glass or small capillary ends. A novel scheme due to Schwarz (*482*) consists in supporting the small quantity of liquid to be titrated (1 drop) on a metal ring which is one of the electrodes. The other electrode tip and the buret make contact with the drop, which is agitated very gently by a magnetically operated vibrator to which the ring electrode is fastened. Micro hydrogen electrodes have been described by Frediani (*149*) and by Löbering (*342*) and micro quinhydrone electrodes by Mikawa (*376*), LaMer and Armbruster (*319*) for studies of heavy water solutions, Fuhrmann (*161*), and Itano (*256*).

A list of typical detailed applications of microtechnique in potentiometric titrations follows:

Acidimetry, using glass electrode (Partridge and Bowles, *427*)
Amino nitrogen (Roche and Roche, *462*)
Bromide, 0.25 mg. in the presence of 25 mg. of chloride (Vladimirov and Epstein, *603*)
Chloride, 0.25 ml. of 0.0001 *N* solution in alcohol or acetone titrated within a few per cent of the correct value (Schwarz and

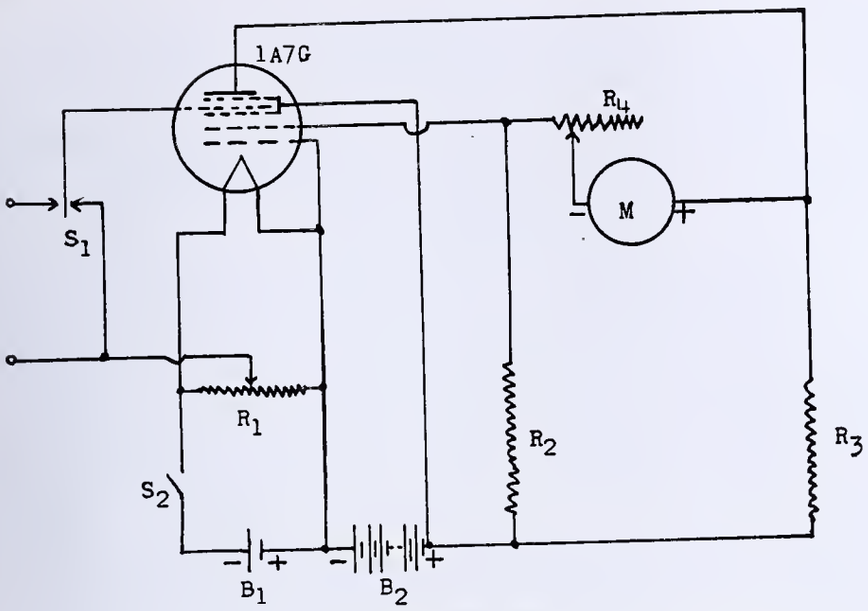


FIGURE 5. CIRCUIT OF CONTINUOUS-READING POTENTIOMETRIC INDICATOR (*176*)

B_1. 1.5 volts
B_2. 45 volts
R_1. 1000 ohms
R_2. 2000 ohms
R_3. 2500 ohms
R_4. 50,000 ohms
M. 0 to 50 microamperes

This circuit is easy to construct and has given good service in the hands of numerous workers.

Schlösser, *484*). For concentrations of chloride greater than 2.5 mg. per liter, maximum error 1.2 per cent (Bruevich and Vartolomeeva, *76*)

Lead, titrated with ferrocyanide (Schwarz, *482*)

Mercury, titrated with potassium iodide in range 1 microgram to 1 mg. (Schwarz and Kantor, *483*)

Silver in the range of a few micrograms (Zürcher and Hoepe, *630;* Spychalski, *543*)

Sodium, via one of the triple uranyl acetates and ceric sulfate (indirect titration of U^{IV} to U^{VI}), (Linder and Kirk, *336;* Kryulov and Kolarova, *314*)

Thallium (Berg and Fahrenkamp, *42*)

Titanium (Klinger *et al.*, *297*)

The paper by Schwarz (*482*), in which the titration of a single drop of liquid is described, covered the titration of halides with silver nitrate, sulfuric acid with sodium hydroxide, lead with ferrocyanide, ferricyanide with iodide, iodate, and thiosulfate, and bromate with arsenite. Prior to the period now under review some of these methods as well as others had been studied on a micro scale (cf. *299*, page 149; Hahn, *210;* and Zintl and Betz, for details).

CONCENTRATION CELLS. The use of suitable concentration cells makes possible a micro- or macroestimation of substances. Both titration procedures and direct measurements of the e. m. f. values of concentration cells may afford useful analytical information.

Johnson and Low (*267*) developed the work of Johnson (*266*) to make possible the estimation of the end point of the adjustment process in atomic weight studies. Furman and Low (*169*) developed a concentration-cell method for estimating traces of chloride in various salts. They noted effects of iodide ion upon the silver–silver chloride electrode. Hahn (*208*) noted that bromide ion affected the potential of the calomel half-cell and suggested that this type of effect might be used for the determination of bromides in chlorides. Low and Pryde (*344*) developed a concentration-cell method based upon the ferric-ferrous electrode for the indirect estimation of fluorides in various solutions. Drewski (*122*) used the concentration-cell technique for the estimation of iodine numbers. Valmari (*596*) used a somewhat similar idea for the estimation of nitrate and nitrite and the indirect estimation of potassium in the cobaltinitrite; a calibration curve was established with known mixtures. Sergeev and Yavorskiĭ (*488*) determined pH by trying various buffers until a galvanometer showed no deflection when connected between antimony electrodes in the buffer and the unknown, respectively. Oxygen in air may be determined by its effect upon the polarization of the carbon electrode of a dry cell, according to Moiseev (*380*).

ELECTRODES FOR pH MEASUREMENT. A complete review of this field is beyond the scope of this paper. A selection of important papers and reviews has been made.

Antimony Electrode. Good bibliographies of earlier work on this electrode are to be found in papers by Vellinger (*600*) and Guéron (*198*) and in Böttger's monograph (*47*). Papers that deal with the theory of the electrode and with precision measurements are those of Ball (*32*), Hovorka and Chapman (*249*), Parks and Beard (*425, 426*), Perley (*428*), and Wulff, Kordatzki, and Ehrenberg (*626*). Relative to the standard hydrogen electrode at 25° C., $E_{Sb} = 0.2552 + 0.05893$ pH from pH 2 to 8, according to Hovorka and Chapman (*249*). This work is in good agreement with work of Perley (*428*). King (*295*) has measured the salt error of the antimony electrode.

Gas Electrode. Krueger and Kahlenberg (*311*) studied the effects of various gases—hydrogen, oxygen, argon, and helium—upon metal electrodes. They reported small definite effects due to the inert gases as well as to oxygen and hydro-

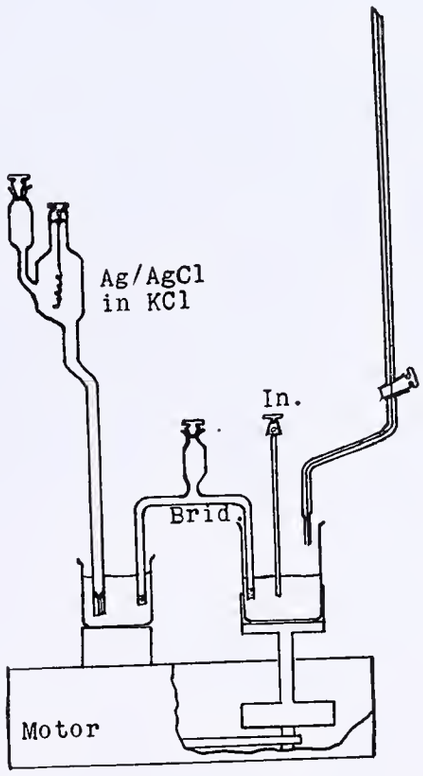


FIGURE 6. TYPICAL ASSEMBLY FOR POTENTIOMETRIC TITRATIONS ON MICRO SCALE

The beaker, holding 5 ml. of solution, is turned to cause stirring. The electrodes are of conventional design but of small dimensions.

gen. It is by no means certain that the observed effects were not due to trace impurities or electrode strain, etc.

Glass Electrode. Fundamental information is to be found in the papers by MacInnes and associates (*350–355*), Dole and associates (*112–120, 174*), Gross and Halpern (*195, 196*), and Laug (*331*). The measurement of the pH of distilled water has been studied by Burton *et al.* (*78*). Metal-connected glass films function as glass electrodes according to Thompson (*576*). Shielding by a solution is described by Adler (*5*). Detailed information is to be found in a monograph by Dole (*112*).

Hydrogen Electrode. A review of the applications has been given by Guéron (*198*). Because of the practical advantages that are associated with the use of the glass electrode, relatively little new work on the hydrogen electrode has appeared in the last decade.

Mercury–Mercuric Cyanide Electrode. Tomíček and Pribil (*586*) have shown that this electrode measures changes in hydrogen-ion activity in alkaline solutions.

Oxygen Electrode. The mechanism of this electrode has been studied by Hoar (*242*), who considers the well-known irreversibility as due to a film of oxide that is rather impervious except for cracks and pores. Tartar and associates (*567, 568*) have concluded that adsorption effects play a large role in the behavior of the electrode.

Quinhydrone Electrode. Important studies of this electrode have been made by Harned and Wright (*224*), Hovorka and Dearing (*250*), and Morgan, Lammert, and Campbell (*321, 322, 381, 382, 383*). The reproducibility and reliability of the electrode under proper conditions have been amply confirmed. Coons (*95*) has adapted the electrode to continuous pH measurement.

Tellurium–Tellurium Oxide Electrode. Studies by Bravo (*56*), Getman (*183*), and Tomíček and Feldmann (*580*) show that this electrode may be used for measurements in the range pH 5.3 to 11.5.

Tungsten and Molybdenum Electrodes. These electrodes which have been found in the past to be approximate pH indicators have been reinvestigated by Brintzinger and Rost (*62*). By empirical calibration with the aid of buffers it was found possible to measure pH within 0.1 pH unit.

Electrodes for Acid-Base Titrations. It is obvious that the better pH indicators are the most reliable electrodes to use when establishing the course of pH *vs.* milliliters of reagent. If the interest is merely in estimating end points, many simplified devices may be used.

Antimony Electrode. The use of this electrode in the titration field has been further studied by Böttger and Szebellédy (*49*), Britton *et al.* (*70*), Hahn (*205*), and Kauko and Knappsberg (*276*). The latter studied the effect of partial pressure of oxygen upon the behavior of the electrode.

Bimetallic Electrode. Numerous studies of possible combinations have been tested:

Aluminum *vs.* Rose metal (French and Hamilton, *151*)
Copper *vs.* tungsten (Teis and Vagner, *569*)
Ferroalloys and various alloys and metals—e. g., iron-nickel *vs.* molybdenum; iron-nickel *vs.* tungsten; also other ferroalloys (Banchetti, *36*). Brass, copper amalgam, iron oxide, pyrites; titanium, silver, iron, nickel, lead, copper, and tin in various pairs have been tested by Boltunov and Vorsina (*55*).
Gold *vs.* rhodium in fused salt baths (Lux, *345*)
Platinum *vs.* Carborundum (Khlopin, *289*)
Platinum *vs.* chromium (Wolf, *621*)
Platinum *vs.* graphite (Tikhonov, *577*)
Platinum-nickel (Atanasiu and Velculescu, *22*)
Platinum-antimony, platinum-tantalum (Boltunov and Krogius, *54*)
Platinum-nichrome (Mazzuchelli, *368*)
Platinum *vs.* V2A steel, ferroalloys, etc. (Banchetti, *36*)
Silicon *vs.* silicon carbide (Boltunov and Isakova, *53*)
Tungsten *vs.* nickel (Furman and Low, *168*)

These systems must be used with caution in working with dilute solutions. Many of them function well for titrations of strong acids and bases. In general, it is best to use one electrode which is known to be a good indicator electrode—e. g., antimony—and another which is a reference electrode—e. g., silver—in solutions containing chlorides.

The *germanium–germanium dioxide electrode* may be used for acid-alkali titrations (*415*).

A *glass electrode* of low resistance has been developed by Johnson (*268*). The glass electrode has been used very frequently for all types of titrations (cf. *72, 73*). A number of specific instances are pointed out in the summary of potentiometric acid-alkali titrations.

The *oxygen electrode* has been studied further by Butler and Armstrong (*79*).

A *palladium hydride electrode* has been found suitable for titrations of organic bases dissolved in ethanol or acetone (*475*).

The attraction of a *silk* thread toward one electrode as the solution passes from acid to alkaline has been proposed as an electrometric indication (*338*).

The *tellurium–tellurium oxide electrode* may be used for titrations (Brouwer, *75, 580*).

The *tungsten electrode* has been studied by Britton and Dodd (*64*).

Applications

Summary of Potentiometric Applications of Acid-Alkali Titrations

Precipitations. Aluminum, using antimony indicator electrode (Kanning and Krath, *272*)
Aluminum, in presence of nitrates (Madzhagaladze, *356;* Stefanovskiĭ, *546*)
Aluminum, titration, using galvanometric method (Martin, *362*)
Aluminum, effect of various anions (Whitehead *et al.*, *611*)
Copper (Beebe, *39*)
Gold (Britton and Dodd, *65*)
Iron (Kanning and Krath, *272;* Shisakov, *497;* Zaides, *627*)
Lead as $Ph(OH)_2Pb(CNS)_2$ in presence of other ions (Hayek, 225)
Magnesium, antimony electrode, (Malvea and Withrow, *359;* Berraz and Christen, *43;* Itano and Tsuji, *258*)
Mercuric ion (Britton and Wilson, *73*)
Mercurous ion (Bennett, *41*)
Oxalates of lead, barium, etc., adsorption of hydrogen or hydroxyl ion (Tannaev, *561*)
Platinum (Britton and Dodd, *66*)
Uranium (Britton and Young, *74*)
Zinc (Prytz, *438*)
Reactions in Aqueous Solvents. Acids, amino and polypeptides (Balson *et al.*, *34, 35*)
Acids, dibasic (Ingold and Mohrhenn, *251*)
Dicarboxylic, unsaturated (Ashton and Partington, *12*)
Fatty (Lottermoser and Ghose, *343*)
Imino (Litzinger and Pickett, *339*)
In alkaloids (Drewski, *123*)
In colored liquids (de Lingo and Thaler, *337;* Thaler and de Lingo, *572;* Minnaev, *378*)
Alkali, in phenates, unsaturated oxygen electrode (Kargin and Usanovich, *273*)
Alkalinity, in sea water (West and Robinson, *610*)
Amino acids, polypeptides (Balson *et al.*, *33*)
Ampholytes (Konikov, *309*)
Antipyrine, antimony electrode (Gurevich, *199*)
Boric acid, quinhydrone electrode (Wilcox, *613, 614*)
Carbonates (Aumeras and Marcon, *27*)
Carbon dioxide, glass electrode (Wilson *et al.*, *618*)
Carbonic acid and sodium bicarbonate (Liander, *335*); glass electrode (Dorello and Rowinski, *121*)
Chromic acid, glass electrode used (Nuess and Rieman, *414*)
Dichromate, platinum electrode (Ruiss and Babalova, *467*); platinum–lead oxide electrode (Sabinina and Moralev, *472*)
Fumaric, maleic, and succinic acids (Cattelain and Couchet, *81*)
Gallic and gallotannic acids, differential method with hydrogen or tungsten electrodes (Sunthankar and Jatkar, *555*)

Hypochlorite, platinum electrode (Abribat, *3*)
Nitrogen, formol titration, antimony electrode (Roche and Roche, *461*)
Organic acids, volatile (Craig, *96*)
Phosphoric acid and phosphates, antimony electrode (Vengerova *et al.*, *601*)
Phosphoric acid, quinhydrone electrode (Sanfourche, *476*)
Polypeptides (Balson *et al.*, *34*, *35*)
Proteins, animal and vegetable (Errera *et al.*, *129*)
Silica, indirect, through potassium silicofluoride and calcium chloride, antimony electrode (Tarayan, *565*)
Sodium benzoate, bicarbonate, and salicylate in mixtures (Izmailov and Shvartsman, *260;* Pinkhof method, to pH of buffer reference electrodes)
Strong acids, galvanometric titration (Sergeev, *487*)
Sulfurous, selenious, and alpha-hydroxyalkyl sulfonic acids, glass electrode (Rumpf, *468*)
Sulfur, in steel (Thanheiser and Dickens, *575*)
2,5-Diaminotoluene-4-thiosulfuric acid, hydrogen electrode (Bogert and Sevag, *51*)
Thiosulfate, formed from sulfur and sulfurous acid (Müller and Melhorn, *390*, *391*)

Acid-Alkali Reactions in Nonaqueous Solvents. Acetic acid medium, determination of amino acids (Nadeau and Branchen, *407*)
Acetone-water medium. The ionization constants of weak acids—e. g., acetic and dichloroacetic—are made smaller while hydrochloric acid remains highly ionized (Izmailov and Bel'gova, *259*). Sulfuric acid in presence of lactic (Shkodin, *498*)
Alcohols, *N*-butanol, change in ionization constants (Hantzsch, *222;* Evans and Davenport, *131;* Wooten and Ruehle, *624*). Butyl Carbitol (Halford, *220*)
Alcohols, as medium for amino acid titration (Ogston and Brown, *421;* Neuberger, *412;* Balson *et al.*, *33*)
Alcohols, acetone, benzene, hexalin, and mixtures as media, using antimony or tellurium electrodes (Tomíček and Feldmann, *580*)
Alcohol-acetone (Shafershtein *et al.*, *489*)
Alcohol-anisole (Demarest and Rieman, *101*)
Benzene as medium (LaMer and Downes, *320*)
Cellosolves (glycolmonalkyl ethers) as media (Ruehle, *466*)
Propionic acid as medium for the titration of organic bases (acetanilide, acetamide, urea, acetoxime), (Terjesen and Sandved, *571*)
Oils, acidity in (Evans and Davenport, *130;* Clarke, Wooten, and Compton, *92;* Ralston *et al.*, *445*)

The foregoing section gives several illustrations of a new trend in research on the determination of acidity—namely, the deliberate use of a nonaqueous or a mixed solvent to spread the ionization characteristics of two or more solutes, so that a differential titration is possible in the medium and either impossible or less accurate in aqueous medium. The use of anhydrous acetic or propionic acid to enhance the relative sharpness of the titrations of bases which are very feebly ionized in water is another noteworthy advance in technique.

Titrations Involving Precipitation

Special Electrodes. The silver electrode for halide precipitations and the like and the platinum oxidation-reduction indicator electrode for ferrocyanide precipitations still are the chief indicating electrodes in this field. Mention has been made of acid-alkali processes above. Changes in pH due to adsorption during the precipitation of oxalates and sulfates of lead, calcium, strontium, and barium have been observed by Tannaev and Mirianashvili (*564*). Atanasiu (*14*) has proposed the platinum-nickel and the platinum–silicon carbide couples as suitable for ferrocyanide precipitations, and the platinum-nickel, platinum–silicon carbide, and platinum-graphite couples for halide and silver titrations. Second-class electrodes, as, for example, silver–silver chloride for halides or silver–silver sulfide for zinc, cyanide, or nickel titrations, are discussed by Hiltner (*231*, *232*). Chromium, molybdenum, and tungsten electrodes in contact with their insoluble salts are useful in the estimation of chromium, molybdenum, tungsten, and various heavy metals, according to Brintzinger and Jahn (*59*). It has been observed by Böttger and Schall (*48*), Kolthoff and Wang (*308*), and Obrucheva (*420*) that a platinum wire, after being used, becomes a good indicator for silver ions, perhaps because of an oxidation-reduction process involving a higher oxide of silver.

Third-Class Electrodes have been studied especially in connection with determination of calcium. The following list gives reference to separate publication in this field.

$Ag/Ag_2C_2O_4/CaC_2O_4,Ca^{++}$; $Zn/ZnC_2O_4,CaC_2O_4,Ca^{++}$; $Pb/PbC_2O_4,CaC_2O_4,Ca^{++}$; $Ag/Ag_3(PO_4)_2,Ca_3(PO_4)_2,Ca^{++}$; $Hg/Hg_3(PO_4)_2,Ca_3(PO_4)_2,Ca^{++}$; $Pb/Pb(IO_3)_2,Ca(IO_3)_2,Ca^{++}$ (Velíšek, *599*)
$M_1/CuC_2O_4,CaC_2O_4,Ca^{++}$ (M_1 is Pt, Au, or Ag); (Nierstrasz and Tendeloo, *416*)
$Hg(Pb)/PbC_2O_4,CaC_2O_4,Ca^{++}$ (Denina and Caris, *102;* Nierstrasz and Tendeloo, *416*). This system is said to function in the presence of chlorides which interfere with the majority of the above electrodes.
$Pb/PbSO_4,CaSO_4,Ca^{++}$; $Ag/Ag_2C_2O_4,CaC_2O_4,Ca^{++}$; $Hg(Zn)-ZnC_2O_4,CaC_2O_4,Ca^{++}$; $Hg/Hg(IO_3)_2,Zn(IO_3)_2,Zn^{++}$; $Hg/Hg_2I_2,-Tl,Tl^+$ (Velíšek, *598*)
Pt(Hg)/HgS,PbS,Pb^{++} (Ripan-Tilici, *457*)
Theory of third-class electrodes (LeBlanc and Harnapp, *332*)
Membrane electrodes formed of silver iodide and silver bromide, or of silver iodide and silver sulfide, and sealed to glass tubes with picein cement have been tested as indicating electrodes in silver and halide titrations by Skobets and Kleibs (*509*)

Tendeloo (*570*) has used a membrane of calcium fluoride in connection with the estimation of concentrations of calcium that are equal to or greater than 1 milliequivalent per liter. The electrode was applied in the study of gelatin and milk.

Applications of Potentiometric Method to Precipitations. Chromicyanide and cobalticyanide as reagents (Czaporowski and Wiercinski, *100*). Cobalticyanide was found to be useful for the titration of silver, cupric, and mercurous ions, but not for cadmium, cobalt, zinc, and nickel. The reactions of chromicyanide were too slow.
Cerium, lanthanum, and thorium determined as oxalates (Atanasiu, *16*, *17*, *19;* Jantsch and Gawolowski, *262*)
Chromate, with barium (Brintzinger and Jahn, *58*, *59*)
Ferrocyanide with mercuric, nickel, or cobalt (Masaki, *364*)
Ferrocyanide as reagent. Carbonate, indirect by adding excess of lead solution, and back-titration with potassium ferrocyanide (Ringbom, *450*)
Cadmium (Tannaev and Diaparidze, *562*)
Cerium, lanthanum, thorium (Atanasiu, *15;* Atanasiu and Velculescu, *21*). Acetone-water or alcohol and water (30 per cent alcohol) used (Shemyakin and Volkova, *494*)
Cerium in 30 per cent alcoholic solution (Spacu, *540*)
Cobalt and nickel (Atanasiu and Velculescu, *23*)
Copper (Fisher and Mavrin, *138*)
Copper, titrated with sodium sulfide (Jha, *263;* Prased and Jha, *435*)
Potassium, indirect (Nikolskiĭ and Lavrov, *417*)
Zinc (Brennecke, *57;* Fisher, *137;* Joassart and Leclerc, *265;* Kamienski, *270;* Kamienski and Karczewski, *271;* Saitō, *473*, *474;* Stefanovskiĭ, *547;* Tannaev, *561*)
The general types of precipitates that are formed with silver, lead, copper, cadmium, zinc, cobalt, nickel, and manganese salts when precipitated with hydroferrocyanic acid have been studied by Britton and Dodd (*67*).
Fluoride, precipitated by calcium ion (Ryss and Bakina, *471*); precipitated by cerous ion (Allen and Furman, *9*)
Lead, precipitated with sulfide (Maheshwari and Jha, *357*); precipitated with phosphate (Shakhkeldian, *491*)
Lead nitrate as reagent (Ringbom, *450*); a general study including details as to the determination of sulfate, carbonate, sulfite, chromate, tungstate, and molybdate (*500*)
Mercuric nitrate as reagent (Tomíček and Procke, *587*)
Mercurous salts (Michalski, *373*); iodide as reagent (Spacu and Spacu, *529*). Determination of arsenic with mercurous solution (Spacu, *537*)
Molybdenum, with lead nitrate (Ringbom, *450;* Senyuta, *485*)
Phosphate, with uranyl acetate (Atanasiu and Velculescu, *26*). The precipitate is HUO_2PO_4, temperature 60–70° C., pH 5.5 to 6. The same precipitate is formed with mono-, di-, or tribasic alkali phosphate.
Potassium, indirect, by its effect upon the titration of lithium ferrocyanide with heavy metals (Tannaev and Diaparidze, *563*)

Silver (Moser *et al.*, *385;* Raub, *448;* Weiner and Schmidt, *605*); determination of silver for coinage purposes (Robinson and Hugg, *460*). Gold *vs.* carbon treated with nitric acid as electrode system. Spychalski (*543*) titrated Bredig solution for silver with bromide or iodide with an accuracy of 0.5 to 1 per cent of the silver present. Hiltner and Gittel (*234*) developed a systematic potentiometric scheme for estimation of silver, bismuth, lead, copper, and cadmium in the same solution.

Silver nitrate as reagent. Arsenate, in acetate buffer plus alcohol (Hanson *et al.*, *221;* Spacu, *537*)

Azide (Moskovich, *386;* lead azide in 2 per cent barium nitrate solution, 0.04 *N* in nitric acid, and buffered with 0.2 *N* sodium acetate .

Bromide, in presence of chloride (Flood and Sletten, *142*). The inflection for the bromide should be 15 mv. from the prolonged chloride curve. An empirical correction can be made accurately by a graphical method.

Chloride, effect of electrolytes on curve (Orlov, *424*)

Effects of acids (Guzeli, *202*)

Electrode system, silver *vs.* graphite (Khlopin, *280–282*)

Silver–silver chloride *vs.* quinhydrone, pH 3 (Itano, *257*)

In atomic weight studies (Johnson, *266;* Johnson and Low, *267*)

In brines (Hoff-Jørgensen, *245;* Tremblay, *592*)

In bromide mixtures (Schütza, *481*)

In sap (Neller, *410*)

In sea water (West and Robinson, *610*)

In soils (Snyder, *516*)

Cyanamide (Sinozaki, *508*)

Cyanide (see complex formation)

Cyanines, through alkyl iodides (Calzavara, *80*)

Cyanates (Ripan-Tilici, *454*); in presence of cyanide (Ripan-Tilici, *455*)

Ferrocyanide (Tomíček and Hubrova, *583*)

Hydrochloric acid, in alkaloids (Shafershtein *et al.*, *489*)

Hypophosphorous acid (Jung and Uspenskaya, *269;* Wolf *et al.*, *622;* Grundmann and Hellmick, *197*). The solution is neutralized to phenolphthalein and then disodium hydrogen phosphate is added. The halides, cyanide, and thiocyanate are precipitated before the hypophosphite reacts.

Iodide, accuracy of reaction at extreme dilutions (Kolthoff and Lingane, *302*). Precision studies accurate to 0.003 per cent (Lange and Berger, *327*)

Mercaptans. Mercaptans are precipitated prior to chloride (Tamele and Ryland, *559;* Tamele *et al.*, *560*). The latter paper deals with the use of aqueous alkaline solutions.

Mercaptobenzothiazole (Spacu, *532*)

Molybdate (Spacu, *533*)

Oxalate (Atanasiu, *18*)

Phosphate, as silver thallium phosphate (Spacu and Drăgulescu, *524*). As silver phosphate (Michalski, *372*)

Selenocyanate (Spacu, *536*). Determination of selenocyanate in presence of thiocyanate (Ripan-Tilici, *453*); in presence of cyanide (Ripan-Tilici, *456*); in presence of halides (Spacu, *538*); in presence of iodide (Spacu and Spacu, *531*, p. 179); in presence of chloride and bromide (Spacu and Spacu, *531*, p. 248); in presence of thiocyanate (Spacu, *536*)

Selenite (Ripan-Tilici, *458*)

Sulfide, with cyanide, thiocyanate, and chloride (Raines and Putning, *444*). Four breaks in e. m. f. are found, the first after silver sulfide is precipitated, the second after complex cyanide formation. The third follows the combined precipitation of cyanide and thiocyanate, and the fourth after the chloride has precipitated. Bursuk and Zan'ko (*77*) determined sulfide, thiocyanate, and chloride in a single titration with succession of end points.

Sulfide as reagent for heavy metals (Hiltner and Grundmann, *239*)

Sulfite (Spacu and Drăgulescu, *525*, *526*)

Tetrathionate, trithionate (Ishikawa and Murooka, *253*)

Thiocyanate. Indirect determination of cadmium via thiocyanate, $Cdpy_2(CNS)_2$ (Spacu and Spacu, *530*, p. 263)

Cobalt (Spacu, *535*)

Copper (Spacu and Spacu, *530*, p. 99)

Nickel (Spacu and Spacu, *529*, p. 270)

Copper direct with thiocyanate (Hiltner and Grundmann, *237*)

Thiosulfate and chloride (Petit, *429*)

Vanadates (Britton and Robinson, *71*). Metavanadate (Spacu, *539*)

Sulfates, precipitation with barium ion (Christensen *et al.*, *91;* Visyagin, *602;* Orestov, *422*); with lead, indirect (Shakhkeldian, *492*)

Tungstate, as barium tungstate (Brintzinger and Jahn, *58*); as lead tungstate (Noda, *418*).

Complex-Formation

Alkaloids. Maricq (*360*) has further extended the applications of the reagent potassium iodomercurate (K_2HgI_4) in the field of alkaloid chemistry to include stovaine, novocaine, pyramidone, antipyrine, and cinchophen. The alkaloid is precipitated with excess of the reagent and the excess is titrated, after filtration, with standard mercuric chloride.

Aluminum may be titrated with a standard solution of 0.5 *N* sodium fluoride to form sodium fluoaluminate in 50 per cent alcoholic solution saturated with sodium chloride according to Tarayan (*566*). The ferric-ferrous system is used for indirect indication with a platinum-calomel electrode system.

The potentiometric determination of *copper* in steels by the cyanide titration method is said to be more rapid than the colorimetric or the electrolytic method (Weihrich, *604;* Piccinini, *431*).

Glucose in simple solutions or in blood may be determined indirectly by the iodomercurate method, according to Maricq (*361*).

Fluoride may be titrated with uranous solution (U^{IV}) and at the completion of the reaction: $5F^- + U^{++++} + K^+ = KUF_5$ there is a jump of the potential of the uranyl-uranous electrode. Calcium, aluminum, iron, and phosphates interfere. The pH of the solution is controlled by a buffer of 0.5 *N* sulfanilate saturated with sulfanilic acid (Flatt, *140*). According to Ryss and Bakina (*471*), fluoride may be titrated with a standard calcium solution after saturating the solution with sodium fluosilicate, using the quinhydrone electrode as indicator.

Spacu and Drăgulescu (*527*) have studied the reaction between *mercuric chloride* and ammonium sulfite. The formation of two compounds is indicated, NH_4HgSO_3Cl and $(NH_4)_2Hg(SO_3)_2$. Mercury may be determined by titration with potassium iodide (Hiltner and Gittel, *235*). Spacu and Murgulescu (*528*) titrate iodomercurate ion with $Cu(en)_2$ sulfate or nitrate as reagent. The reaction is: $HgI_4^{--} + Cu(en)_2 = Cu(en)_2HgI_4$. A mercury electrode and a calomel half-cell are used. The reaction is sluggish near the end point.

The potentiometric determination of *nickel* by the cyanide method has been repeatedly studied. Bohnholtzer (*52*) used the conventional silver-calomel combination with a salt bridge. Chatterjee and Jha (*83*) found that the quinhydrone electrode could be used to indicate the formation of nickel cyanide when nickel was titrated with standard cyanide solution. The pH at the end point is about 7.5. Hiltner and Grundmann (*236*, *238*) recommended the use of the silver iodide and silver sulfide electrodes in connection with the cyanide method for nickel. Hiltner and Seidel (*241*) applied the cyanide method to the estimation of nickel in nonferrous alloys after the electrolytic removal of copper and lead. Khlopin (*283*) applied the galvanometric method of indication for the estimation of nickel in steels with standard cyanide solution. He also developed a back-titration method, using nickel as the final reagent after adding excess of standard cyanide to the ammoniacal solution (*285*, *290*). Weihrich (*604*) recommends the use of silver and silver iodide as the indicator electrode and gives tables of factors for converting various weights of copper to their equivalent amounts of nickel in this process.

Many organic acids enter into complex formation with ferric chloride, and Treadwell and Wettstein (*590*) have shown that certain of these processes may be followed potentiometrically—for example, the ratio of ferric ion to acid is 2 to 3 for citric acid, 1 to 3 for benzoic acid, 1 to 2 for malic acid, and 1 to 1 for oxalic acid. The platinum-calomel system

is used and air is excluded. The addition of alcohol or sugar is useful.

Wick (*612*) made a thorough study of the application of the potentiometric method to silver cyanide plating baths. Weiner and Schmidt (*605*) have also studied this question.

The reaction of sulfite ion with mercuric chloride solution has been studied potentiometrically by Spacu and Drăgulescu (*525*, *526*).

Oxidation-Reduction Reactions

The applications in this field are arranged approximately in the order of relative oxidizing power of the reagents with a numbered section for each reagent or group of similar reagents.

1. Permanganate Processes. The estimation of manganese after oxidation to the permanganate stage has been used repeatedly in steel analysis (cf. Section 13). The titration of the permanganate with ferrous solution in the presence of fluoride has been applied to the estimation of manganese in iron castings by Zan'ko and Stefanovskiĭ (*629*). The decomposition of permanganates may be studied in concentrated acids with a platinum–mercurous sulfate–mercury electrode system according to Chloupek (*88–90*). The effect of the presence of fluorides upon the reaction between arsenious and antimonous solutions or iodide or hydrogen peroxide and potassium permanganate has been studied by Pugh (*439*, *441*).

Iron. The determination of iron in feldspar has been considered by Lyle (*348*). Ryabchikov and Silnichenko (*470*) propose the use of cuprous chloride for the reduction of the iron, in the presence of sodium chloride and hydrochloric acid. Upon potentiometric titration two breaks are obtained, one after oxidation of the excess of cuprous salt and the other after re-oxidation of the iron.

Hydrogen Peroxide and Other Peroxides. Müller and Brenneis (*403*) found the permanganate method to be more suitable than many other methods (cf. Reichert *et al.*, *449*). Pugh studied the titration of permanganate with hydrogen peroxide in the presence of fluosilicate (*440*) as a buffer. There is one fairly sharp break at the end of the reduction to manganese dioxide and a sharper break in potential when the latter is reduced to manganous salt.

The potentiometric titration of *iodide* to iodine monochloride with standard permanganate is suitable in the presence of chloride or bromide, according to Flatt and Boname (*141*) and Hahn (*212*).

Nitrites may be titrated with permanganate, although the reaction is slow, according to Jimeno and Ibarz (*264*).

Nitroprusside complexes may be studied by potentiometric titration with permanganate (Masalskiĭ and Chernuiĭ, *366*).

Platinum. Bivalent platinum may be estimated with potassium permanganate or potassium bromate (Stelling, *549*). Grinberg and Ptitzuin (*191–193*) have used the potentiometric method for the study of various platinum and iridium complexes.

Uranium. This process has been reinvestigated by Luyckx (*346*).

Zinc. Tannaev (*561*) has proposed the titration of excess ferrocyanide with permanganate for this indirect estimation.

Titrations in Strongly Alkaline Solutions. Tomíček (*579*) studied the estimation of arsenite, antimonite, selenite, and tellurite. The direct titration of selenite was not successful.

2. Auric Chloride. That this reagent is useful in the titration of tocopherols was shown by Karrer and Jaeger (*274*). Certain carotenoids interfere. Some of the members of the group consume eight equivalents per mole, others two, and still others are not oxidized.

3. Cerate Systems. The sulfato ceric system has proved to be reliable and useful. General reviews of the applications have been given by Janssens (*261*), Willard and Young (*617*), and Furman (*164*).

Ceric (IV) *vs. Arsenious* (III). Lang and Zwerina (*325*) determine cerium with 0.1 *N* arsenite, using either iodine monochloride or manganous salt as catalyst.

Arsenic and antimony may be determined successively by titration with ceric sulfate, with addition of iodine monochloride after the antimony has been oxidized. The method is selective for antimony, provided the concentration of the arsenious acid is not too high (Furman, *163*).

Arsenite and ferrocyanide may be determined in the same solution (Lang, *323*).

The *ceric vs. ferrous* reaction has been recommended again for the estimation of cerium (Weiss and Sieger, *606*).

Various *combinations of elements* that are of interest in steel analysis may be determined in whole or in part with standard ceric solution: manganese, chromium, and vanadium (Dickens and Thanheiser, *110*). Vanadium, uranium, and iron (vanadium in ores) (Levenson and Kochmarev, *334*). Molybdenum, after reduction with stannous solution (Stehlik, *548*), with mercury (Furman and Murray, *172*), or with silver (Birnbaum and Walden, *44*).

Uranium (IV) after reduction of uranyl solutions may be titrated with ceric sulfate, as was shown by Ewing and Wilson (*134*) and by Furman and Schoonover (*173*). This reaction has been applied to the indirect potentiometric estimation of sodium by Furman, Caley, and Schoonover (*167*), and Kryulov and Kolarova (*314*).

Zinc may be estimated by the indirect ferrocyanide process (Sturges, *554*).

Nitrato and Perchlorato Cerates. Smith and Getz (*511*, *512*, *513*) have shown in a series of systematic studies that nitrato cerate ion, $Ce(NO_3)_6^{--}$, and the perchlorato cerate ion, $Ce(ClO_4)_6^{--}$, function at still higher potentials than the sulfate complex. With these reagents there is a better differentiation in the successive titration of ferrous iron and vanadyl ion in mixtures than with the sulfato reagent. The oxidation of organic compounds proceeds more smoothly and regularly, which makes possible improved methods for the titration of oxalate and other substances. Smith and Duke (*510*) were able to work out a rapid method for glycerol by oxidation with perchlorato cerate at 50° to 60° C.

4. Bichromate Processes. In the main the reaction bichromate *vs.* ferrous ion has been further studied or applied to direct and indirect estimations, as follows:

Study of asymmetry of the titration curve (Winter and Moyer, *619*). The reaction has also been studied by Shakhkeldian (*490*)

Barium, indirect estimation (Fisher, *137*)

Chromium (Khlopin, *288*, *290*). Platinum-tungsten system more exact than visual methods. In ferrochrome (Khlopin, *286;* Spindek, *542*); in plating baths (Khlopin, *278*)

Ferrous sulfate solutions (Shisakov, *497*)

Glycerol (*437*)

Iron, in ores (Sosnovskiĭ, *519*)

Oximes, indirect estimation of metals (Ishimaru, *254*)

Sulfite, sulfoxylate (Löbering, *341;* Mutschin, *406*)

5. Halogens and Their Oxy-Compounds. Periodate, estimation of glucose (*446*)

Potassium bromate. 8-Hydroxyquinoline (Smith and May, *514*)

8-Hydroxyquinoline, anthranilic acid (Kitajima, *296*)

Beta-naphtholsulfonic acids (Forrester *et al.*, *146–148*, *223*)

Platinous complex oxalates (Grinberg and Ryabchikov, *194*)

Thio acids, RSH (Hellström, *227*)

Thiocyanate, arsenite, and antimonite (Nakazono and Inoko, *409*)

Thionalide, indirect estimation of thallium (Berg and Fahrenkamp, *42*)

Tin (Raikhinstein, *443*)

Potassium iodate. Antimony (Furman and Miller, *170;* Singh and Ilahi, *503*)

Arsenic (Schoonover and Furman, *480;* Singh and Ilahi, *503*)

Cuprous thiocyanate (Hope and Ross, *248*)

Hydrazine (Stelling, *550*)

Iodide (to iodine chloride) (Flatt, *139*)

Iodide complexes, cadmium indirect (Kiba, *291*)

Iron (Singh, *501*)

Phenyl hydrazine and semicarbazide (Furman and Miller, *171*)

Stannous ion (Spacu and Drăgulescu, *521;* Singh and Ilahi, *503*)

Thiocyanate, tetrathionate, etc. (Singh and Ilahi, *504*)

Potassium chlorate. Estimation of various reductants: arsenic (III), antimony (III), ferrous, iodide, and thallous ions (Singh and Singh, *507*)

Estimation of phenol, *p*-nitroaniline, diphenylamine, and hydroquinone (Singh and Singh, *507*, p. 346)

Stannous ion (Kulvarskaya, *316*)

Estimation of chlorates and hypochlorites, using osmium tetroxide catalyst (Gaukhman and Stefanovskiĭ, *178*)

Chloramine T as oxidant. Estimation of antimony, arsenic, stannous, ferrous, ferrocyanide, and iodide ions (Tomíček and Sucharda, *588*)

Indirect determination of oxidants, titration of hydroquinone, hydrazine, potassium iodide, sodium bisulfite, sodium nitrite (added from buret) (Singh and Rehman, *506*)

Thallium (Aguado, *7;* Fresno and Aguado, *152*)

Hypochlorite as oxidant. Bromide (Afanase'v *et al.*, *6;* Chirkov and Spikel'man, *87*)

Various reductants: antimony, arsenic, ferrous, iodide, and thallous ions (Singh and Singh, *507*)

Iodine to iodine chloride, bromine to bromine chloride (Chirkov and Schnee, *86*)

Hydrazine, urea (Tomíček and Filipovic, *581*, p. 415)

Thiocyanate, thiosulfate, thallium, cyanide, selenite, and tellurite (Tomíček and Filipovic, *581*, p. 340)

Determinatlon of hypochlorite (Albribat, *3*)

Hypobromite as oxidant. Tomíček and Jasek (*584;* see also *581*) have studied the applications of this rather unstable reagent. In the estimation of thiosulfate, sulfite, and thiocyanate the results are 1 to 4 per cent high.

Bromine as oxidant. Hypophosphite (Blaser and Halpern, *45*)

Phosphite and thiosulfate (Nakazono, *408*)

Thiosulfate, reaction with bromine or potassium bromate (Cernatescu and Ralea, *82*)

Iodine as oxidant. Antimony (Furman and Miller, *170*) and tin (Pevtsov, *430*)

Mercurous salt (Michalski, *374*)

Tetraphenyl arsonium chloride, indirect estimation of metallic ions (Willard and Smith, *616*)

Thionalide, indirect estimation of metals (Kiba, *291*)

Thiosulfate (Fresno and Valdes, *160*). Indirect applications: antimony (Spacu and Drăgulescu, *522*); arsenic (Spacu and Drăgulescu, *523*); iodate (Spacu, *534*, *540*); oxygen (Perley, *428*); and thallium (Hollens and Spencer, *246;* Cuta, *99*)

Water, Karl Fischer reaction: $I_2 + SO_2 + 3C_5H_5N + H_2O = 2(C_5H_5NHI) + C_5H_5NOSO_2$ (Almy *et al.*, *10*)

Estimation of iodine, application to medicinals (Gjaldbaek, *187*)

Miscellaneous. Halogen solutions and sodium thiosulfate (Fresno and Valdes, *160*)

Chlorine electrode (Rius and Arnal, *459*). Electrode is not suitable for determination of chlorine in hypochlorous acid solution

Potassium iodide as reductant for palladium (Müller and Stein, *392*)

6. Vanadic Solutions as Oxidants. It has been shown that most of the more powerful reductants may be estimated with this oxidant: Ferrous, molybdenum (III, V), tungsten, uranium, vanadium (II, III), stannous, cuprous, and titanous solutions; hydrazine, hydroxylamine, hydrogen sulfide, phosphites, and sulfites (Syrokomskiĭ and Antropov, *556;* Syrokomskiĭ and Klimenko, *557*).

7. Hydrogen Peroxide, Permonosulfuric Acid, and Persulfuric Acid. Estimation of hydrogen peroxide by various methods (Reichert *et al.*, *449*).

Estimation of persulfuric and permonosulfuric acids, and hydrogen peroxide.

Permonosulfuric acid is reduced by excess of standard arsenite and potassium bromide in a solution 1.4 to 1.5 *N* in sulfuric acid. The excess of the arsenite is estimated with standard bromate.

Hydrogen peroxide is estimated by adding an excess of standard arsenite and sodium hydroxide. After 2 minutes the solution is acidified and the excess of arsenite is titrated.

Persulfuric acid. After the hydrogen peroxide reaction an excess of arsenite is added and after heating 40 minutes at 100° C. with the solution 1.4 to 1.5 *N* in sulfuric acid the excess of arsenite is titrated with bromate after cooling (Isida and Yukawa, *255*).

Müller and Holder's Procedure (*389*). Permonosulfuric acid may be titrated selectively in the presence of hydrogen peroxide with arsenite if the permonosulfuric acid is titrated directly with arsenite after neutralizing sulfuric acid with bicarbonate. After the determination of Caro's acid (H_2SO_5) sodium acetate is added to buffer and nitrate with bromine in potassium bromide solution to estimate hydrogen peroxide.

Bodin (*46*) estimates the hydrogen peroxide with potassium permanganate. The permonosulfuric acid is titrated by the potassium iodide–thiosulfate procedure after adding a few drops of iodine. To estimate the persulfuric acid a catalyst of cuprous iodide in potassium chloride is added and the iodine liberated from potassium iodide is titrated with thiosulfate.

Denisov (*103*) used the platinum-tungsten electrode system in connection with Gleu's method (*188*). This is the estimation of Caro's acid by the bromide-arsenite-bromate procedure; the estimation of hydrogen peroxide by potassium permanganate after adding manganese sulfate; indirect estimation of persulfuric acid by reduction with excess of standard arsenite in acidified solution at 100° C.

8. Nitrite as Reagent. Reaction between nitrite and hydrogen peroxide (Jimeno and Ibarz, *264*)

Diazotation. Aromatic amines (Singh and Ahmad, *502*)

Benzidine (Atanasiu and Velculescu, *20*); indirect determination of sulfate (Atanasiu and Velculescu, *25*)

Nitrite-ferrocyanide reaction for estimation of nitrite (Romon, *464*)

9. Potassium Ferricyanide as Reagent. Chromium by oxidation with ferricyanide in alkaline solution (Kutovskii and Kholmyanskaya, *317*)

Cobalt in steels and alloys (Dickens and Maassen, *104*, *105;* Scherbakov and Kholcheva, *479;* Tomíček and Freiberger, *582*)

Glucose in sodium carbonate medium (Britton and Phillips, *69*)

Manganese (Tomíček and Kalny, *585;* cf. *105*, *106*)

Sulfides. The sulfide is oxidized quantitatively to free sulfur (Scagliarini, *477*)

Uric acid. The reaction occurs best at pH 10.5 (Beccari, *38*).

10. Oxidation by Cupric Salts. Glucose, titrated with Fehling's solution (Britton, *63*). Hydroxylamine and hydrazine (Britton and Königsten, *68*). A nitrogen atmosphere is used. Hydroxylamine is oxidized to N_2O, and hydrazine to nitrogen. Indigo, sodium hydrosulfite. Air is excluded and Fehling's solution is used (Strafford and Stubbins, *552*).

Reduction by Cuprous Solutions. Chlorate and hypochlorite (Troberg, *593*); gold and platinum (Müller and Tänzler, *393*)

11. Miscellaneous Studies with Less Powerful Reductants. A number of the applications that belong in this section have been given under bichromate (ferrous reaction), peroxides (arsenite as reductant), and iodine (potassium iodide as reductants).

Arsenite for estimation of lead dioxide (Lang and Zwerina, *326*)

Ferrocyanide (See Section 9)

Hydroquinone, amino phenols. Estimation of gold (Ryabchikov and Knyazheva, *469*); estimation of iridium (Bogdanov and Krasikov, *50*)

Substituted hydroquinones (*436*)

Uranous sulfate, for ferric ion or bichromate (Ducloux, *124*)

Hydrazine, estimation of bichromate and vanadate (Holst, *247*); estimation of octavalent osmium (Crowell, *97*).

Sulfite, applied to estimation of bichromate, iodine, ferricyanide, cupric ion, and hydrogen peroxide (Singh and Malik, *505*)

12. More Powerful Reductants. *Vanadyl Sulfate in Alkaline Solution.* Chromate (Fresno and Mairlot, *154*, *157*, *158*)

Chromate and ferricyanide (Fresno and Mairlot, *155*, *159*)

Ferricyanide (Tomíček, *578*)

Gold (Fresno and Mairlot, *156*)

Gold, copper, silver, permanganate, chromate, and ferricyanide (Fresno and Mairlot, *158*)
Mercury (Fresno and Lafuente, *153*)
Selenite and tellurite (Tomíček, *578*)

Stannous Solution as Reagent. Chromium, vanadium, molybdenum (Trebiatowski, *591;* Sosnovskiĭ, *518*). Chromium and iron (Müller and Haase, *388*)
Nitroso-β-naphthol (Belen'kiĭ and Sokolov, *40*)
Molybdenum (Fogel'son and Kalmuikova, *144;* Krüll, *312;* Stehlik, *548;* Sosnovskiĭ, *520*)
Rhenium (Höleman, *244*). The action of various reductants and ReO_4^- was also studied by Höleman (*243*).
Titanium, iron, and molybdenum (Martynchenko and Shimko, *363*)

Sodium Hydrosulfite ($Na_2S_2O_4$). Copper, mercury, and silver (Murooka, *405*)

Potassium Tungsten Ennachloride. Permanganate, ceric ion, bromate, bichromate, ferric, and cupric ions (Uzel and Pribil, *595*)

Titanous Chloride or Sulfate. Dipicrylamine (potassium, rubidium, cesium indirect) (Kiba, *292*)
Food colors (Evenson and Nagel, *132, 133*)
Iridium (Woo and Yost, *623*)
Molybdenum (Stehlik, *548;* Wirtz, *620*)
Murexide (Kuhn and Lyman, *315*)
Palladium (Müller and Stein, *392*)
Uranium (Matula, *367*)

Chromous Solution as Reagent. Chromium, vanadium, and molybdenum (Zan'ko and Shylakman, *628*)
Copper (Miloslavskiĭ and Dolgova, *377*)
Iron and molybdenum (Brintzinger and Rost, *62*)
Iron, molybdenum, and titanium (Martynchenko and Shimko, *363*)
Iron and vanadium (Brintzinger and Rost, *60, 61*)
Osmium (Crowell and Brumbach, *98*)

Vanadous Solution. Estimation of ceric ion (Banerjee, *37*)
Estimation of cupric, ferric, silver, or bichromate ions (Maass, *349*)

13. Miscellaneous Applications of Oxidation-Reduction Methods, Principally in Steel Analysis. Application of systematic potentiometric analysis: aluminum, chromium, titanium, uranium, iron, manganese, cobalt, and nickel (Steuer, *551*). Silver, bismuth, lead, copper, cadmium, and iron-zinc groups (Hiltner and Gittel, *233, 234*). Lead, copper, nickel, zinc, and manganese (Hiltner and Seidel, *241*)

A general review of steel methods is given by Dickens and Thanheiser (*109*)

Steels and Ferroalloys. Cerium, manganese, chromium, and vanadium (Lang and Faude, *324*)
Chromium (Khitarov, *277;* Dickens and Thanheiser, *108*)
Chromium and vanadium (Fogel'son and Kalmuikova, *145;* Heczko, *226;* Werz, *607*)
Chromium, vanadium, and manganese (Adamovich, *4;* Hiltner and Marwan, *240;* Khlopin, *287*) polemic
Chromium, vanadium, molybdenum, and titanium (Gerke and Kardakova, *182*); Zanko and Shylakman, *628*)
Iron and alloys (Dickens and Thannheiser, *107*)
Manganese (Akhumov and Vasile'v, *8;* Avrunina and Zan'ko, *28, 29;* Genis *et al.*, *181;* Khitarov, *277;* Khlopin, *279, 284;* Soloveva, *517*)
Molybdenum (Dickens and Brennecke, *104;* Fogel'son and Kalmuikova, *143;* Werz, *608*)
Molybdenum and copper in steel (Schaefer, *478*)
Molybdenum and titanium in steel and alloys (Klinger *et al.*, *297*); in ores (Krüll, *312*)
Molybdenum in ferromolybdenum (Rabinovich, *442*)
Sulfur (Thanheiser and Dickens, *573*)
Vanadium (Eisermann, *127;* Thanheiser and Dickens, *574;* Dickens and Thanheiser, *107;* Fogel'son and Kalmuikova, *145;* Gutman and Mikeeva, *200;* Gutman and Piradyan, *201;* Fogel'son and Kalmuikova, *144;* Pinskaya, *432*)

Review of Methods for steel, etc. (Dickens and Thanheiser, *111*)

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Measuring the Specific Surface of Particulate Substances

A Water Vapor Adsorption Method

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SINCE the publication in 1921 of a photomicrographic method (*3*) of determining particle size, there has been considerable demand for a more rapid means for making such measurements. Though the authors agree that other procedures for making particle size determinations are desirable, they do not believe that simply a reduction in the time of measurement is the most important point involved. The real necessity for developing a new method of particle size measurement is that many particulate substances are not amenable to microscopical analysis. Gardner (*2*) lists approximately 2000 pigments, probably not more than 40 per cent of which could be measured with the microscope.

Photomicrographic Limitations

If a material such as a pigment is to be measured microscopically it must have, first, a suitable particle size. The individual particles must not be so small that they cannot be resolved and photographed clearly. Second, the pigment must possess a relatively high degree of uniformity; otherwise a representative sample, at the necessary magnification, cannot be obtained in a single photograph. Third, for white light and ultraviolet microscopy the material should preferably have a refractive index that permits the particles to be seen distinctly in suitable mounting media.

A water vapor adsorption method has been developed for determining the specific surface of particulate substances. No high-vacuum equipment is required, as the measurements are made at atmospheric pressure. This method makes possible determination of the surface shape factor and investigation of the particle size of materials too fine-grained to be measured with the microscope.

A table of the specific surfaces of a large number of particulate substances which were measured is included and checks with the microscope are given wherever possible. Methods of comparing microscopical measurements with adsorption measurements are discussed.

Though many pigments comply with the second and third requirements, they are sometimes so small in size that the individual particle cannot be seen with the usual microscopical equipment. Such pigments include the gas blacks, lithol reds, peacock blues, etc. The individual particles of these pigments can be detected with a microscope using a dark-field condenser or with an ultramicroscope, but these methods are not suitable for linear measurement of fine-grained materials, though they are successfully used for counting the number of particles in a given volume.

Electron microscopy is very well suited for the measurement of particles in the range between 0.01 and 0.1 μ, but this technique is too specialized and the equipment too costly for the average laboratory interested in particle size and specific surface measurement.

Notwithstanding the limited field of the photomicrographic method, it probably gives as complete an analysis of the subject as any method generally used at present. Its great advantage lies in the fact that it produces a frequency distribution curve from which the uniformity coefficient and all the various average diameters can be calculated. The method also involves certain quantities known as "shape factors", which are simply the proportionality constants to be used in conjunction with the horizontally measured diameters (*3*), so that these diameters can be employed for determining specific surface and the number of particles per gram of material.

It has been stated elsewhere (*4*) that the important unsolved problem in the subject of particle size determination has been to measure the surface shape factor, and that the solution would probably be found in an adsorption method. This statement was made from the microscopist's point of view. It could just as readily have been stated that the important problem is to measure specific surface—i. e., the number of square meters of particle surface per gram of material, or the square meters of surface per solid cubic centimeter of material.

Adsorption Method

The authors report here some work done on the development of a comparatively simple procedure for obtaining specific surface by an adsorption method. The measurement of specific surfaces of finely divided materials has been studied in great detail by Emmett and his co-workers in their classic work on the low-temperature adsorption of nitrogen and other gases. Adsorption from solution has also been employed for the surface measurements of finely divided materials (*5*). The work described here concerns the adsorption of water vapor at room temperature in a system which has a full atmosphere of pressure.

Some work was being done on the determination of the moisture content of various particulate substances. The method being studied was essentially one in which the material was mixed with calcium carbide which reacted with any water present. The amount of acetylene produced by this reaction was supposed to be proportional to the amount of water in the particulate substance. While attempting to check the accuracy of this method with a sample of iron blue which had a definite known moisture content, it was found that no pressure was produced. This condition was noted by James Massarene, one of the authors' co-workers. Further investigation showed that the gas which was generated produced no pressure because it was adsorbed by the sample. As a consequence, acetylene was first used in the adsorption apparatus but later it was found more convenient simply to use water vapor. The idea embodies measuring the number of molecules of water adsorbed on a known quantity of material and then multiplying this number by the cross-sectional area of the water molecule. Wherever possible, checks were made with the photomicrographic method of measurement.

The apparatus and method used in the determination of specific surface given in this paper depend on the fact that adsorption takes place on solid surfaces (*6*).

Apparatus

Essentially the apparatus consists of two adsorption flasks, a sensitive differential manometer, and a system for introducing the gas or vapor to be adsorbed.

The adsorption flasks, *A* and *B* in Figure 1, are connected through the differential manometer, *C*, with stopcocks, *a*, between each flask and the manometer.

Into the stoppers, which have ground-glass joints, are sealed stopcocks, *b*, and the three-way cocks, *c*. The stems of cocks *b* go well down into the flasks while those of cocks *c* are near the top, so that good circulation is obtained when any gas or vapor is passed through the system.

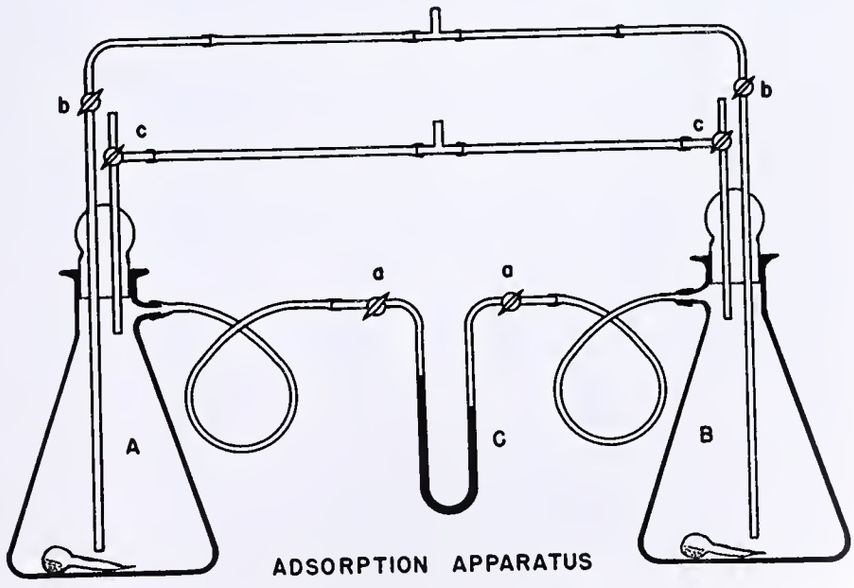

FIGURE 1

All connections for the introduction of gas or vapor, for drying, and for evacuation are made at *b* and *c*.

The differential manometer is of the ordinary inclined type. Its function is to indicate the change in pressure which takes place upon adsorption in either of the flasks, the pressure in the other being maintained constant. Dibutyl phthlate is employed as the manometer liquid because of its low vapor pressure, low specific gravity, and good wetting of the manometer tube. The insolubility of the gases or vapors in the manometer liquid is of importance, and in this respect, too, dibutyl phthalate is a good choice.

The use of each of the flasks for making adsorption measurements on different samples of the same material is only incidental to their original purpose. In an earlier apparatus employing only one flask connected to an open-end manometer, variations in atmospheric pressure caused drifting of the manometer. Originally the second flask was attached to the other end of the manometer to act as a constant reference pressure chamber to eliminate the drift in the manometer. Later this reference pressure chamber was used for adsorption also, giving the symmetrical system as shown in Figure 1.

The gas or vapor can be introduced either by circulation through flasks *A* and *B*, or where pressures less than saturation are required, by partial evacuation of the system and introduction of the gas or vapor to restore any desired pressure. All the present work was done at atmospheric pressure.

This whole system is maintained at a constant temperature in a water bath. Thermoregulator and heating coils are employed, and the bath is stirred continuously.

Sample Preparation

Small thin-walled glass bulbs are made for receiving the sample to be measured, and for sealing the sample from all gases until adsorption is to take place. These bulbs are made small enough to enter the mouths of flasks *A* and *B* easily.

The material, the specific surface of which is to be measured, is given a preliminary drying before weighing the sample in order to eliminate the bulk of the moisture. Samples of the material are then placed in the small glass bulbs and weighed carefully, so that the weight of the sample is known. When the samples have been weighed, the open ends of the bulb are drawn down to fine thin-walled capillaries which are left open. The samples are then ready for the final drying procedure.

Two procedures have been used for the actual drying, equally good results having been obtained by each method. One procedure is to heat the sample bulbs at 185° C. for 18 hours, remove from the oven, and place immediately in a desiccator at room temperature, through which air is circulated after having been dried over calcium chloride and phosphorus pentoxide. When the samples have reached room temperature, they are removed from the desiccator and sealed immediately.

In the second procedure, the bulbs are placed in a Pyrex desiccator and heated at 185° C. for 18 hours with the pressure reduced to 300 mm. of mercury throughout the heating. Air, dried over calcium chloride and phosphorus pentoxide is passed into the desiccator when the heating has been completed and the drying tubes are maintained connected to the desiccator until the samples have reached room temperature and are at atmospheric pressure. The bulbs are then removed and sealed immediately.

The samples as prepared have a pressure within the bulbs equal to atmospheric pressure. This is necessary because the actual specific surface determinations are made with a full atmosphere of pressure in adsorption flasks *A* and *B*. Because of this, the samples must be cooled to room temperature before finally sealing, so that there will be no pressure difference between the bulbs and the flasks and therefore the breaking of an empty bulb similarly prepared will produce no pressure change in the system.

During most of this investigation water vapor was used for the actual adsorption. In some cases it is impossible to use water vapor, where reactions may take place (as for cement, gypsum, etc.) and where the material is hygroscopic. Where water vapor cannot be used, other vapors such as chloroform have been employed.

The final step in the preparation of the sample is the sealing of the bulb after removal from the dry atmosphere of the desiccator. During the short period of time between the removal of the bulb from the dry atmosphere and the sealing of the capillary tip, water molecules may enter the capillary. However, the bulb is sealed in an interval which is brief in comparison to the time required for molecules to diffuse from the tip of the capillary to the sample, so that if water vapor does enter the capillary, any adsorption by the sample will take place after the capillary is sealed.

The introduction of these few water molecules into the sample bulb is equivalent to a corresponding increase in the final number of water molecules available for adsorption in the adsorption flasks. The pressure in the sample bulb, as placed in the adsorption flask, may be somewhat lower than atmospheric pressure, owing to the adsorption of these few water molecules. When the sample bulb is broken in the adsorption flask there will be a small pressure decrease in addition to that produced by the adsorption taking place in the flask. This initial pressure decrease is equal to that which would have resulted from the adsorption of these first few water molecules if they had been adsorbed by the exposed sample in the adsorption flask instead of in the sealed sample bulb. From this point, the remainder of the adsorption proceeds as usual.

The total adsorption by the sample, as indicated by the manometer, is equal to that which would take place in an atmosphere having an added number of water molecules equal to the number sealed into the sample bulb, except that the adsorption would have taken place in two installments to give the same final total adsorption.

Water Vapor Adsorption

Samples of the particulate substance, prepared as outlined above, are placed in flasks *A* and *B*. Air is circulated through the two flasks to eliminate gases or vapors which may be present from any previous measurement. The water vapor is now introduced into *A* and *B* by reducing the pressure to 350 mm. and then restoring the system to atmospheric pressure by the addition of water-saturated air. The system is allowed to come to temperature equilibrium, then the sample in one of the flasks is exposed to the water vapor by shaking the flask to break the bulb. The pressure change is noted when equilibrium has again been attained; then the sample in the other flask is similarly exposed and this second pressure change is noted.

When the sample is first exposed to the water vapor, the pressure decreases very rapidly, owing to adsorption. If the pressure continues to decrease slowly for a considerable period after this initial rapid change, it is assumed to be due to causes other than monomolecular adsorption (condensation in capillaries, etc.) and has been treated as such in evaluating the specific surfaces of the samples.

Up to the instant the sample bulb is broken, the sample is sealed off from the surrounding atmosphere of water vapor. When the bulb is broken, the sample is exposed immediately to the molecules to be adsorbed; no time is required for diffusion from one part of the system to another. The agitation the sample receives facilitates the adsorption by preventing the formation of a mound of material at the bottom of the flask.

From the manometer readings obtained in the adsorption experiment, the specific surface of the sample may be computed if we also know the volume of the adsorption system, the sensitivity of the differential manometer, the mass of the sample, the cross-sectional area of the molecules adsorbed, and the temperature at which the measurements are made.

The characteristics of the apparatus which determine the relationship between specific surface and manometer readings are as follows:

Volume of adsorption flask, liters	1.20
Specific gravity of dibutylphthalate	1.05
Slope of inclined manometer	1/10.9
Temperature of apparatus, ° C.	25

The number of molecules in this system at 25° C. and atmospheric pressure is

$$N = \frac{1.20\ (6.06)10^{23}\ (273)}{22.4 \qquad 298} = 298 \times 10^{20}$$

Each millimeter displacement on the inclined dibutylphthalate manometer is equivalent to

$$\frac{1.05}{13.5\ (10.9)} \text{ mm. of Hg} = 0.00714 \text{ mm. of Hg}$$

which corresponds to a change in the number of molecules of gas or vapor in the system by

$$\frac{0.00714}{760}\ (298 \times 10^{20}) = 0.279 \times 10^{18}$$

Taking $10A^2$, $(10^{-19}m^2)$, to be a representative figure for the cross-sectional area of the adsorbed water molecule, it follows that each millimeter decrease in pressure as indicated on the inclined manometer corresponds to the coverage of 0.0279 square meter by water molecules adsorbed in a monomolecular layer. Thus, the relationship for specific surface as a function of manometer displacement and mass of sample becomes

$$\text{Specific surface} = 0.0279\ \frac{\Delta p}{m} \text{ square meter per gram} \qquad (1)$$

for this apparatus.

In a large number of measurements made for various masses of the same particulate substance, it was found that the adsorption followed the Freundlich adsorption isotherm very closely. The decrease in pressure due to the adsorption by the sample is very nearly proportional to the mass of the sample when the pressure change is small. In Figure 2 are some representative curves showing how $\Delta p/m$ becomes nearly constant at small values of m. All the authors' determinations were made with small samples, so that the deviation from the constant relation between specific surface and $\Delta p/m$ was very small.

Average Particle Diameters

In making comparisons of adsorption measurements and microscopical measurements it is well to consider briefly the various average diameters for nonuniform particulate substances (*4*).

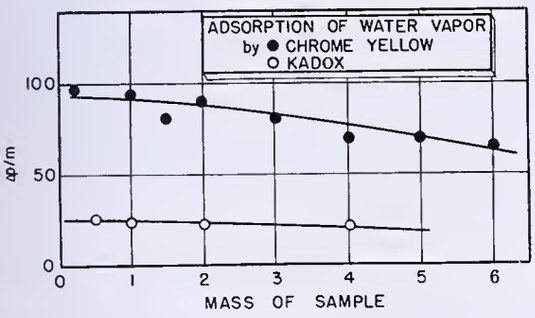

FIGURE 2

The average diameter related to specific surface, S, is d_3. This diameter is equal to $\Sigma nd^3/\Sigma nd^2$, where n is the frequency of particle diameter d. The surface shape factor is σ' and the volume shape factor is ν'. The density of the particle is ρ. The diameter related to average volume is

$$D = (\Sigma nd^3/\Sigma n)^{1/3} \qquad (2)$$

A series of six samples of fine-grained zinc oxide was measured in pairs, taking the average measurement of the pair in each case. The relationship

$$d_3 = \sigma'/\nu'\rho S \qquad (3)$$

was employed to determine d_3 from the specific surface, assuming $\sigma'/\nu' = 6$, which is the same as assuming the particles to be spheres or cubes. This gave the following:

ZnO Sample	Specific Surface	d_3 by Adsorption	d_3 by Microscopy
Grams	*Sq. m./gram*	*Micron*	*Micron*
0.50	5.7	0.19	0.17
1.00	5.7	0.19	0.17
1.50	5.4	0.20	0.17
	Average 5.6	0.19	0.17

A list of some of the other materials measured appears in Table I. These materials are merely representatives of the large classes of which they are samples, and the results are not necessarily significant for the whole class. The d_3 by adsorption was determined from specific surface measurements, the microscopic d_3 was determined by the photomicrographic method (*4*), and the D was determined by the slit ultramicroscope.

Accuracy of Method

In the major part of this study, specific surface determinations were checked by making measurements on samples of the same material from each side of the adsorption system. For each determination the samples had been prepared together and therefore received the same treatment. The results of one side of the system checked the other within 2 or 3 per cent, and in a great many cases within 1 per cent, indicating adequate reproducibility for the method.

In the series of measurements made on the fine-grained zinc oxide, all the samples had been prepared together, receiving the same treatment, but specific surface measurements were made at different times. These checked within 1 per cent for two of the groups of samples, and within 5 per cent for a third group. The mass of the samples in these groups varied.

This method of specific surface measurement is an indirect one, and in order to discuss its accuracy, it is necessary to consider the assumptions made. The pressure decreases in the system are measured directly, and from this, specific surface is computed on the following assumptions:

1. *The surface of the material is completely covered by a monolayer of adsorbed molecules.* In the extensive work done by Emmett (*1*) and his co-workers it is shown that the lower pressure end of the linear portion of experimental adsorption isotherms corresponds to monomolecular adsorption.
2. *No condensation takes place on the surface of the material, in any interstices between the particles, or in any pores the material may have.* Conditions of the adsorption have been arranged so that the possibility of condensation has been minimized. The adsorption is carried out in an atmosphere having a vapor pressure of water lower than 50 per cent of the saturation pressure. The sample is intentionally small, so that no mound of exposed material will be formed when the bulb is broken, and therefore condensation between particles will be at a minimum. The pressure change in the system is noted as soon as temperature equilibrium is attained, so that any pressure change due to condensation, which takes place much more slowly than adsorption, is practically excluded in the determination of specific surface. The rate at which adsorption proceeds is so much greater than the rate of condensation that the latter will not have progressed to a great extent when the adsorption is completed.
3. *The cross-sectional area of the absorbed molecule is known.* The error in this value for the water molecule is probably in the order of 10 per cent or less. Figures in the literature vary between $9A^2$ and $11A^2$. Computations from water at maximum density and assuming a cubical molecule give $9.6A^2$ for this area. W. D. Harkins, in a private communication, suggested $10A^2$ as a good figure based on what appears in the literature, and the authors have used this value in their computations.

Wherever possible, the results obtained on the basis of these assumptions were checked by the photomicrographic method. The microscopical measurements of the d_3 diameters were made independently by E. F. Fullam, one of the authors' coworkers, and consequently were not influenced by the results obtained by their absorption method. The d_3 diameter is the

Table I. Specific Surfaces and Average Diameters of Some Common Pigments

Sample	Specific Surface	d_3 by Adsorption	d_3 by Microscopy	D by Microscopy
	Sq.m./gram	*Microns*	*Micron*	*Micron*
ZnO (Green Seal)	2.4	0.45	0.44	...
Heated 0.5 hour at 800° F.	2.3	0.47	0.47	...
Heated 0.5 hour at 1100° F.	2.3	0.47	0.50	...
Heated 0.5 hour at 1400° F.	1.8	0.59	0.59	...
Heated 0.5 hour at 1700° F.	1.7	0.63	0.66	...
Gas black 1	330.	0.010	...	0.025
Gas black 2	110.	0.033	...	0.025
Gas black 3	150.	0.023	...	0.040
Gas black 4	95.	0.036	...	0.050
Gas black 5	110.	0.031	...	0.050
Gas black 6	34.	0.10	...	0.060
Gas black 7	59.	0.058	...	0.060
Pigment black 8	12.	0.29	0.26	0.200
Whiting	2.6	0.85	...	...
Asbestine	4.1	0.51	...	...
Lithopone	2.3	0.61	0.43	...
Blanc fixe	2.2	0.62	...	...
Barytes	0.59	2.3	...	...
Titanium dioxide	8.2	0.19	0.43	...
Antimony oxide	0.61	1.7	0.61	...
Basic carbonate white lead	1.1	0.80	0.66	...
Eosine toner	41.	0.073	...	...
Viridine green lake	69.	0.035	...	...
Hansa yellow toner	2.6	1.5	0.21	...
Rhodamine toner	12.	0.18	...	...
Iron blue	26.	0.13	...	...
Orange mineral	0.76	1.3	0.43	...
Chrome yellow medium	3.9	0.26	0.32	...
Madder lake	53.	0.071	...	...
Red lake C barium	15.	0.23	...	...
Lithol red	36.	0.083	...	...
Ultramarine blue	13.	0.20	0.59	...
Peacock blue	83.	0.036	...	...
Lithol rubine	26.	0.12	...	...

average of a great many measured "horizontal diameters" (*3*) and is obtained by the equation given above. The close agreement between the microscopic measurements and the adsorption measurements made on particles which the authors believe to be nonporous indicates that these assumptions are well made in most cases. On the basis of complete correctness of these assumptions, the specific surface values would deviate about 3 per cent from the actual surface available for the adsorption of water molecules owing to apparatus calibration errors. This error is in addition to any introduced by incomplete correctness of the assumptions made.

Comparison Data

The values of the D diameters were given by the Binney and Smith Co., which also supplied the eight samples of blacks. These D diameters were obtained by counting the number of particles in a given volume of suspension in which the total volume of solids was known. From such data the volume of the particle of average volume was calculated. It is invariably assumed in this type of measurement, with the slit ultramicroscope, that the particles are spheres; then $\pi D^3/6$ becomes the average particle volume and v' equals $\pi/6$.

It is obvious from what has preceded that no definite relation can exist between the d_3 and D diameters reported here. If D had been calculated from the equation $D = (\Sigma nd^3/\Sigma n)^{1/3}$, where d is the horizontal diameter, it would follow that the authors' d_3 would always be greater than D (*4*); but under the present circumstances the D diameters were obtained by a different method, so that no conclusion can be made as to the relative values of d_3 and D.

In checking the results of the adsorption method with the microscope one has the choice of comparing either the specific surfaces or the average particle sizes. In either case an assumption has to be made that the particles are spheres or some other definite form. The adsorption method measures specific surfaces; the microscopic method measures d_3 and D. Assuming the particles to be spheres, d_3 can be converted to specific surface by the expression

$$S = 6/\rho d_3 \qquad (4)$$

D, however, bears no relation to specific surface and therefore cannot be employed except as an approximate value for d_3. Again, if particle sizes are to be compared, the specific surface obtained from the adsorption method is then used for calculating d_3 from Equation 3, particles still assumed to be spheres.

Since "particle size" is referred to more generally than "specific surface", the latter method was used in securing the data for Table I.

Discussion of Results

An examination of the tabulated measurements shows that the zinc oxides gave the best results, checking remarkably well with the microscope. The blacks also checked satisfactorily, considering the fact that the d_3 and D diameters are not strictly comparable. The first noticeable discrepancy appears to be with titanium dioxide. There can be no question here that a possible error in the microscopical measurement accounts for the difference between 0.19 and 0.43 micron. With a material as uniform as the titanium dioxide particle appears to be, a microscopical error of 0.24 micron is out of the question. The fact that the adsorption method gives a particle size that seems to be too small cannot be accounted for by an insufficiently dried surface, for that would give results of an opposite nature. The large specific surface which is inconsistent with microscopical measurement is explained on the basis of photographs taken with the electron microscope, which show the "smooth particle" as measured by light microscopy to be a firm aggregate of small tetragonal crystals irregularly arranged. This firm aggregate, which the light microscope shows as the ultimate particle, cannot be broken down by any commercial grinding process. This structure has a considerably larger surface than is indicated by the light microscope.

In the cases of antimony oxide and orange mineral, the specific surfaces are undoubtedly too small; this is probably due to the lack of a sufficiently dried surface before adsorption takes place. In the case of ultramarine, the assumption that the particles are spherical could readily be far enough from the truth to account for the discrepancy found here. The hansa yellow particles grow rapidly at elevated temperatures, and have actually grown during the drying process, so that comparison with the microscopical value is not valid. In regard to the remaining substances, none of the adsorption figures seems to be out of line with microscopical observation.

It is realized that this paper is only a preliminary step toward establishing a simple adsorption method for the determination of specific surface. The authors believe that the instrument itself leaves little to be desired in regard to simplicity and dependability.

Acknowledgments

The authors wish to acknowledge their indebtedness to Interchemical Corporation for making this investigation possible; to W. D. Harkins for many valuable suggestions; and to James Massarene, C. W. Jerome, and E. F. Fullam of the research staff for their helpful assistance in carrying out this work.

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The Cloud Point of Varnish Resins in Drying Oils

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The temperature at which a heated mixture of varnish resin and a selected mineral oil clouds on cooling is a useful index of the relative solubility of the resin. The solubility achieved by heating mixtures of resin and drying oil may be measured by mixing the drying oil and resin with a suitable mineral oil and determining the temperature at which the mixture clouds. The changes observed on heating ester-type resins are believed to be due to an ester interchange of the resin and the drying oil, and to the poorer solvent power of the bodied oil. With pure phenolic and some hydrocarbon resins, the effect of the decreased solvent power of the bodied drying oil is the controlling factor.

SEVERAL tests have been developed to measure the solvent power of volatile thinners—for instance, the aniline point and the kauri butanol value (*3*)—but no test has been suggested which will accurately compare the solubility of various varnish resins, or measure the degree of solubility which is attained on heating the resins with drying oils.

The concentration at which a resin clouds in solution on dilution with a volatile solvent has been used as an index of its molecular size (*1, 8*). This method has been used to measure the comparative strength of nitrocellulose solvents (*3*), but as a measure of solubility of a resin it has not been standardized. Such methods have been investigated in this study but they are subject to some qualifications, inasmuch as this cloud point may be hard to duplicate and when the precipitation occurs at low resin concentrations, the values obtained do not always measure the solubility characteristics of the resin accurately. A small amount of relatively insoluble material may indicate poorer solubility than the resin as a whole possesses.

The determination of the cloud point is simple and consists in dissolving the resin at 50 to 70 per cent concentration in a suitable mineral oil by heating. On cooling, a cloud will develop which can usually be duplicated, on reheating and cooling, within one degree. In some cases greater accuracy can be obtained.

The mineral oil used should be chosen for the type of resin to be examined. The aniline point of a mineral oil is a useful index of its solvent power, those with high aniline point having poor solvent power, reflected in high cloud points with a resin, while oils with low aniline point are much better solvents.

While a wide variety of mineral oils have been and may be used, only two mineral oils have been used in this study. One is a viscous paraffinic mineral oil of high (137° C. maximum) aniline point. The other is a white mineral oil (Nujol) of 104° (maximum) aniline point. Various samples of Nujol may vary a degree or two in aniline point and the cloud points will vary accordingly. In general, two mineral oils of the same aniline point will give the same cloud point with a given resin.

Solubility of Rosin–Phenolic Resin Condensates

WW wood rosin was heated with stirring and various amounts of six commercial "heat reactive" phenolic resins, designated as A to F, were added at 125° C. Heating and stirring were continued over a period of one hour when the temperature had reached 250° C. The resin was then poured. In this study a highly paraffinic mineral oil of 137° C. aniline point (maximum) was used as the reference oil.

Determination of Cloud Point

Five to 7 grams of resin are weighed in a 150-cc. beaker to the nearest decigram and the mineral oil is added to bring the total weight to 10 grams. The mixture is then warmed on a hot plate with constant stirring until the resin is thoroughly dissolved. Local overheating should be avoided and the final temperature should not exceed 200° C. The mixture is then transferred to a 15-mm. test tube, immersed in a stirred water bath which has been heated to 10° above the expected cloud point. A glycerol bath may be used for temperatures over 100°. The temperature is allowed to drop at not over 2° per minute. A definite cloud of the oil-resin mixture will develop, and the temperature of the bath at this point is recorded as the cloud point. A slight haze may develop in the case of some resins, but this should not be confused with the true cloud point, which should be read when the mixture becomes opaque.

The determination may be repeated by raising the temperature of the bath 10°, holding until the sample is clear, and repeating the determination.

Figure 1 shows the cloud point of the rosin–phenolic resin condensates with various amounts of phenolic resin. It will be noted that some phenolic resins give much higher cloud point condensates than do others, and that the cloud point increases as the content of phenolic resin increases. Some commercial resins gave condensates which did not cloud at room temperature in the 137° aniline point mineral oil used.

Molecular weight, melting point, and viscosity of a 60 per cent solution in toluene of these condensates were also determined. It was found that the viscosity, molecular weight, and melting point of the condensates which did not cloud at room temperature were ap-

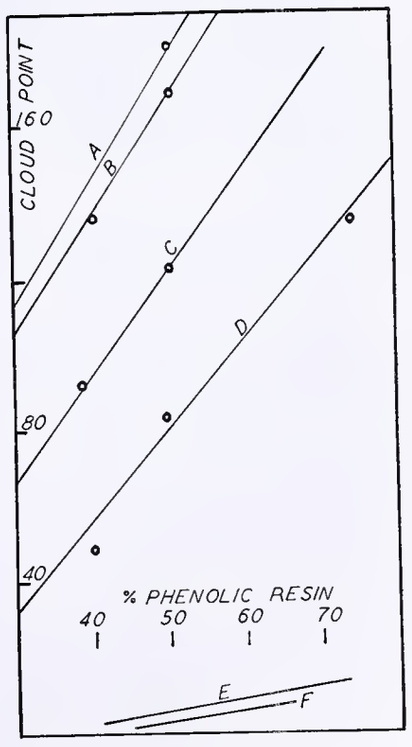

FIGURE 1. CLOUD POINT OF PHENOLIC-ROSIN CONDENSATES
50 per cent resin, 50 per cent mineral oil

preciably lower than the other condensates at the same phenolic content. In the case of the condensates that did cloud, however, the cloud point at the same phenolic resin content did not vary with viscosity, molecular weight, nor melting point, but was a relatively independent property, depending largely on the type of phenolic resin employed.

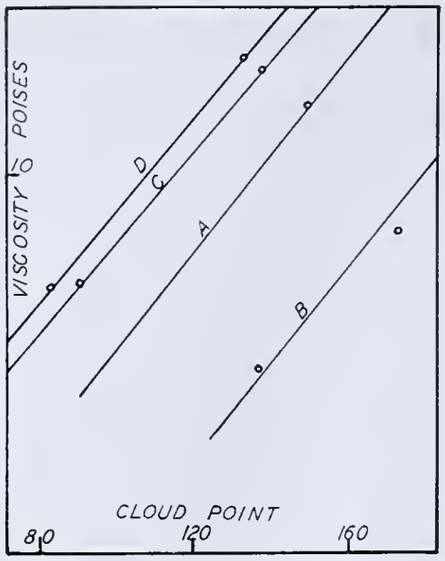

FIGURE 2. VISCOSITY AND CLOUD POINT OF PHENOLIC-ROSIN CONDENSATES

Figure 2 shows the correlation of the viscosity of a 60 per cent solution of the condensates in toluene with cloud point. With a given resin the cloud point increases as the logarithm of the viscosity, but the relationship varies greatly, depending on the phenolic resin used. This difference in solubility is at least in part caused by the nature and size of the alkyl groups in the phenolic compound from which the resin was made.

CLOUD POINT OF ROSIN–PHENOLIC RESINS. The cloud point of nine commercial rosin–modified phenolic resins, designated as G to P, was determined (Figure 3). Resins G to N were difficultly dispersible in the 137° aniline point paraffinic mineral oil, and in these cases a white mineral oil of 104° aniline point (maximum) was used. The cloud point in these cases was taken at several concentrations to determine the variation of cloud point with resin concentration. Usually determination at one concentration (50 per cent) is sufficient to indicate the solubility of a resin.

These curves show the variation that may be found in solubility of resins of this type. The higher melting resins tend to be less soluble but resins of the same melting point show wide variation in solubility. Many commercial resins of this type are lower in cloud point than the resin in Figure 3 (above).

Behavior of Ester-Type Resin in Drying Oils

The behavior in drying oils of the various resins described above was found to agree with their cloud points, the high cloud resins being most difficult to disperse. The cloud point method was tried with drying oil–resin mixtures, and it was found that addition of the paraffinic mineral oil to such mixtures developed a cloud at room temperature which could be dissolved on heating and that cloud points could be determined. These vehicles were clear before addition of the mineral oil and were apparently completely "dispersed". The cloud points give a measure of the extent to which the resin has been dissolved in the oil. It was found that the cloud points decreased as the heating of the oil-resin mixture continued when glyceride resins were used.

In some cases a minimum was reached and the cloud point increased on further heating. The cloud points with a given resin were lower as the ratio of drying oil to resin was increased. This was believed to be caused by an ester interchange between the resin glyceride and the drying oil. Since no simple method was available to measure the ester interchange, the acid interchange between rosin and an acid rosin phenolic resin was measured. All the cloud points of oil-resin mixtures determined in this paper were made on mixtures without volatile thinners. The cloud point may be determined on vehicles containing volatile thinners, but precautions should be used to prevent their loss by volatilization under conditions of the test. The presence of volatile thinners will change the cloud point, depending on the amount and solvent power of these materials.

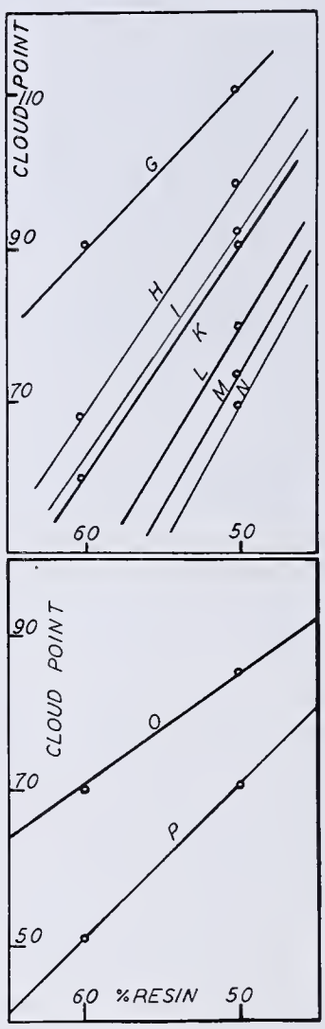

FIGURE 3. CLOUD POINTS OF ROSIN–MODIFIED PHENOLIC RESINS

Above, in white mineral oil
Below, in paraffinic mineral oil

Acid Interchange between Rosin and Oil

Mixtures of WW wood rosin and alkali-refined linseed oil were heated with stirring at 275° and samples taken at intervals. A commercial rosin–phenolic resin condensate was also heated with linseed oil at 275°; in one case the heated mixture was protected by an atmosphere of carbon dioxide.

The free rosin acid was determined by the method of McNicoll (*5*) by determining the decrease in acidity on refluxing the sample with methanol containing *p*-toluenesulfonic acid.

TABLE I. ACID INTERCHANGE OF ROSIN AND ACID PHENOLIC ROSIN RESIN WITH LINSEED OIL

Parts Rosin	Parts Acid Phenolic Resin	Parts Linseed Oil	Time, Hours	Acid No.	Reflux Acid No.	Cloud Point 50% 137° Aniline Point Oil	Remarks
1	...	1	0	85.5	80.6	<0	
1	...	1	0.5	81.8	66.4	<0	
1	...	1	1.0	79.2	58.0	<0	
1	...	1	2.0	78.0	46.3	<0	
...	1	1	0	68.4	56.9	>230	
...	1	1	0.5	53.5	38.5	>230	
...	1	1	1.0	51.9	31.0	>180	
...	1	1	2.0	47.6	24.1	98	
...	1	2	0	41.6	33.5	>230	
...	1	2	0.5	42.0	26.4	84	
...	1	2	1.0	40.0	18.8	0	
...	1	2	2.0	38.0	12.9	0	
...	1	1	0	64.3	55.2	>230	Protected by CO_2
...	1	1	0.5	52.7	38.2	100	Protected by CO_2
...	1	1	1.0	48.8	29.1	52	Protected by CO_2
...	1	1	2.0	42.0	19.4	70	Protected by CO_2

The cloud point was determined as with the resins, except that 10 parts of the oil-resin mixture and 5 parts of 137° aniline point mineral oil were used. Heating was required to bring the mixture into solution.

The results are shown in Table I. The acid number of the rosin–linseed oil mixtures drops slightly on heating but the acid number after refluxing drops considerably, indicating the combination of the rosin acids with the liberation of an equivalent amount of fatty acids. This has been previously suggested (*7*), but apparently the degree of interchange has not previously been measured.

In the case of the rosin–phenolic resin mixture, the drop of the acid number on heating is larger than with rosin. This may be due to loss of "apparent" acidity of the phenolic resin on heating. The "reflux" acid numbers show that the rosin–phenolic resin has combined with the drying oil with the liberation of drying oil acids.

The cloud points in Table I show that rosin is entirely soluble even at the start of the heating. It will be noted that the phenolic resin clouds at progressively lower temperatures as the heating continues. Where the cloud point exceeds 200°, the resin visibly separates from the linseed oil on cooling without the addition of mineral oil.

The cloud points, when the batch was protected by carbon dioxide, are lower, although the amount of interchange is much the same. This is believed to be due to the poorer solvent power of the bodied oil which is formed more rapidly when oxygen is present. This effect is also shown in cases where the cloud point increases on further heating. The cloud point and reflux acid numbers are lower as the amount of drying oil is increased. The excess of drying oil promotes the interchange with the acid resin.

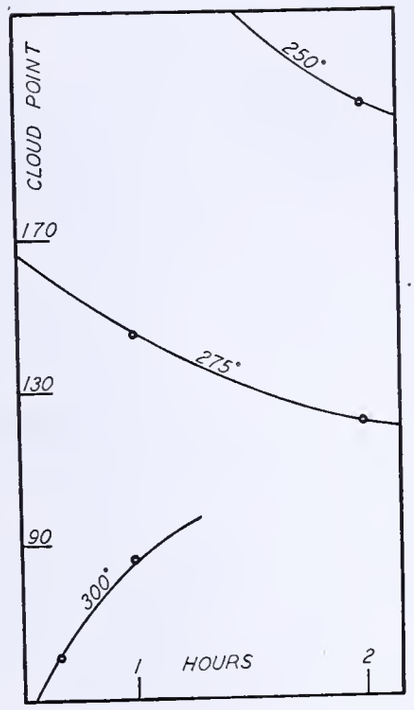

FIGURE 5. CLOUD POINT OF MALEIC RESINS IN LINSEED OIL

TABLE II. CLOUD POINT OF ROSIN PHENOLIC GLYCERIDE IN LINSEED OIL

Concentration of Resin % by wt.	Cloud Point ° C.
1.5	22
1.75	70
2.00	118
2.25	165

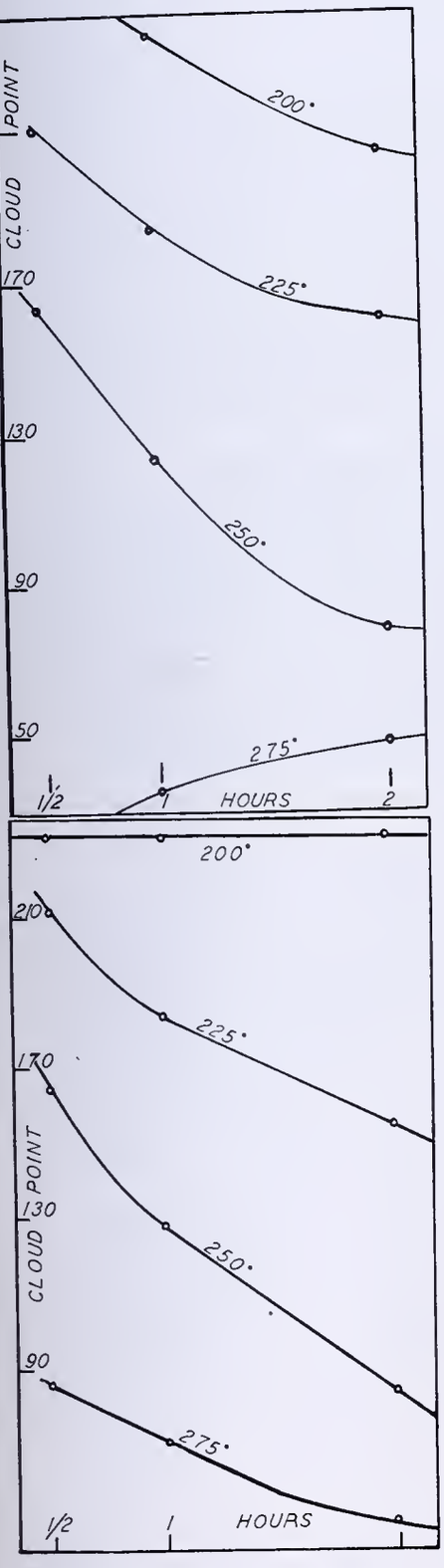

FIGURE 4. CLOUD POINTS
Above, 1 part of resin, 2 parts of oil
Below, equal parts of resin and oil

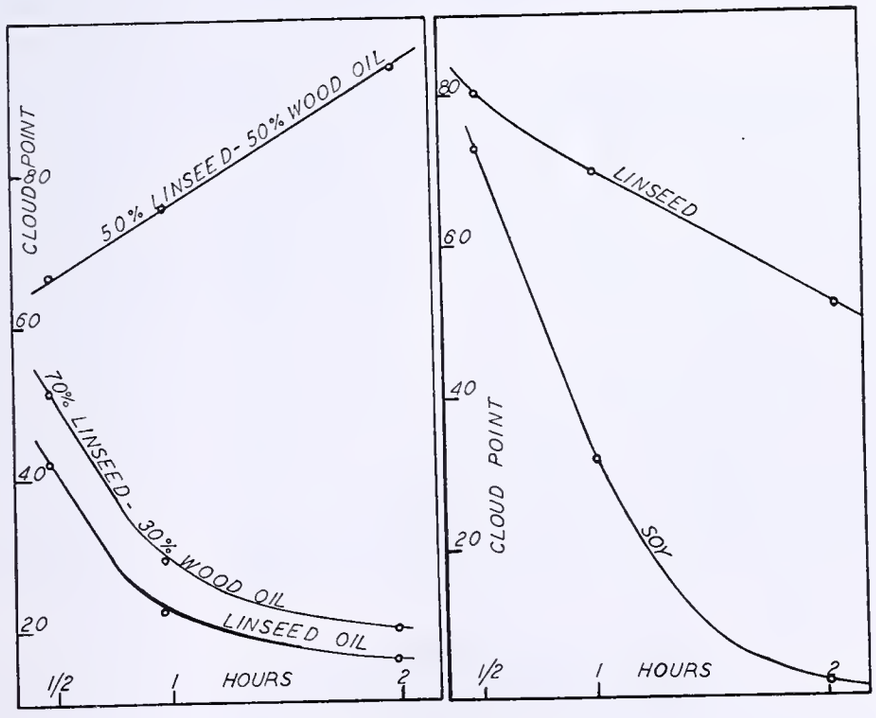

FIGURE 6. CLOUD POINTS
Left, linseed–tung oil mixtures
Right, linseed–soybean oil mixtures

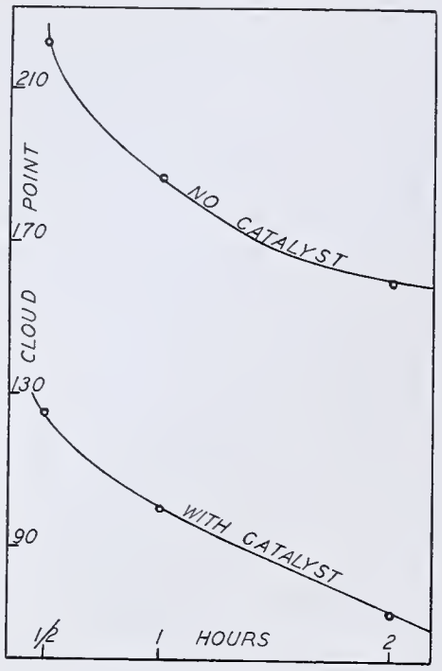

FIGURE 7. EFFECT OF CATALYST

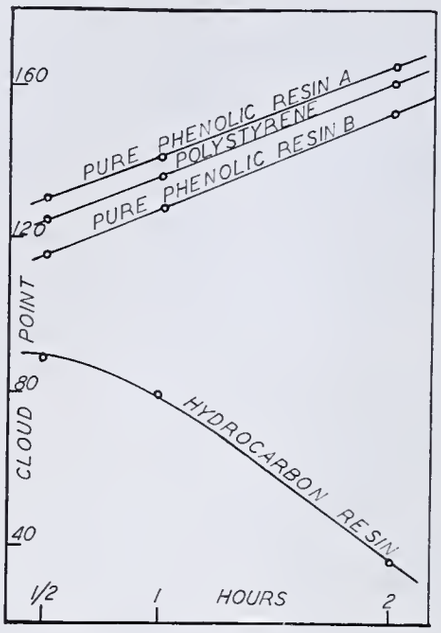

FIGURE 8. CLOUD POINTS IN LINSEED OIL BODIED AT 275° C.

The cloud point results indicate clearly a definite chemical combination between the phenolic resin and rosin. If the phenolic resin were not combined chemically with the rosin, no change in solubility would be expected when interchange with the drying oil acids occurs. It will be shown that pure phenolic resins do not become more soluble on heating with oil.

Varnish Resins and Drying Oils

When the less soluble rosin–modified phenolic resins are heated with drying oils, the changes in solubility may be measured by the cloud point. Figure 4 shows the cloud points of a rosin–modified phenolic glyceride resin heated at various temperatures with nonbreak linseed oil. At 200° C. little change occurs, except when 2 parts of drying oil are used to 1 of resin. At 250° the resin drops in cloud point more rapidly.

At 275° the behavior at the higher linseed oil content is rather different than at the lower. It will be noted that the cloud point increases as heating is continued with two parts of linseed oil to one of resin (Figure 4, above). This increase is believed to be due to the decreasing solvent power of the bodied oil, the oil becoming a progressively poorer solvent as heating is continued. The behavior of this resin is comparable to the acid rosin phenolic condensate and it is believed ester interchange is responsible in part for the changes in solubility of the resin.

Table II shows the cloud point of the resin used in the above study in the same linseed oil when it has been heated with the oil only a very short time. This shows that a small amount, less than 2 per cent, of uncombined resin will cloud. However, it is not believed that this curve is an index of the uncombined glyceride in resin–drying oil mixtures, since the interchanged resin–drying oil glyceride will be a better solvent for the resin, while the drying oil becomes a poorer solvent on heating.

Figure 5 shows the behavior of a maleic resin glyceride. This resin, as measured by cloud point, is less soluble than the rosin–phenolic resin used above, but the high content of ester groups makes it possible to disperse it in drying oils. This shows that cloud point of resins of different types cannot be used in comparing their solubility in drying oils.

Cloud Points in Soybean, Linseed, and Linseed–Tung Oil Mixtures

As noted above, polymerized oils are poorer solvents than unbodied oils. Hence, it might be expected that oils which polymerize more readily will show higher cloud points with the same resin. This has been found to be the case, since soybean oil gives lower cloud points with the same resin than does linseed oil under the same conditions (Figure 6, right). With tung oil, the bodying action is much faster and 30 and 50 per cent mixtures with linseed oil were compared with linseed alone using a phenolic rosin glyceride under the same conditions (Figure 6, left). It will be seen that 30 per cent tung oil has a small but noticeable effect, while in equal parts of tung oil and linseed oil the cloud points are higher and continue to rise as the heating continues. In this case the polymerization raises the cloud point faster than ester interchange lowers it.

Effect of Catalysts

Many materials are known to accelerate ester interchange, and acid catalysts have frequently been used (*2*). Lead soaps are frequently used in varnish and resin preparation for this purpose. Figure 7 shows that the cloud point is lowered when litharge is added to the oil-resin mixture. These varnishes were made from linseed oil and a rosin–modified phenolic resin and heated under the same conditions with and without the addition of litharge.

Pure Phenolic and Hydrocarbon Resins in Drying Oils

Two commercially pure (100 per cent) phenolic resins were heated with alkali-refined linseed oil. The cloud points of the oil-resin mixture are shown in Figure 8. In this case the cloud point increases on heating, owing to the poorer solvent power of the drying oil as it polymerizes. These curves give no indication of a chemical reaction with the drying oil, as has been claimed

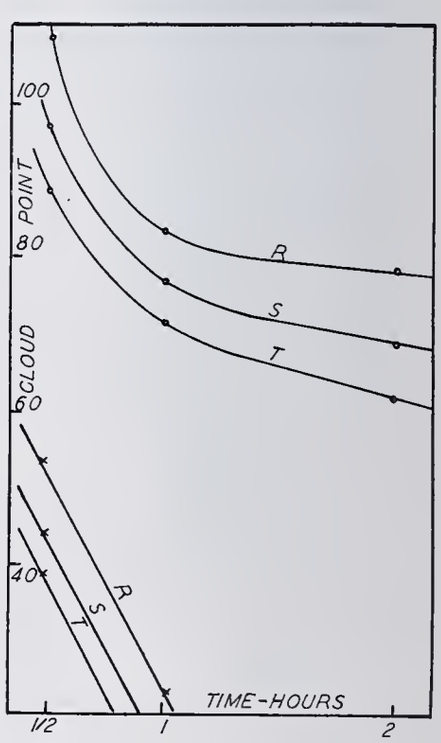

FIGURE 9. CLOUD POINTS OF MODIFIED PHENOLIC RESINS IN LINSEED OIL

O. 2 parts of resin, 1 part of oil
×. 1 part of resin, 2 parts of oil

(4), since there is no time where the cloud point decreases, which would be expected if chemical combination with the oil occurred.

Two hydrocarbon resins, a low molecular weight polystyrene and a coal-tar resin, were heated with linseed oil. The cloud points at various times are shown in Figure 8. The polystyrene–drying oil mixture increases in cloud point in very much the same manner as do the pure phenolic resins. The coal-tar resin decreases in cloud point as heating is continued. This is not believed to be the result of chemical combination with the oil, but rather depolymerization of the larger polymers in the resin. It is known that resins of this type depolymerize at temperatures employed in this experiment (275° C.).

Cloud Point of Resin and Cloud Point of Resin–Drying Oil Mixtures

It has been found that the cloud point of a resin in mineral oil is a good index of its behavior when heated with drying oils. When resins of the same type are compared, resins which give high cloud points in mineral oil will give high cloud points when bodied with drying oils. Figure 9 shows three resins of the same type, R, S, and T (modified phenolic), which were heated with linseed oil. The cloud points in drying oil are in the same order as the cloud points of the resins in mineral oil.

When resins of different classes are compared, the cloud point is of less utility in predicting their cloud points in drying oils. However, if resins of different classes of the same cloud point are compared, the resin which has the greatest reactivity with the drying oil will usually have the greatest solubility and lowest cloud point in the drying oil.

Application of Cloud Point Method

The cloud point determination has been found exceedingly useful in classifying a wide variety of synthetic and natural resins. Its utility extends over a much wider range of resins than has been cited here. Often it is necessary to choose a mineral oil of better or poorer solvent power to match the solubility of the resin.

Some properties of oleoresinous vehicles may be correlated with their cloud points, and the degree of "meta-stability" measured. This property is of considerable importance in controlling the working properties of paints (6).

This report has been offered to suggest the utility of the cloud point technique in studying the solubility characteristics of resins and of resin-oil combinations. It is hoped that the method will find wider utility.

Acknowledgment

The author wishes to express appreciation for the cooperation of V. A. Navikas who conducted much of the experimental work.

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Some Assays of Provitamin A Carotenoids

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An endeavor is made to present the problem of provitamin A carotenoid determination in plant materials in broad perspective. Results on carrots, spinach, tomatoes, apricots, peaches, and prunes are discussed, particularly with reference to preservation practices. The problem is essentially that of β-carotene determination, because α-carotene is at best a minor contributor, and cryptoxanthin is important only in special instances. Each material presents a different problem, sometimes of extraction, but more frequently in effective removal of interfering pigments without vitamin activity. The answer then is found in selection of suitable adsorbents with the proper solvent, and separation is made on small Tswett columns over which β-carotene or cryptoxanthin may be passed without adsorption, but where other pigments are effectively adsorbed.

Because data on vitamins are so frequently interpreted in terms of human needs, and much effort is being devoted to vitamin surveys at the behest of state nutrition committees, the significance of the various findings is briefly evaluated in terms of bioassay and nutrition.

A CONSIDERABLE number of carotenoid assays have been made in this laboratory on certain plant products for a variety of reasons. With carrots and spinach, dehydration problems were involved. In the case of apricots and peaches, dehydration does not yield a product acceptable to the trade, unless the fruit has been exposed to the sun, usually for twelve hours or so, after sulfuring. The nonirradiated product is dull and opaque, rather than translucent, and with peaches in particular, a pale yellow. In cling peaches for canning, an acceptable grading test for maturity was also desired. A further problem with peaches involved the presence of lycopene (23), found in certain European varieties but not here (10). Tomato products were included because of the presence of large amounts of lycopene, relative to carotene, and the authors were interested in attempting a rapid evaluation of the carotene content of juices. Finally, analyses were made on prune samples, thereby including some of the more staple dietary sources of provitamin A carotenoids of plant origin.

These various reasons have been subordinated in assembling the data in an attempt to place in broad perspective the problem of determining provitamin A carotenoids. Difficulties fall into two categories, first, those involving the natural complexity of the pigment mixture, and second, possible changes which the mixture may undergo, either by formation of additional chromophores, as in silage (5), or by bleaching of ex-

Table I. Effect of Drying Temperature on Retention of Carotene in Dehydrated Spinach

Drying Temperature		Drying Time	Retention of Carotene	
			Blanched	Not blanched
° F.	° C.	Hours	%	%
180	82	3.00	89.4	85.9
180	82	3.00	88.7	85.0
180	82	3.25	....	81.7
160	71	4.00	73.1	77.3
150	65.5	4.75	70.2	71.4
140	60	5.75	62.8	57.2
120	49	8.00	....	66.6
120	49	8.00	69.5	65.5

tracts, as in certain legumes (*21*, *25*). Such sources of error may be found in many other products, though normally in less aggravated form.

The materials considered here may be expected to contribute, as normally consumed, one tenth or more of the estimated daily adult requirement of about 5000 international units, 3 mg. of β-carotene. It has seemed worth while to discuss these data briefly in relation to known bioassays, for their nutritional significance.

So far as is known, three carotenoids supply virtually all the vitamin A that terrestrial mammals derive, directly or indirectly, from plant sources—namely, β-carotene, α-carotene (a minor contributor), and cryptoxanthin (important only in special cases). Little is known of requirements for insects (*2*) or other forms of animal life. The mussel is an example of other possibilities (*18*). The other carotenoids of known vitamin A potency for mammals either are of limited and local distribution or they occur in amounts inadequate for dietary significance, and in general it may be said that the problem is essentially one of determining β-carotene except where yellow corn meal is a staple food, and in certain fruits such as the peach.

General Considerations

As recognized by Fraps, Kemmerer, and Greenberg (*4*), no one method is applicable to all cases. A sound procedure demands that the nature of the pigment complex be understood in each product examined. For the most part, as Peterson (*17*) points out, the general problems of extraction, isolation, and determination have not changed greatly. The so-called invert soaps may be used more widely and be placed on a quantitative basis. Solutions of alkyl benzyl ammonium salts have been used by Kuhn, Bielig, and Dann (*8*) to isolate carotenoids from chloroplastin and carotene from carrots.

When an analysis is reported for nutritional use, the extent of absorption and utilization of carotene as distinct from vitamin A (*24*) must be considered, and in such cases a high degree of refinement in method is meaningless. This includes differentiation of α- and β-carotenes in mixtures which rarely contain more than 5 to 15 per cent of the α-component. If such analysis be warranted, it should be accomplished spectrophotometrically, setting up the necessary simultaneous equations for a 2-component mixture on the basis of Beer's law. Measurements should not be made on the individual α- and β-carotenes separated by adsorption because the percentage recovery of the more weakly adsorbed component is usually substantially greater than of the one more strongly held.

Experimental

Extraction procedures have been developed with a view to obtaining suitable extracts for spectroscopic examination without use of unwieldy quantities of solvents, the health menace of which is often overlooked. Although various improvements have been suggested (*1*, *12*, *16*, *25*), the authors have for the most part retained the older procedures. As the principles are well known, brief notes will suffice in some cases.

Spinach. The most reproducible results for fresh spinach are obtained by brief immersion of the 5-gram sample in boiling water. Dehydrated spinach should be thoroughly soaked prior to grinding with sand and acetone. A 0.5-gram sample may thus be extracted with four 25-ml. portions of acetone, transferred to approximately 30 ml. of petroleum ether, and saponified, and xanthophylls removed in the usual way. The petroleum ether is then washed, dried with anhydrous sodium sulfate, made to volume (50 ml.), and filtered in a closed system to obtain a brilliantly clear solution. The concentration of β-carotene is determined by measuring the transmission of the unknown at 480 mμ with a Bausch & Lomb visual spectrophotometer, and a similar measurement is made for a standard β-carotene sample in the same solvent.

The influence of soaking and sample size is clearly shown in the following results, expressed as per cent of β-carotene on a moisture-free basis (*3*). (Results in this paper are reported on a moisture-free basis except for carrot juice and tomato juice, which are more conveniently reported in mg. per 100 ml.) Sample not soaked, 0.5 gram, 0.026; soaked, 2.5 grams, 0.023; 1.5 grams, 0.026; 1.0 gram, 0.029; 0.5 gram, 0.036, 0.036. These compare with values on the same lot, fresh, of 0.038. The fresh samples, ten in number, taken from a local market, varied from 0.027 to 0.045 per cent on the same basis. The temperature of dehydration affected retention considerably. The shorter drying times, at higher temperatures, showed retention of 80 to 90 per cent of the total carotene; the lower temperatures required up to 8 hours, with retention of about 65 per cent. A preliminary steam blanching for 1 minute was also slightly beneficial. Results are shown in Table I. Losses on storage after 2 months at 0° were negligible, at room temperature from 5 to 12 per cent, and at 30° from 40 to 50 per cent. Blanching did not significantly affect losses on storage, though this step was very significant with carrots. Several of the final extracts ready for spectrophotometric analysis were checked by adsorption on magnesium oxide and magnesium carbonate columns for additional chromophores, but none were found.

Carrots. Analyses were made on fresh and dehydrated carrots (the latter containing 2 to 4 per cent moisture) and on canned carrot juice.

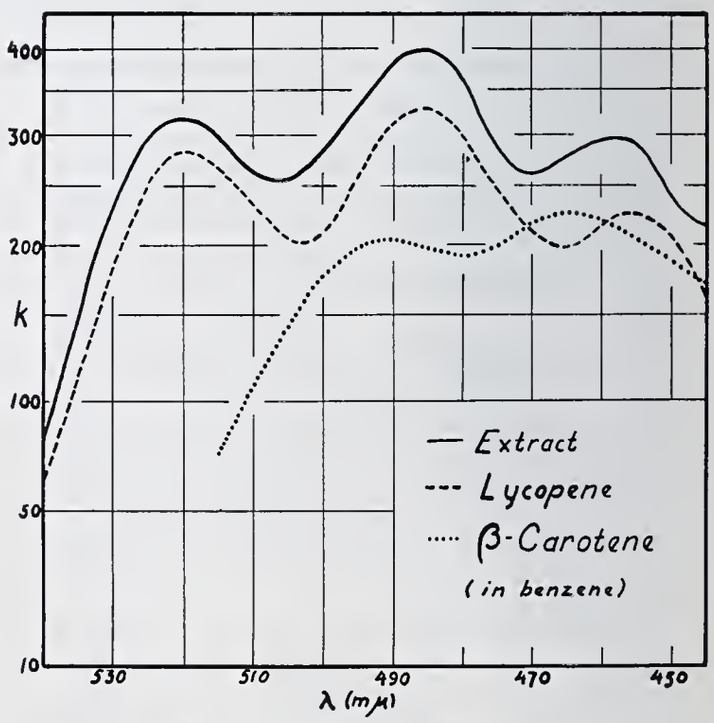

Figure 1. Absorption Constants for Lycopene, β-Carotene, and Tomato Extract in Benzene

Absorption coefficients, k, are given for pure pigments, plot for extract is in terms of E—i. e., kc.

Blanching for 3 minutes in boiling water prior to dehydration was beneficial to carotene retention. The dehydrated material, originally sliced uniformly in disks 3 mm. thick, is not easily extracted without soaking.

Five grams of fresh, or 0.5 gram of dried, carrots are immersed in 5 ml. of boiling water for 3 minutes, and the residue is extraeted as with spinach. In 4 extractions with a total of 75 ml. of solvent the residue is colorless. The extracts are partitioned successively with 50 and 25 ml. of petroleum ether. The second extraction is purely precautionary. The combined petroleum ether layers are washed with water, then 3 times with 85 per cent methanol, in 50-ml. portions. This is followed by water, 3 times (in 50-ml. portions), and the solution is dried with 2 grams of anhydrous sodium sulfate, and made to volume (100 ml.). Five milliliters of canned carrot juice are refluxed for a few minutes with 20 ml. of saturated potassium hydroxide in methanol. The alkali apparently aids disintegration of the finely comminuted particles present. Three 25-ml. portions of acetone suffice for extraction.

It is seen (Table II) that no difficulty is presented in obtaining representative samples from 5 or 6 carrots, total weight about 500 grams.

Table II. Carotene in Carrots, Moisture-Free Basis

	%
Fresh	0.105, 0.104, 0.110, 0.099
Dehydrated, unblanched	0.067, 0.067
Dehydrated, blanched[a]	
Initial	0.105, 0.106[b]
After 40 days' storage[c]	0.104 (0°), 0.101 (22°), 0.079 (30°)

[a] Blanching involves 3 minutes' immersion in boiling water, prior to dehydration.
[b] On basis including solids lost in blanching, this value becomes 0.086.
[c] After 4 months the unblanched samples at room temperature have fallen to 0.055%, the blanched to 0.084% carotene.

Some carotene values in certain laboratory-canned carrot juice samples are as follows: 13.3, 12.8; 11.9, 11.8; 10.6, and 10.5 mg. per 100 ml. of juice. Three commercial brands analyzed gave 9.0, 5.6, and 2.5. The last-mentioned had been strained and, as would be expected, this removed most of the carotene in the pulp. Assuming an average of about 15 per cent solids in these juices, we find the carotene comparable with that of the fresh carrot in most cases. Adsorption tests again revealed the absence of additional chromophores in the final preparations.

Tomatoes. In Figure 1 is shown the absorption spectrum of lycopene, β-carotene, and a tomato extract in benzene solution. It is evident that the lycopene spectrum effectively masks the presence of other pigments. Owing to traces of other carotenoids, the extract cannot be treated as a 2-component system involving only lycopene and β-carotene. Even if this were the case, it is clear by inspection that at least 95 per cent of the absorption is due to lycopene and an error of 1 per cent in the estimation of this component means an error of 20 per cent in the carotene. In the case of the fresh tomato, on which some analyses had been made for another purpose (*6*), considerable difficulty was encountered in securing a representative sample. Wherever possible, the sample should be withdrawn from not less than several hundred grams of macerated well-mixed pulp, and a 50-gram sample is not too small. This requires considerable solvent for satisfactory extraction. In juices, the sampling error is negligible.

Samples of a commercial brand (10, 20, and 30 ml.), well shaken, were extracted exhaustively with acetone (total required about 100 ml. per 10 ml. of juice) and the extracts were transferred to 25 ml. of benzene. The benzene was then thoroughly washed (5 times, 50-ml. water portions), dried with anhydrous sodium sulfate, and adsorbed on magnesium oxide and silica (1 to 1 by weight). The column was 1.5 cm. in diameter and 6 cm. long. The β-carotene appeared in the eluate, and was virtually unadsorbed, and the column was washed with more benzene until this fraction had passed completely into the eluate. This was then made to volume, and the transmission at 490 mμ measured. The results were 0.535, 0.538, and 0.539 mg. per 100 ml. of juice.

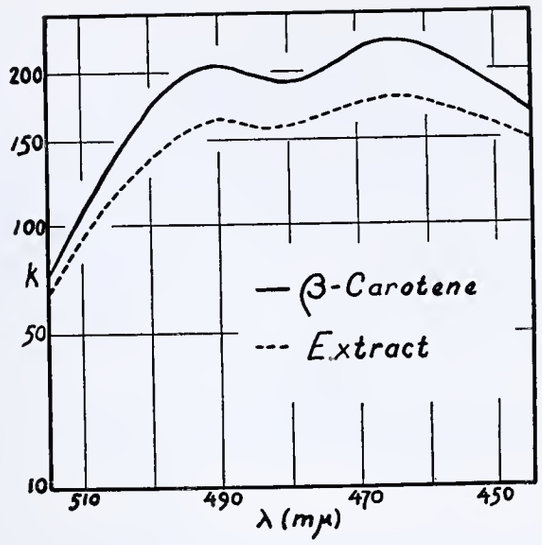

Figure 2. Absorption Constants for β-Carotene and Apricot Extract in Benzene

Results on four commercial brands of juice were 0.535, 0.52; 0.43, 0.43; 0.60, 0.60; and 0.575, 0.60 in mg. of β-carotene per 100 ml. of juice.

On whole tomatoes, results are more variable. A single lot, purchased on the market, may be considered. Sample 1 from 2 halves of a tomato irregularly ripened gave values of 0.0107, 0.004 per cent; two other samples, as homogeneous as practicable, gave 0.0075, 0.0089; and 0.0059, 0.0051 per cent, on a moisture-free basis. Three tomato pastes (of known solid content) gave values of 0.0137, 0.0135, and 0.0127 per cent, dry weight basis.

Apricots. In Figure 2 are given absorption curves for an apricot extract and for β-carotene in benzene. The xanthophylls, lycopene, and γ-carotene do not amount to 10 per cent of the total carotenoid, but again the mixture is too complex for simple spectroscopic assay, though errors in the β-carotene would be small in comparison with a similar assay on tomatoes.

The apricots, fresh or dry, are passed through a fine food chopper, and 10 grams of fresh or 1.0 gram of dried are extracted as for tomatoes. The β-carotene in the eluate is made to volume and estimated as before.

Table III. Carotene in Apricots, Moisture-Free Basis

	%
Fresh (Royals)	0.0212, 0.0200
Sun-dried	0.0168, 0.0164
Dehydrated	0.0165, 0.0160
3 hours ultraviolet then dehydrated	0.0198, 0.0194, 0.0195, 0.0190
Sun-dried, unsulfured[a]	0.0138, 0.0132

[a] From a different lot of apricots. All other dried samples were sulfured.

The effect of light is not to be simply explained. Whether the apricots are sun-dried or dehydrated, carotene losses are comparable (Table III), regardless of the acceptability of the color of the final product to the trade. The ultraviolet source conceivably exerts its effect by rapid inactivation of enzymes, but this phase will not be discussed here.

Peaches. Carotenoids of the following varieties have now been examined: (freestone) Elberta, Muir, Lovell, Foster, Late Crawford, (cling) Phillips, and Halford. The first three have been examined after dehydrating and also sun-drying, and Elbertas after canning. In previous work, carotenoids were isolated from vacuum-dried peach powder (*10*) and owing to low yields only a small quantity of fresh Lovells were included for comparison. Varieties grown here apparently

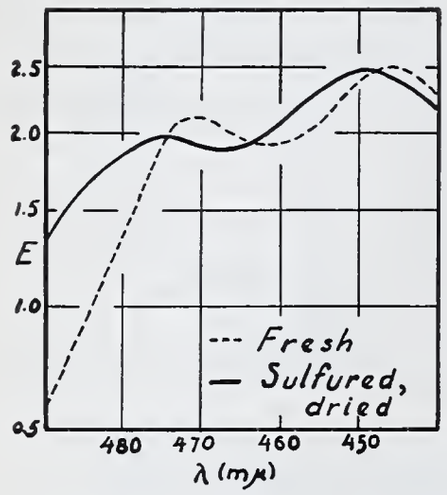

FIGURE 3. EFFECT OF SULFURING AND DRYING ON ABSORPTION OF PEACH CAROTENOIDS

Curves in terms of *E* are adjusted for equality at their main maxima.

do not contain measurable quantities of lycopene. When added to the extent of 3 per cent of the pigment mixture, it can be detected, but it cannot comprise 9 or 10 per cent of the mixture, as observed in European varieties (*23*). As a result of the more extensive work, certain modification of previous findings is necessary. In the first place, mature fresh and canned fruit yields an extract with lutein- or xanthophyll-type absorption maxima. This is also true of fruit sun-dried in the absence of sulfur, but the dried sulfured product is relatively much richer in β-carotene and similar components, as shown by a shift in the absorption maxima (Figure 3). In drying, the brunt of the loss is borne by the xanthophyll fraction.

Secondly, cryptoxanthin is not present in the amount previously indicated (*10*) in all of the above varieties. The carotenoids fractionated from fresh Fosters, for example, gave essentially the same chromatogram, but the supposed cryptoxanthin gave absorption maxima about 3 mμ too far to the blue, and it was more weakly adsorbed than genuine cryptoxanthin on a test column of magnesium oxide in benzene. Finally, as a result of studies on immature fruit, it became apparent that a labile component was present, having absorption characteristics similar in some respects to neoxanthin (*21*)—i. e., with maxima further to the blue than those of lutein. With inclusion of this labile fraction, the β-carotene fraction is less than 10 per cent of the total carotenoid in the fresh peach, and about 20 per cent in the sulfured dried peach, where much of the xanthophyll has been destroyed, including all the very labile fraction. Excluding this latter fraction, an approximate estimate of total carotenoid may be made within 10 to 15 per cent by the following procedure:

A petroleum ether extract is prepared from 10 grams of fresh, or 3 to 5 grams of dried peaches, in the same way that the benzene solutions were prepared from tomatoes and apricots. The absorption maximum for the band at the longer wave length is between 472 and 481 mμ, depending upon whether xanthophylls predominate. The total concentration is estimated by assuming an average specific absorption coefficient of 220, in liters per gram cm., at the individually located maximum for each extract. In actuality, after transmission measurements had been made, the solution was adsorbed on a column of magnesium carbonate (Merck), and the β-carotene was collected in the eluate very rapidly as it is unadsorbed. The details are thus in essence identical with those for carotene in apricots, except for solvent and adsorbent differences. Although these data are available, it is thought of greater interest to report the total carotenoid, based on the assumption just noted, because the effect of treatment causing heavy losses in total pigment is not reflected nearly so strikingly in the carotene itself. The β-carotene may be sufficiently precisely estimated as 10 per cent of the total for fresh, and 20 per cent for the sulfured dried, from the values in Table IV.

Results with fresh, green, borderline, and dead ripe Halford and Phillips peaches gave values of the same order of magnitude as for the fresh freestone varieties, but were 20 to 40 per cent lower for green fruit, where chlorophyll was readily detected.

PRUNES. Trade-dried prunes with 18 per cent moisture or less provide a peculiar extraction problem. The fresh Imperial prune is as readily extracted as peaches or apricots. Nor is any difficulty experienced with the processed dried prune, *ca.* 30 per cent moisture, but prune powder *ca.* 3 per cent, and dried prunes from the yard *ca.* 18 per cent moisture or less yield no coloring matter to acetone or petroleum ether. A yellow solution is obtained with 95 per cent ethanol but the coloring matter is not extracted with petroleum ether. A similar difficulty in preparing extracts from prunes was found in the Department of Home Economics and was obviated by soaking in water. A brief immersion of the sample for 1 to 2 minutes in boiling water is sufficient.

The prune was not investigated in great detail, but it was clear from the absorption curve for the total carotenoid extract in petroleum ether that the mixture approximated the same general picture as in peaches. Prunes are dried whole, usually after a brief lye dip. Dehydration is more common than in other fruits because the crop is harvested later in the season and there is in general poorer drying weather. Normally they are not sulfured.

Analyses reported here are based on the procedure for peaches, with samples from 2 to 8 grams; the total carotenoid in petroleum ether is passed over a short column (1.5 × 5 cm.) of magnesium carbonate, and the β-carotene in the eluate is made to volume and estimated. Analyses on a sample of fresh Imperial prunes gave 0.0041, 0.0037 per cent β-carotene on a moisture-free basis. Two commercial brands (processed) gave 0.0010, 0.0011, 0.00105; 0.0015. Another sample of the second brand gave 0.0025, 0.0030. The former were evidently older packages, and the flesh was definitely browner than in the second sample.

TABLE IV. ESTIMATED TOTAL CAROTENOID IN PEACHES, MOISTURE-FREE BASIS

	Elberta	Muir	Lovell
	%	%	%
Fresh[a]	0.0154	0.0171	0.025
	0.0185	0.0189	0.024
Sun-dried, unsulfured[b]	0.0078	0.0079	...
Sun-dried, sulfured[c]	0.0053	0.0050	0.0058
Dehydrated, sulfured[c]	0.0055	0.0040	0.0058

[a] Approximate ratios of xanthophylls to carotene: [a] 10, [b] 6, [c] 5, to 1.

Discussion

An important source of error lies in the labile nature of carotene. It not only involves the reference standard, but it also impairs the value of a checking procedure where known amounts are added to an unknown sample. The spectroscopic constants for carotene, melting point 182–3°, can be reproduced within 1 to 2 per cent at the maxima.

Little need be added with respect to methods. Where adsorption is necessary, the authors have preferred to use a readily available adsorbent (Micron brand magnesium oxide, with Hyflo-Supercel as diluent, and Merck's magnesium carbonate) in actual Tswett columns of small dimensions, and to employ a solvent which in the individual cases permits the desired separation and allows the fraction in which they are interested—the β-carotene—to pass through unadsorbed.

The underlying assumption is that there is no loss of the completely unadsorbed pigment. Pure β-carotene in benzene on magnesium oxide and in petroleum ether on very dry magnesium carbonate is in actuality slightly adsorbed. Recoveries range from 75 to 95 per cent, when known quantities are added to test columns, depending on the amount added and the rapidity of elution. There is no adsorption, however, of this pigment from crude extracts under otherwise comparable conditions, owing to the presence of colorless impurities which affect the development of the chromatogram. Where

β-carotene was added, not in excess of that present in the aliquot of the unknown, recoveries of 95 to 97 per cent were obtained. If more than double the amount in the unknown was added, between 80 and 92 per cent was recovered, and seemingly the value was determined in part by the additional length of time the zone was in contact with the adsorbent.

It is generally recognized that adsorbents are highly variable in property, and the magnesium carbonate, for example, will work satisfactorily only for relatively small quantities of pigment, per unit weight of adsorbent. The authors suspect that contamination with traces of ethanol or similar solvents is, however, the most frequent cause for unsatisfactory results. They can only emphasize that these adsorbents have served their purpose, and for general applicability, they feel the technique to be more desirable than that involved in adding adsorbent directly to the solution (*4*). Where it has been positively identified, cryptoxanthin may be readily washed off a column of magnesium carbonate by changing the eluting solvent from petroleum ether to benzene. In view of the differences in this fraction in different peach varieties, they have not included data so far obtained. Some preliminary experiments with corn meal and with genuine cryptoxanthin indicate that there should be no serious difficulty in applying the procedure to the peach and prune.

By courtesy of A. F. Morgan, the authors have compared their data with bioassays on similar fruit made during the last decade (*13*, *14*, *15*, *19*). There are no serious discrepancies, though losses of carotene in the authors' dried apricots are much less severe. In large measure this appears to be due to small scale operations, to a short time of storage (one week), and particularly to prompt sulfuring after cutting and pitting. In all yellow peaches tested by bioassay, the differences in carotene content are within 30 per cent of each other. In a tomato paste from the "pear tomato", there was only an 8 per cent difference in the carotene. The authors have therefore not been greatly interested in varietal differences. The gene concerned with plastid pigments affects chiefly the lycopene, according to LeRosen, Went, and Zechmeister (*9*).

A more serious but well-known discrepancy lies in the relative inefficiency of yellow vegetables, notably carrots, when compared with green sources (*20*)[1]. It is consequently not evident that varietal studies designed to increase the carotene content will have utilitarian value. Within a given class of foods—e. g., dried fruits or green vegetables—there seems to be satisfactory agreement between data such as the authors' and bioassays, which is lessened when different classes are compared.

It may be deduced from the arguments of Wald, Carroll, and Sciarra (*24*) that a daily adult requirement of 5000 units—i. e., 3 mg. of β-carotene—is based on a wrong unit of time, because the daily diet is too variable; a weekly basis is more stable. Also, the excretion of single large doses ordinarily begins within 1 to 2 days and is at a maximum from 3 to 5 days, ceasing after 5 to 7. In any event, absorption of carotene must be distinguished as a process not necessarily involving utilization, and even the addition of bile salts (*7*) cannot be assumed to ensure this.

It is unfortunate that bioassays on a given fruit are usually made with A-deficient rats. The results are in practice interpreted as bioassays for man or livestock rarely at such an advanced stage of depletion, and no allowance is made for differences in the respective digestive tracts and their capacities for making the carotene available. It has been pointed out to the authors that in the poultry industry there has been a rather precise standardization of poultry needs based on β-carotene and alfalfa, the chief supplement used. The retention of carotene *in situ* involves many factors which are not easily disentangled. In the experimental section on carrots, the authors reported on dehydrated disks originally 3 mm. thick. This thickness permits rapid dehydration, but a thinner slice retains less carotene. The value of 0.055 per cent for the unblanched sample after 4 months does not give a true picture. The surfaces are almost devoid of color. On soaking, it becomes apparent that the central part of the carrot disk is still well colored. Carotene retention, at least in some instances, is tied up with the fate of antioxidants, and its disappearance on storage appears to follow an autocatalytic curve. One cannot therefore place great reliance on a single determination.

These points are emphasized because of the ease with which reasonable human diets can be formulated in excess of 20 to 25 mg. of carotene per week from plant sources alone. For example, 100 grams of carrots should provide about 10 mg., 100 grams of spinach 3 to 5 mg., 100 grams of prunes 1 to 3 mg., 500 ml. of tomato juice or its equivalent 2 to 3 mg., etc., but these figures do not have necessarily the same nutritional significance. There are omitted from this survey many good sources such as sweet potatoes (*11*), pumpkins, etc., more intermittently consumed, and green vegetables except spinach. Minor sources such as orange juice (*22*) may in the aggregate represent a considerable fraction of the requirement.

The problem of greater urgency therefore is physiological in nature, and that for the food technologist is at present relatively simple, that he retain in a processed food a reasonably high proportion of its original endowment of carotene. Here the broad underlying principles are well appreciated: in canning, exclusion of air, and in dehydration, blanching and storage at low temperature or in inert gases.

Acknowledgment

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Adsorption of Vapors by Crystalline Solid Surfaces

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The important problem of the determination of the surface area of a finely divided powder has been considered by the intercomparison of values obtained from the adsorption of water, propyl alcohol, and benzene vapors at 25° C., and the adsorption of nitrogen gas at −195.5° C. The solids utilized were the common pigments, titanium dioxide, stannic oxide, zinc oxide, and pulverized quartz.

It is concluded that reliable values of the surface area of a crystalline powder may be obtained from vapor adsorption data at room temperature. Thus the area per gram of one of the titanium dioxide powders obtained from the adsorption of nitrogen at −195.5° C. was 8.3 square meters; the adsorption of water vapor at 25° C. gave 9.1, propyl alcohol 8.9, and benzene 9.3 square meters, respectively. A preliminary value from the electron microscope for this powder gave 10.7 square meters per gram.

NUMEROUS reports of vapor phase adsorption on porous solid structures such as the charcoals and silica gel exist in the literature (*6*, *8*). Studies of adsorption on plane crystalline surfaces have been generally avoided, however, owing to the extremely small adsorption that is observed when powders prepared by ordinary methods are utilized. As a consequence of recent industrial developments, nonporous crystalline powders of high specific area have become available, and the need for suitable methods to measure surface areas of such materials has arisen. In 1931, the study reported in this paper was begun with this purpose in view. An experimental investigation of the adsorption of water, propyl alcohol, and benzene vapors by crystalline titanium dioxide, quartz, zinc, and stannic oxides was completed. Langmuir isotherms were observed for the adsorption of propyl alcohol on all these solids up to three-fourths saturation pressure of the vapor. The adsorption of water, however, gave a sigmoid-shaped curve to which the simple Langmuir form could not be fitted.

More recently other workers (*1*, *9*, *10*, *11*) have found an S-shape for the isotherm for water, and sometimes other vapors. This same type of isotherm was also found in the important researches of Brunauer and Emmett (*3*) for the low-temperature adsorption of nitrogen, oxygen, argon, carbon monoxide, carbon dioxide, and butane on many crystalline solids. An adequate theory of this type of isotherm has been given (*4*). The sigmoid form is assumed to result from the building up of a polymolecular film by the adsorption of molecules held by essentially van der Waals forces. Langmuir's kinetic condensation-evaporation mechanism is generalized, and a variety of analytic expressions result. The theory has been extended (*2*) to include other species of adsorption isotherms, including those types observed for adsorption on porous solids. A valuable result of this work has been that a simple and reliable procedure for the determination of the surface areas of powders has been established.

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The theory of Brunauer, Emmett, and Teller (*4*) has been applied to the authors' data and surface areas have been computed. Striking agreement between values obtained from the van der Waals adsorption of nitrogen gas at −195.5° C., and values from the adsorption of water, propyl alcohol, and benzene vapors at about 25° C. has been found. A quantitative measurement of the adsorption of water vapor at room temperatures may afford a convenient procedure for the evaluation of surface area of powders.

Experimental

The adsorption of vapors on powders of metallic oxides was measured in the apparatus shown in Figure 1.

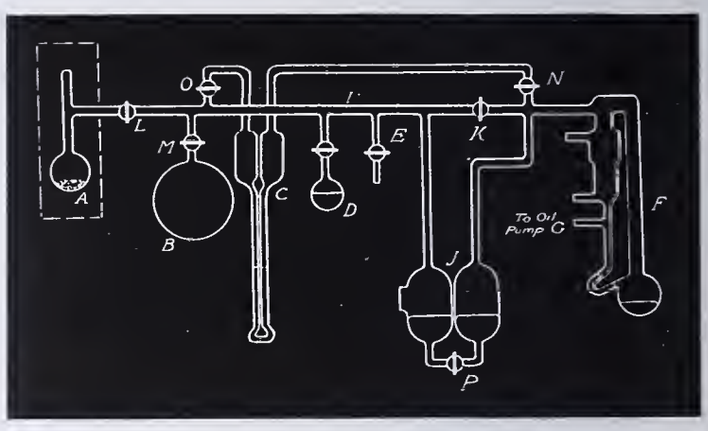

FIGURE 1. APPARATUS FOR STUDYING ADSORPTION OF VAPORS ON CRYSTALLINE NONPOROUS POWDERS

Pressure difference constitutes the measuring scale. The volume of flask *B* was determined directly; from this the volume of the remainder of the apparatus, except that of the adsorbing flask, *A*, was determined by measuring the new pressure of a little air which was allowed to expand from *B*, at a known low pressure, into the apparatus. Boyle's law was used to calculate the new volume. The volume of *A* was measured directly by filling with water at a known temperature and determining the weight difference. Pressures of water vapor and air were measured with a *n*-dibutyl phthalate manometer, *C*. Stanolax, instead of phthalate, was used to measure the pressures of vapors of *n*-propyl alcohol and benzene. Vapor was admitted to the apparatus from flask *D*. A mercury vapor pump, *F*, and a Hyvac oil pump at *G* were used to evacuate the apparatus.

After a series of experiments with these vapors was completed, the optical monometer, *J*, of the type described by Carver (*5*) was attached to the apparatus to study the vapor of butyric acid. Only a few preliminary experiments were done with this vapor.

The titanic oxide was supplied by the Titanium Pigment Co., Inc. The silica was made by pulverizing quartz. The zinc oxide and stannic oxide were reagents of C. P. quality. Each powder was heated in a high vacuum to temperatures which ranged from 350° to 450° C., depending on the substance, for about 10 hours (usually overnight) preparatory to vapor adsorption. Permanent gases in the liquids were pumped off before each experiment was started. The liquids used were ordinary distilled water, *n*-propyl alcohol of C. P. quality which was redistilled, and recrystallized benzene of C. P. quality. Experiments were run in duplicate.

Vapor was admitted to the apparatus while stopcocks *K* and *L* were closed. After the pressure was read, *L* was opened and adsorption was allowed to proceed until equilibrium was reached. The system was considered to have reached equilibrium when the manometer level remained constant for 15 minutes. *L* was then

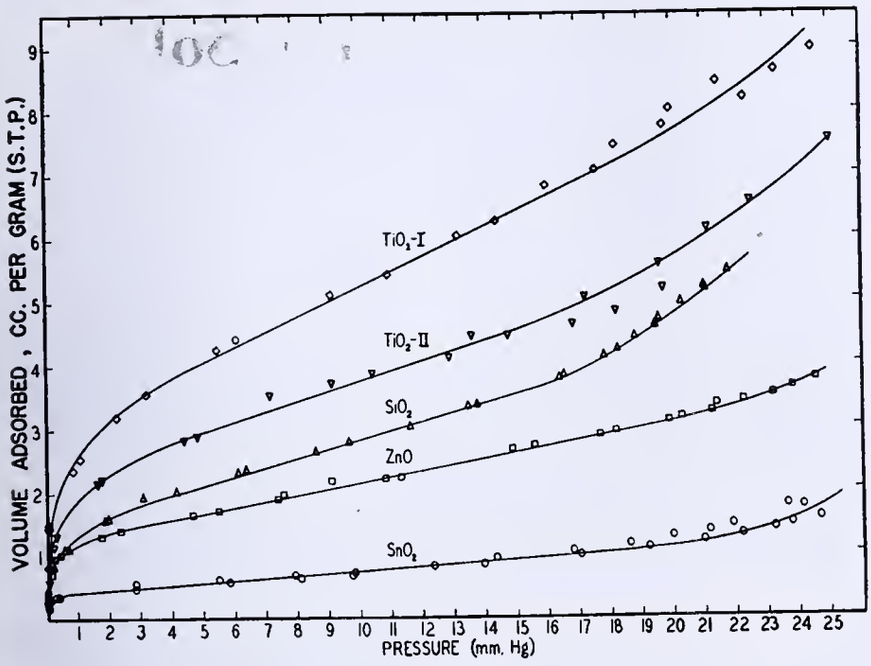

FIGURE 2. ISOTHERMS FOR ADSORPTION OF WATER VAPOR ON CRYSTALLINE POWDERS AT 25° C.

TABLE I. ADSORPTION OF WATER ON TITANIC OXIDE

(t = 31° C.)

P, mm. Hg	$n \times 10^8$ per gram	v, cc. at S. T. P.
0.007	1.5	0.37₅
0.023	3.6	0.90
0.13	6.6	1.65
0.84	10.4	2.60
2.21	14.1	3.52
5.44	18.8	4.70
9.08	22.8	5.70
13.12	26.8	6.70
16.00	30.2	7.55
18.20	33.1	8.28
19.95	35.6	8.90
21.48	37.6	9.40
33.38 = p_0		

closed and the process was repeated. The calculations are based on the perfect gas law, $PV = nRT$, which is deemed sufficiently accurate for vapors at these temperatures as long as one works appreciably below the vapor tension of the liquid. At the highest pressure attained, $p/p_0 = 0.80$, unquestionable accuracy cannot be expected.

The number of moles of vapor adsorbed per gram of powder and the volume of adsorbed vapor under standard conditions were calculated with due regard to the quantity of vapor present initially and finally in each section of the apparatus. A typical set of results is given in Table I.

Blank experiments were performed to show that no observable decrease in pressure resulted from absorption of any vapors by the manometer fluid or the stopcock grease. Curves of duplicate experiments are identical at low pressures. In some cases they diverge at higher pressures, in part, no doubt, because errors are additive under the method adopted. There is no divergence below a relative pressure (p/p_0) of 0.25. Desorption points were obtained on each isotherm, and agreed satisfactorily with those determined in adsorption. Desorption points are not shown in the figures, however.

Results

The adsorption of water vapor on five powders is shown in Figure 2. Typical S-shaped isotherms have been obtained, the low-pressure portion of the isotherm being concave to the pressure axis, the higher pressure region convex to the pressure axis, and the intermediate region approximately linear with respect to pressure. The data have been fitted to Equation A (4).

$$p/v(p_0 - p) = 1/v_m c + (c - 1/v_m c)p/p_0 \quad \text{(A)}$$

where, for the authors' data, p = pressure in mm. of mercury, p_0 = saturation pressure of the vapor, v = volume of vapor adsorbed in cc. at standard temperature and pressure, v_m = volume of vapor adsorbed when the entire adsorbent surface is covered with a complete monomolecular layer, $c = (a_1 b_2/b_1 a_2)\ \exp.\ (E_1 - E_L)/RT$, where a_1, b_1, a_2, and b_2 are constants, E_1 = heat of adsorption in the first layer, and E_L = heat of liquefaction of the vapor in cal. mole^{-1}. A plot of Equation A for water vapor on the adsorbents is shown in Figure 3. Between relative pressures of 0.05 and over 0.35 the plots are closely linear. From them the values of v_m and c were obtained by the methods of least squares. From c approximate values for $E_1 - E_L$ were calculated (Table II).

The adsorption of propyl alcohol vapor on the same crystalline powders is shown in Figure 4, and in Figure 5 are shown the data for the adsorption of benzene vapor on titanium dioxide I, titanium dioxide II, and quartz. These isotherms resemble the well-known Langmuir type over most of their course. Increased adsorptions at high relative pressures (benzene), however, may suggest the beginning of a polymolecular film. The data, when plotted according to Equation C (4),

$$p/v = p_0/v_m c + p/v_m \quad \text{(C)}$$

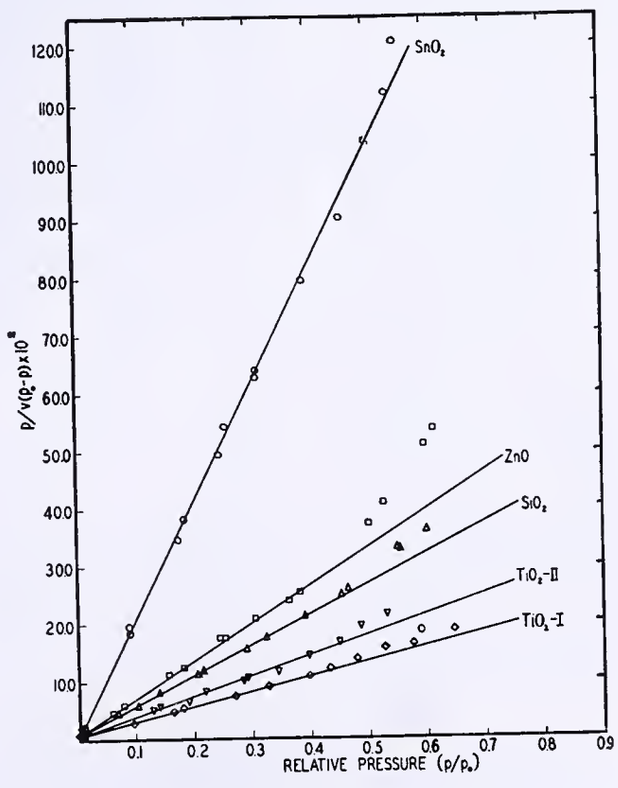

FIGURE 3. ADSORPTION DATA FOR WATER VAPOR PLOTTED ACCORDING TO EQUATION A

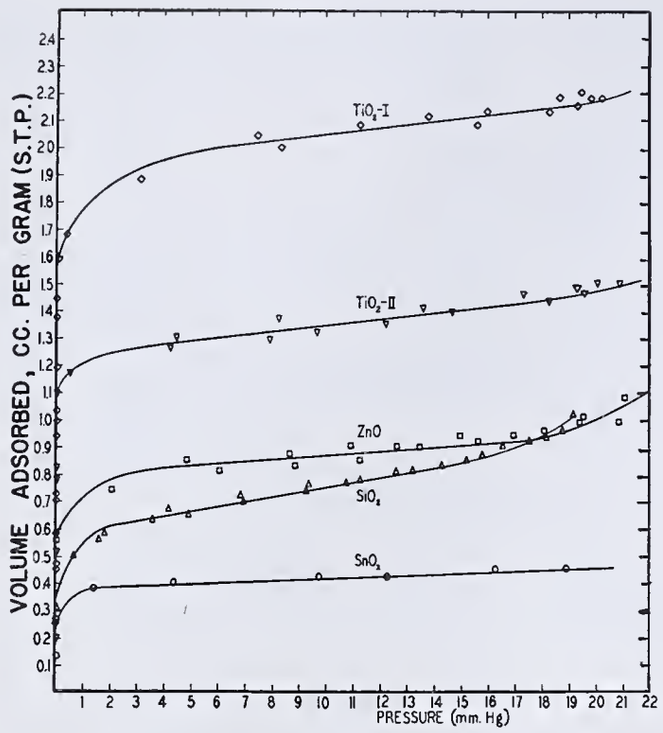

FIGURE 4. ISOTHERMS FOR ADSORPTION OF PROPYL ALCOHOL VAPOR ON CRYSTALLINE POWDERS AT 25° C.

gave good straight lines. Values of c and v_m calculated by least squares are listed in Table II.

In addition to the vapor adsorptions, isotherms for the adsorption of nitrogen gas at −195.5° C. were determined for both samples of titanic oxide. The apparatus in which these experiments were conducted was patterned closely after that described by Emmett and Brunauer (*3*), and their suggested technique was followed. Temperatures were accurately determined by a calibrated copper-constantan thermocouple in conjunction with a White double potentiometer and sensitive galvanometer. The curves obtained were typical of van der Waals adsorption, and were used to determine values of the total surface area per gram given in Table III.

Discussion

It is possible to utilize the values of v_m given in Table II to arrive at an estimate of the surface extent of a crystalline

TABLE II. VALUES OF CONSTANTS FOR ADSORPTION OF VAPORS AT 25° C. ON CRYSTALLINE SURFACES

Powder	v_m Cc./gram	c	$E_1 - E_L$ Cal. mole^{-1}
	Water vapor		
TiO_2-I	4.28	48.6	2400
TiO_2-II	3.17	48.9	2380
ZnO	1.52	64.3	2500
SiO_2	1.96	46.7	2300
SnO_2	0.48	....[a]	....[a]
	Propyl alcohol vapor		
TiO_2-I	2.41	32.1	2080
TiO_2-II	1.65	32.3	2080
ZnO	1.07	23.0	1840
SiO_2	1.18	....[a]	....[a]
SnO_2	0.52	25.5	1940
	Benzene vapor		
TiO_2-I	1.44	77.0	2640
TiO_2-II	1.13	56.4	2450
SiO_2	0.36	33.4	2330

[a] In these cases calculated values of c and $E_1 - E_L$ were considerably different from the other agreeing values in these columns and are therefore omitted. By the present method of calculation the value of v_m determined by position of the knee of the adsorption isotherm is the most reliable of the three, while c and $E_1 - E_L$ are very sensitive to slight changes in the slope of the isotherm beyond its knee.

TABLE III. SURFACE AREA VALUES

(Square meters per gram)

Powder	$N_2(L)$	$H_2O(L)$	$C_3H_7OH(M)$	$C_6H_6(L)$
	16.2	10.6	20.0	30.5
TiO_2-I	13.5	12.4	13.0	11.9
TiO_2-II	8.3	9.1	8.9	9.3
ZnO	....	4.4	5.8	....
SiO_2	....	5.6	6.3	3.0
SnO_2	....	1.4	2.8	....

powder, provided a reasonable choice of the area per adsorbed molecule can be made. If the suggestions of Emmett and Brunauer (*3*) are followed, and one assumes that the adsorbed molecules are in the closest possible packing—namely, hexagonal two-dimensional packing—one may apply the formula

$$\text{Area per molecule} = 1.091\,(M/Nd)^{2/3}$$

where M is the molecular weight of the vapor, N is Avogadro's number, and d may be taken as the density of either the solidified or liquefied gas at the temperature of the adsorption experiment. Thus, for water at 25° C., 11.1 and 10.6 Å.^{2} per molecule are calculated, respectively; for propyl alcohol, 27.0 Å.^{2} per molecule; and for benzene, 30.5 Å.^{2} per molecule. These values, with the exception of propyl alcohol, have been

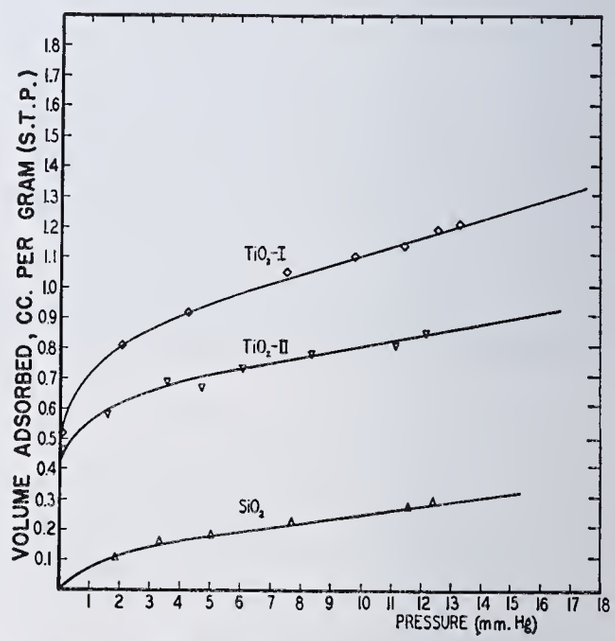

FIGURE 5. ISOTHERMS FOR ADSORPTION OF BENZENE VAPOR ON CRYSTALLINE POWDERS AT 25° C.

used in the construction of Table III. In the case of the alcohol, this value appears high, and the limiting area for close-packed hydrocarbon chains vertically oriented as obtained from insoluble films of long-chain alcohols has been used instead. This fact is indicated by M (M = monolayer); L denotes that the density of the liquefied vapor has been used.

The concordance for the surface areas in the case of the titanium dioxides is as satisfactory as is to be expected, considering the uncertainty involved in the somewhat arbitrary manner in which the area per adsorbed molecule was chosen. Table III seems to indicate that for specific areas greater than 5 square meters the method of vapor adsorption is of approximately the same degree of reliability as low-temperature adsorption. More recent work (*7*) wherein a basically different technique for the determination of the amount adsorbed is used, supports this contention.

Owing to the relatively small size of the water molecule, it is evident that a larger number of them is necessary to form a

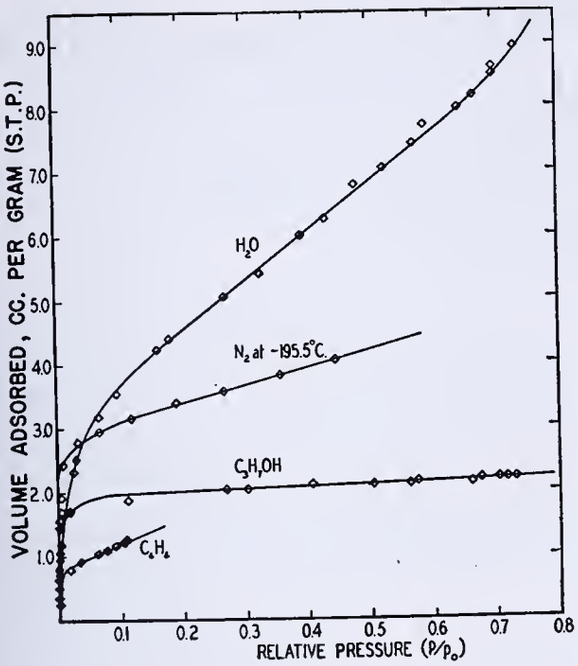

FIGURE 6. COMPARISON OF ADSORPTION ISOTHERMS OF VAPORS ON CRYSTALLINE TITANIUM DIOXIDE (ANATASE, TiO_2-I)

Curves for adsorption of water, propyl alcohol, and benzene vapors at 25° C.

monomolecular layer on a unit surface; hence, greater volumes of water vapor than nitrogen gas are observed to be adsorbed on the same solid surface. This fact should serve to make for greater accuracy in surface area determinations when water vapor is employed if the smaller equilibrium pressures may be read without increased percentage error.

It is of interest to speculate on the cause of the great difference between the character of the adsorption of propyl alcohol, and of water and benzene vapors. The distinction is illustrated by Figure 6 where the abscissa is taken as the relative pressure, p/p_0, in order to bring the isotherms to a common basis. The flatness of the curve for propyl alcohol might be interpreted as signifying that adsorption to form second and higher layers does not occur until pressures near saturation are reached. Since it is probable that the alcohol molecule is nearly vertically oriented on the polar solid surface with its hydroxyl group down, the uppermost surface of an adsorbed monolayer would present an oil-like or hydrocarbon surface on the average. On such a surface, the condition for condensation to form multilayers, $E_2 \cong E_L$, might not be satisfied; rather, it may be that $E_2 < E_L$, since the configuration of the molecules in the first layer differs strongly from the structure of a unimolecular liquid layer. In the classification suggested by Brunauer, Deming, Deming, and Teller (*2*), the adsorption isotherm for propyl alcohol on anatase is a combination of their Types I and III.

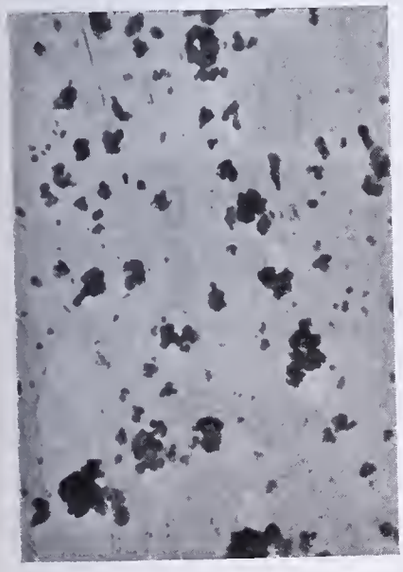

FIGURE 7. ELECTRON MICROSCOPE PHOTOGRAPH OF TiO_2-I (× 14,600)

Figures 7 and 8 show the electron microscope photographs of the two titanium dioxides, obtained with the R. C. A. electron microscope of the Research Laboratories of Interchemical Corporation by E. F. Fullam, who calculates the specific area of 20.9 square meters per gram for titanium dioxide I and 10.7 for titanium dioxide II for the particles as spheres from the statistical determination of d_3 which is $\Sigma nd^3/\Sigma nd^2$. The value for titanium dioxide II is in as good agreement as may be expected with the values of Table III; that for titanium dioxide I is not in as good agreement, and leaves a discrepancy to be clarified by further work.

FIGURE 8. ELECTRON MICROSCOPE PHOTOGRAPH OF TiO_2-II (× 14,600)

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Determination of Fluorine

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HOFFMAN and Lundell (*1*) as well as Specht (*2*) have described procedures for the determination of fluorine, in which lead chlorofluoride is precipitated, filtered, and dissolved in nitric acid and the chloride is determined by the Volhard method. These procedures may be somewhat improved by the use of fritted Gooch-type glass-disk crucibles of medium porosity (Corning Glass Works 32960-30M) for the filtration.

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Detection of Second-Hand White Cotton Filling Materials

Used in Articles of Bedding and Upholstered Furniture

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THE Bedding Regulations of the Department of Health of Ontario came into force on December 28, 1938. Under these regulations, "new" as applied to any filling material means any undyed material which has not been previously manufactured (except felted) or used for any purpose but does not include converted material. "Converted material" as applied to any filling material means any otherwise new material which has been dyed or colored or spun into yarn or knit or woven into fabric but not further manufactured than cut up, torn up, broken up, shredded, or felted. "Second-hand" as applied to any filling material means any material which is neither new nor converted.

For administrative purposes it is necessary to be able to detect the presence of second-hand filling materials. A review of the literature shows that little information has been published in this field.

In 1932, Wynne and Donovan (*5*) of the Maryland State Department of Health reported that new and second-hand white cotton could be distinguished by the difference in the color of the fluorescence exhibited by these materials under a suitable source of ultraviolet light. They concluded that this test was quick and simple, could readily be demonstrated, and in the hands of an experienced person gave fairly accurate results in a large majority of cases. In 1933, Burke and Kane (*1*) of the Bedding Division of the New York State Department of Labor developed a chemical method to differentiate new from second-hand cotton fillings. They found that new cotton fillings had a water-soluble sulfate content of less than 0.25 per cent expressed as sodium sulfate and that second-hand cotton fillings were characterized by a sulfate content in excess of this amount. As they found that new cotton did not contain sufficient sulfate in any form to account for this increase, they concluded that the additional sulfate found in second-hand cotton fillings must have come from an outside source.

In 1935, Moskowitz, Landes, and Himmelfarb (*3*) of the Bedding Division of the New York State Department of Labor published data on the ammonia, urea, and sodium sulfate content of new and second-hand cotton filling materials. They reported that unused cotton materials had both an ammonia and urea content of less than 0.030 and 0.010 per cent, respectively, whereas used cotton fillings had in most cases an ammonia content above 0.030 per cent, a urea content above 0.010 per cent, or both. Racicot of the Massachusetts Department of Public Health developed qualitative methods for the detection of urea and creatinine (*4*). Later Racicot and Lythgoe (*4*) determined the ash, oil, ammonia, and urea content of white cotton wastes; they stated that the urea content of new white cotton wastes was very unlikely to exceed 0.0028 per cent. In 1940, Fetherolf (*2*) of the Pennsylvania Bedding Law Division published data on the pH values of the aqueous extracts of white cotton filling materials. He reported that new undyed cotton materials had in no single instance exhibited a pH value of less than 6.0, whereas samples removed from known second-hand mattresses had not approached 6.0 in any one case.

The various tests which have been devised to recognize second-hand white cotton filling materials may be divided into two groups: those which show alteration in the cotton itself, such as the fluorescent, staining, and reduction tests, and those which detect contamination from body excretions, such as ammonia, urea, sulfate, and creatinine determinations. It is believed that second-hand white cotton filling materials have been analyzed for phosphates and chlorides, but to the knowledge of the authors, no study of this subject has been reported, although under the Rag Flock Act of 1911 the chlorine content is used in Great Britain as a test for washed material.

It is difficult to determine the significance of the pH test and little can be said until more is known about the reason for the shift in the pH values.

Excluding converted material, this paper deals with the results of the analyses of all the white cotton filling materials which were received in this laboratory during the past two years, except for a few which were omitted because (1) samples were too small for complete analysis, (2) they contained sufficient dirt or oil to mask the true fluorescence, or (3) the fluorescence was not sufficiently uniform to be properly classified. Included in this report are the remaining 400 samples, among which are specimens from the United States, Brazil, Egypt, Russia, India, China, and Java.

Methods

FLUORESCENT TEST. The equipment consisted of a 400-watt Hanovia analytic model quartz mercury vapor lamp installed in a dark room. The lamp was fitted originally with a red-purple Corex A No. 986 molded glass filter 16.25 cm. (6.5 inches) square and 5 mm. thick. This filter was broken and was replaced by one of the same type ground and polished to a thickness of 3 mm. Although these two filters transmitted rays of the same wave lengths (2480 to 4359 Å.), the thinner filter transmitted a higher percentage of the total radiation.

The first filter did not necessitate protection for the eyes but the second demanded the use of goggles. Nine different types made by the Willson Products, Inc., were tried. Willsonite goggles (shade C) were chosen as the most suitable as, under ultraviolet light, it was found that the difference in appearance of the samples tested was accentuated by their use. It was readily possible to separate samples of white cotton into at least five groups, depending upon the intensity of their violet fluorescence. This is difficult if not impossible without the use of goggles.

In practice, each sample of white cotton is placed in one of five groups which are numbered from 4 to 0. In group 4 are placed those samples which exhibit a deep violet fluorescence. Groups 3, 2, and 1 contain samples which show progressively decreasing amounts of violet fluorescence, and into group 0 are put samples which exhibit no violet fluorescence.

pH TEST. Two and one-half grams of white cotton filling material are immersed in 50 ml. of distilled water and kneaded with a thick glass rod until thoroughly wetted and the hydrogen-ion concentration of the aqueous extract is determined to the nearest 0.1 pH by means of a Coleman Model 3A pH electrometer.

CHEMICAL TEST. *Ammonia and Urea Determinations.* Five grams of white cotton filling material are placed in a 400-ml. beaker, 150 ml. of distilled water are added, and the whole is boiled for 20 minutes. The solution is filtered by suction through a Büchner funnel and the sample is boiled again with 150 ml. more of distilled water. One milliliter of 0.1 *N* hydrochloric acid is added to the pooled filtrate, which is then evaporated down to about 5 ml; 1 ml. of 0.1 *N* sodium hydroxide is added, and the solution is washed into a 15-ml. graduated Pyrex centrifuge tube, the volume being made up to 12.5 ml. with distilled water. Two aliquots of 5 ml. each are pipetted into Pyrex culture-tubes (250 by 25 mm.).

To aliquot No. 1, 5 ml. of buffer solution (6.0 grams of potassium dihydrogen phosphate and 9.1 grams of anhydrous disodium hydrogen phosphate made up to 1 liter with distilled water) and one eighth of a crushed Squibb urease tablet are added. After standing 20 minutes at room temperature, 7 ml. of saturated borax

solution and 7 drops of caprylic alcohol are added. The culture tube is immediately inserted into a steam-distilling apparatus and the liberated ammonia is distilled over through a block-tin condenser into a 50-ml. Erlenmeyer flask containing 10 ml. of 2 per cent boric acid and 3 drops of methyl red (0.05 gram of methyl red dissolved in 100 ml. of 95 per cent ethyl alcohol). The solution is steam-distilled for 5 minutes, during the last 2 minutes of which the flask containing the boric acid is lowered so that the end of the condenser is above the level of the solution. The solution is titrated with 0.01 *N* sulfuric acid, using a microburet, until the color is a pale pink. Then it is heated nearly to boiling, and the titration is continued until a full pink color is reached.

To 5-ml. aliquot No. 2, 7 ml. of saturated borax solution and 7 drops of caprylic alcohol are added. The tube is inserted into the steam-distilling apparatus, and the solution is distilled and titrated as above. Blank determinations on the reagents must be run. The titration values from the blanks are subtracted from the values obtained in the analysis of the sample to get the corrected volumes to be used in calculating the results.

CALCULATIONS. Percentage of ammonia in sample = ml. of sulfuric acid (No. 2 corrected) × 0.0085

Percentage of urea in sample = ml. of sulfuric acid (No. 1 corrected minus No. 2 corrected) × 0.015

Sulfate Determination. The residual solution from the steam-distillation in the ammonia determination (No. 2 above) is transferred from the culture tube to a 250-ml. beaker and diluted with 100 ml. of distilled water. The solution is acidified with hydrochloric acid heated to boiling and treated with 5 ml. of 5 per cent barium chloride solution. The solution is allowed to stand overnight; the precipitate is filtered off, washed, and ignited in the usual way. The water-soluble sulfates are expressed as sodium sulfate.

CALCULATION. Percentage of sodium sulfate = mg. of barium sulfate × 0.0304

Chloride Determination. Five grams of white cotton filling material are extracted twice with 150 ml. of boiling distilled water as in the determination for ammonia. The filtrate is concentrated to about 100 ml. and 6 ml. of concentrated nitric acid (density 1.42) are added. The solution is further concentrated until it becomes clear and light yellow in color. It is then filtered into a 250-ml. volumetric flask and the precipitate washed with distilled water. Ten milliliters of 0.1 *N* silver nitrate are added and the solution is allowed to stand at room temperature, with frequent shakings, for at least 20 minutes, then made up to 250 ml., and filtered. The first 50 ml. of the filtrate are discarded. If the filtrate is not perfectly clear the solution is refiltered through the same filter paper, as finely divided silver chloride will act like silver nitrate in the titration with potassium thiocyanate. A 100-ml. aliquot of the filtrate is titrated with 0.1 *N* potassium thiocyanate solution, using a microburet. Ten milliliters of a 2 per cent solution of ferric ammonium sulfate are added to act as an internal indicator. The end point is taken as the first appearance of a permanent red color. The soluble chlorides extracted from the cotton are expressed as sodium chloride.

CALCULATION. Percentage of sodium chloride = (4.00 minus ml. of 0.1 *N* potassium thiocyanate) × 0.292

Phosphate Determination. The initial steps are the same as those in the chloride determination, except that 15 instead of 6 ml. of concentrated nitric acid are used, no silver nitrate is added, and the solution in the 250-ml. flask is not filtered before taking the 100-ml. aliquot. The 100-ml. aliquot is heated to 70° C. and 25 ml. of ammonium molybdate solution are slowly added with constant stirring. (The ammonium molybdate solution is prepared by dissolving 100 grams of pure molybdic acid in a mixture of 400 ml. of water and 80 ml. of strong ammonia, density 0.90. When the molybdic acid is dissolved the solution is slowly poured with constant stirring into a mixture of 400 ml. of strong nitric acid, density 1.42, and 600 ml. of distilled water.) It is then allowed to stand on a hot plate at a temperature of 40° to 60° C. for 2 hours with occasional stirring, and then at room temperature for an hour longer. The solution is filtered, and the precipitate is washed with 1 per cent potassium nitrate until the washings are neutral to litmus. The filter paper and precipitate are transferred to a beaker containing 25 ml. of 0.1 *N* sodium hydroxide. When the precipitate has been completely dissolved the solution is diluted with distilled water and titrated with 0.1 *N* nitric acid, using phenolphthalein as the indicator.

CALCULATION. Percentage of P_2O_5 = (25.00 minus ml. of 0.1 *N* nitric acid used) × 0.0062

TABLE I. CORRELATION BETWEEN AMMONIA CONTENT AND INTENSITY OF VIOLET FLUORESCENCE

No. of Samples	Intensity of Violet Fluorescence	Ammonia Content: Highest % found	Ammonia Content: Average % found	Ammonia Content: Lowest % found	Below 0.0125%: No. of samples	Below 0.0125%: % of samples	Above 0.0125%: No. of samples	Above 0.0125%: % of samples
61	4	0.011	0.004	0.000	61	100.0	0	0.0
158	3	0.017	0.005	0.000	155	98.1	3	1.9
25	2	0.032	0.013	0.003	14	56.0	11	44.0
111	1	0.091	0.031	0.012	1	0.9	110	99.1
45	0	0.094	0.043	0.015	0	0.0	45	100.0

TABLE II. CORRELATION BETWEEN UREA CONTENT AND INTENSITY OF VIOLET FLUORESCENCE

No. of Samples	Intensity of Violet Fluorescence	Urea Content: Highest % found	Urea Content: Average % found	Urea Content: Lowest % found	Below 0.0105%: No. of samples	Below 0.0105%: % of samples	Above 0.0105%: No. of samples	Above 0.0105%: % of samples
61	4	0.010	0.003	0.000	61	100.0	0	0.0
158	3	0.015	0.004	0.000	154	97.5	4	2.5
25	2	0.035	0.008	0.001	19	76.0	6	24.0
111	1	0.080	0.011	0.002	69	62.2	42	37.8
45	0	0.071	0.015	0.001	19	42.2	26	57.8

Results

As mentioned above, the samples were separated into five groups (numbered from 4 to 0), depending upon the intensity of their violet fluorescence. A representative sample from each group was chosen and kept as a reference standard, and the intensity of the violet fluorescence of any individual sample was determined by comparison with the fluorescence of these standards. It has been shown, by others, that new white cotton has the power of exhibiting a violet fluorescence under ultraviolet light and that the loss of this property is associated with second-hand material.

The results of the ammonia determinations are summarized in Table I and include the high, average, and low amounts for each of the five groups. The concentration of ammonia steadily increases as the intensity of the violet fluorescence decreases. The highest amount of ammonia found in groups 4 and 3 was 0.017 per cent and the lowest amount found in groups 1 and 0 was 0.012 per cent. A study of the individual results led to the adoption of 0.0125 per cent as the standard for the ammonia determination.

As shown in Table I, 216 out of 219 or 98.6 per cent of the samples in groups 4 and 3 had substandard concentrations, whereas 155 out of 156 or 99.4 per cent of the samples in groups 1 and 0 had an ammonia content greater than 0.0125 per cent. Samples in group 2 were not compared with the ammonia or other determinations for reasons discussed below.

Table II gives the results of the urea determinations. It will be observed that the urea content on the average gradually rises as the intensity of the violet fluorescence falls. However, the results are not consistent, as both high and low values have been encountered in the samples of the last two groups. It has been suggested that the low concentrations might occur as a result of the slow conversion of urea into ammonia in the cotton by enzyme action.

The largest quantity found in groups 4 and 3 was 0.015 per cent, which is considerably in excess of the amounts occurring in many samples in groups 1 and 0. Thus the choosing of a standard for urea presents some difficulty and a figure of 0.0105 per cent was finally adopted. A study of Table II

shows that 98.2 per cent of the samples in groups 4 and 3 and 56.4 per cent of the samples in groups 1 and 0 have a substandard urea content. Thus less than one half of the samples, in groups 1 and 0, contain excess amounts of urea.

Table III reports the results of the sodium sulfate determinations. A decrease in the intensity of the violet fluorescence is accompanied by an increase in the percentage of sodium sulfate. It has been pointed out that some foreign cottons, before shipment to this continent, are treated with hygroscopic sulfates to prevent loss of moisture in transit; thus an occasional sample of foreign cotton containing a relatively high percentage of sodium sulfate might be expected. In the authors' experiments, however, the greatest amount found in groups 4 and 3 was 0.32 per cent, which is considerably less than the quantity obtained from many of the samples in groups 1 and 0. As the majority of the samples in the first two groups had a sodium sulfate content below 0.255 per cent and as most of the samples in the last two groups had a content above 0.255 per cent, this figure was chosen as the standard.

As shown in Table III, 213 out of 219 or 97.3 per cent of the samples in groups 4 and 3 had substandard concentrations, whereas 151 out of 156 or 96.8 per cent of the samples in groups 1 and 0 were above the standard amount.

Table IV reports the results of the sodium chloride determinations. Although from group 4 to group 0 there is a gradual rise on the average in the amount of sodium chloride found, some samples in the first two groups showed relatively high values, mainly among the samples of foreign cottons. It is possible that some of these materials had been treated with hygroscopic chlorides prior to their shipment to this continent and this factor was taken into consideration in choosing the standard. The figure finally adopted was 0.135 per cent. As shown in Table IV, 95.4 per cent of the samples in groups 4 and 3 had a substandard content, whereas, in groups 1 and 0 only about two-thirds of the samples contained more than 0.135 per cent.

Table V gives the results of the phosphate determinations. It is apparent that there is no significant difference between the amounts of phosphate found in the samples showing a deep violet and those exhibiting a weak violet fluorescence. For this reason the phosphate test was abandoned after one hundred determinations had been made.

Table VI gives the results on the one hundred pH analyses which have been made. Although at the present time, the information available is only sufficient to permit the presentation of a preliminary report, it appears that in most cases a decrease in the intensity of the violet fluorescence is accompanied by an increase in the acidity of the aqueous extracts. As 6.1 was the lowest pH value found in groups 4 and 3, 6.05 was chosen as the tentative standard. Thus all samples in groups 4 and 3 had pH values greater than 6.05 and over 90 per cent of those in groups 1 and 0 had values below 6.05.

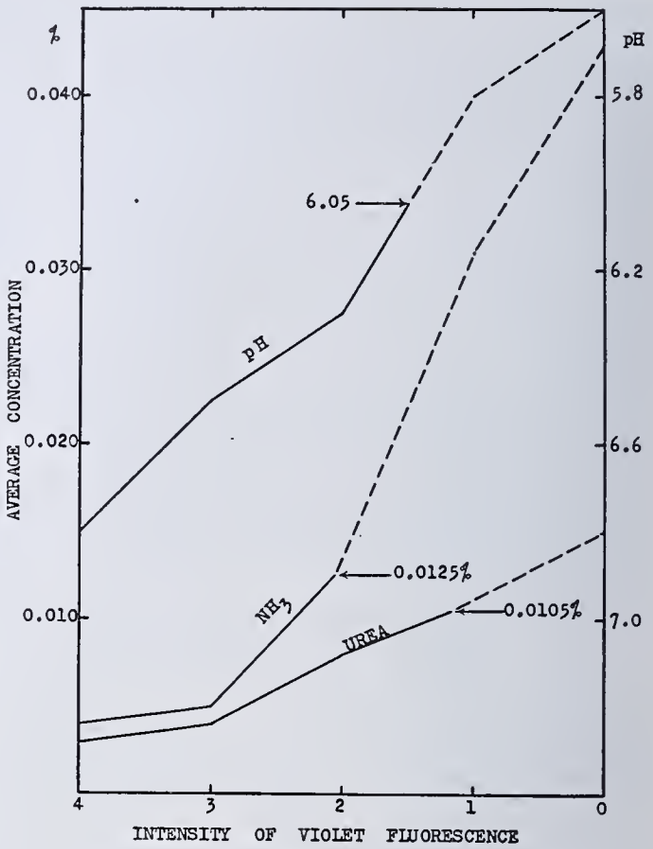

FIGURE 1

Discussion

In previous papers dealing with the detection of second-hand white cotton filling materials, attempts have been made, in most cases, to determine at the time of collection the new or second-hand nature of the samples to be analyzed. Prior to any experimental work, samples were classified, in part, according to information supplied by manufacturers, wholesale dealers, supply houses, etc., or according to the opinion of an independent expert.

Grading samples as new or second-hand on information obtained from manufacturers, wholesale dealers, supply houses, etc., has already been severely criticized by others. It has been pointed out that the trade is likely, in some instances, to submit material far different from what is expected. The second method, that of placing absolute reliance on the opinion of an expert, is also open to question, as it places too much faith in the ability of anyone to grade correctly all doubtful samples by visual inspection alone. Such information should be treated with reserve, as any error would tend to invalidate the interpretation of the results.

In this paper, the samples were collected by a bedding inspector from numerous retailers, manufacturers, felters, and supply houses. In most

TABLE III. CORRELATION BETWEEN SODIUM SULFATE CONTENT AND INTENSITY OF VIOLET FLUORESCENCE

No. of Samples	Intensity of Violet Fluorescence	Sodium Sulfate Content Highest % found	Average % found	Lowest % found	Below 0.255% No. of samples	Below 0.255% % of samples	Above 0.255% No. of samples	Above 0.255% % of samples
61	4	0.27	0.14	0.01	60	98.4	1	1.6
158	3	0.32	0.17	0.02	153	96.8	5	3.2
25	2	0.37	0.24	0.03	14	56.0	11	44.0
111	1	0.84	0.36	0.20	4	3.6	107	96.4
45	0	0.71	0.44	0.20	1	2.2	44	97.8

TABLE IV. CORRELATION BETWEEN SODIUM CHLORIDE CONTENT AND INTENSITY OF VIOLET FLUORESCENCE

No. of Samples	Intensity of Violet Fluorescence	Sodium Chloride Content Highest % found	Average % found	Lowest % found	Below 0.135% No. of samples	Below 0.135% % of samples	Above 0.135% No. of samples	Above 0.135% % of samples
61	4	0.17	0.07	0.01	60	98.4	1	1.6
158	3	0.23	0.09	0.02	149	94.3	9	5.7
25	2	0.20	0.11	0.04	19	76.0	6	24.0
111	1	0.30	0.16	0.06	43	38.7	68	61.3
45	0	0.58	0.18	0.11	11	24.4	34	75.6

TABLE V. CORRELATION BETWEEN PHOSPHATE CONTENT AND INTENSITY OF VIOLET FLUORESCENCE

No. of Samples	Intensity of Violet Fluorescence	Phosphate Content (P_2O_5) Highest % found	Average % found	Lowest % found
16	4	0.10	0.06	0.04
36	3	0.14	0.07	0.05
9	2	0.08	0.06	0.05
25	1	0.10	0.07	0.05
14	0	0.10	0.08	0.06

TABLE VI. CORRELATION BETWEEN pH VALUE AND INTENSITY OF VIOLET FLUORESCENCE

No. of Samples	Intensity of Violet Fluorescence	pH Values Highest found	Average found	Lowest found	Above 6.05 No. of samples	Above 6.05 % of samples	Below 6.05 No. of samples	Below 6.05 % of samples
15	4	7.1	6.8	6.4	15	100.0	0	0.0
30	3	7.0	6.5	6.1	30	100.0	0	0.0
5	2	6.9	6.3	5.7	3	60.0	2	40.0
25	1	6.7	5.8	5.2	3	12.0	22	88.0
25	0	6.1	5.6	5.2	1	4.0	24	96.0

cases, opinions concerning the new or second-hand nature of the materials were obtained by the inspector at the time of collection. To eliminate any possible error, all information received from outside sources was disregarded (except data concerning the geographical origin of the foreign cottons), as was the personal opinion of any member of the staff connected with the enforcement of the bedding regulations. Thus the authors were not influenced by any preconceived ideas about the new or second-hand nature of the samples which were tested but were able to base their conclusions directly on the experimental results.

It is of the utmost importance to be able to tell whether a sample of white cotton contains second-hand material. Three methods of testing are available. The first is the chemical test to detect contamination from body excretions, the second is the use of ultraviolet light to detect some alteration in the cotton itself, and the third is the pH test.

CHEMICAL TEST. An ideal single chemical determination, to distinguish between a sample of new and second-hand material, would be one which fulfills the following conditions: the results for new cotton should always be negative, and the results for second-hand cotton should always be positive and sufficiently high to make the evidence conclusive. So far none of the methods available meets the first requirement.

In assessing the relative merits of the chemical determinations, reported in this paper, it is evident that they are not all of equal value. Figures 1 and 2 show the average percentage concentration of ammonia, urea, sodium sulfate, sodium chloride, and phosphate plotted against the intensity of the violet fluorescence.

The authors consider that the ammonia determination is the best of the chemical methods which were investigated, as it most closely approaches the ideal conditions mentioned above. It will be seen from Figure 1 that, as the intensity of the violet fluorescence decreases, there is an initial gradual rise followed by a sharp increase in the concentration of ammonia. Next in importance is the sodium sulfate determination. As shown in Figure 2, there is a steep rise in the sodium sulfate curve which is similar to the one for ammonia except that the initial slope is somewhat greater.

The urea and sodium chloride determinations are of less value as, on the average, the concentrations of these compounds vary within narrower limits. This is apparent from a study of the curves in Figures 1 and 2. As stated above, the phosphate determination was abandoned after one hundred analyses had been made.

The standards adopted for ammonia, urea, sodium sulfate, and sodium chloride were 0.0125, 0.0105, 0.255, and 0.135 per cent, respectively. In some samples none of these standards were exceeded, while in others excess quantities of one or more of these compounds were found. The results are shown in Table VII. In group 4, 100 per cent and in group 3, 98.8 per cent of the samples had substandard values in all or all but one of the four chemical determinations, while in group 1, 97.3 per cent and in group 0, 100 per cent of the samples exceeded two or more of these standards. Thus when none or only one of the standards is exceeded, the sample is not considered to contain second-hand material by the chemical test.

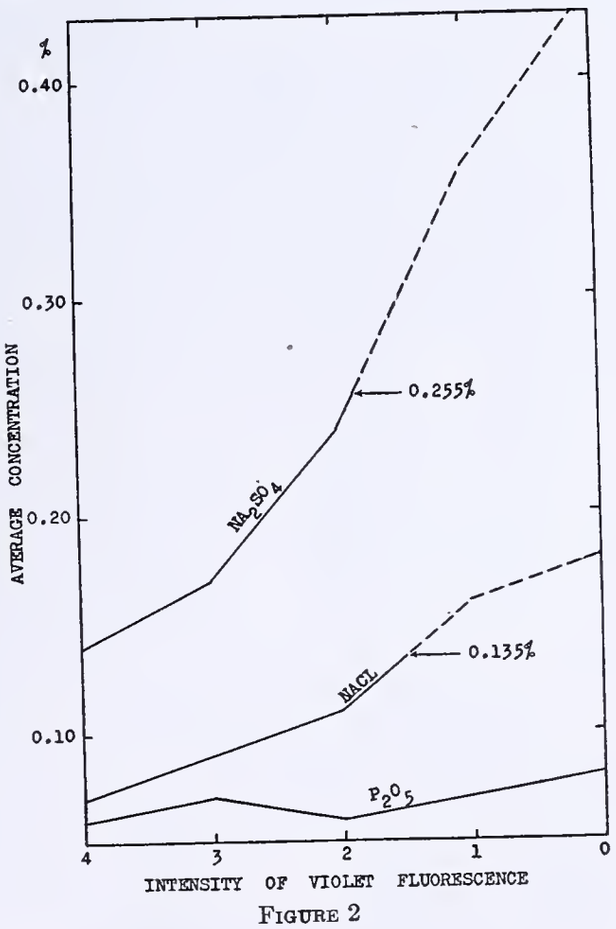

FIGURE 2

TABLE VII. CORRELATION BETWEEN NUMBER OF CHEMICAL STANDARDS EXCEEDED AND INTENSITY OF VIOLET FLUORESCENCE

Samples Which Exceed None, One Only, Two Only, Three Only, and All Four of Ammonia, Urea, Sodium Sulfate, and Sodium Chloride Standards

No. of Samples	Intensity of Violet Fluorescence	None No. of samples	None % of samples	One Only No. of samples	One Only % of samples	Two Only No. of samples	Two Only % of samples	Three Only No. of samples	Three Only % of samples	All Four No. of samples	All Four % of samples
61	4	59	96.7	2	3.3	0	0.0	0	0.0	0	0.0
158	3	139	88.0	17	10.8	1	0.6	1	0.6	0	0.0
25	2	12	48.0	3	12.0	1	4.0	7	28.0	2	8.0
111	1	1	0.9	2	1.8	32	28.8	42	37.9	34	30.6
45	0	0	0.0	0	0.0	6	13.3	18	40.0	21	46.7

Table VIII. Correlation between Chemical and Fluorescent Tests

No. of Samples	Intensity of Violet Fluorescence	Classification by Chemical Test[a] New: No. of samples	New: % of samples	Second-Hand: No. of samples	Second-Hand: % of samples
61	4	61	100.0	0	0.0
158	3	158	100.0	0	0.0
25	2	15	60.0	10	40.0
111	1	3	2.7	108	97.3
45	0	0	0.0	45	100.0

[a] Based on results of ammonia, urea, sodium sulfate, and sodium chloride determinations.

As has been pointed out, new foreign cottons may have been treated with hygroscopic sulfates and/or chlorides. If a sample of cotton has been treated with both these chemicals, excess amounts of both sodium sulfate and sodium chloride will be found and the sample classed as containing second-hand material by the chemical test. Thus, to say that a sample contains second-hand material when any two of the four standards are exceeded might be open to some criticism. In Table VII, in group 3 one of the 158 samples exceeded "any two" standards and one sample exceeded "any three" standards. In both these samples the ammonia content was below 0.0125 per cent, which means that in neither case was the ammonia standard exceeded. For these reasons a sample is considered to contain second-hand material according to the chemical test when the ammonia and at least one other of the three standards are exceeded.

Fluorescent Test. The fluorescent test as done in this laboratory has been found very satisfactory. In the authors' opinion, a sample is considered to contain second-hand material when the intensity of the violet fluorescence is 1 or 0. On the other hand, a sample with an intensity of violet fluorescence of 2 might or might not contain second-hand material. For this reason group 2 was not included in the correlation with the chemical determinations, as the results were valueless for choosing the chemical standards. A sample is not considered to contain second-hand material when the intensity of the violet fluorescence is 4 or 3.

Table VIII shows the correlation between the chemical and fluorescent tests. In groups 4 and 3, neither test showed the presence of second-hand material in any sample. Group 2 contains different types of materials which give approximately the same intensity of violet fluorescence. These types can readily be separated, under ultraviolet light, into those samples which suggest or do not suggest the presence of second-hand material. In every case the accuracy of this separation was substantiated by the results of the chemical test. In groups 1 and 0, 153 of the 156 samples showed the presence of second-hand material by both the fluorescent and chemical tests. Thus in 397 of the 400 samples analyzed, when the fluorescent test indicated the presence of second-hand material, the chemical test indicated the presence of contamination from body excretions. Hence the fluorescent and chemical tests are of equal value in detecting the presence of second-hand white cotton. However, it is thought that both these tests should be made before a sample is graded.

From the results of the experiments on the 400 samples which were tested, the authors consider that a sample of white cotton contains second-hand material when the following three conditions are fulfilled:

1. The intensity of the violet fluorescence is of the order of 2 or less.
2. The concentration of ammonia exceeds 0.0125 per cent.
3. Either the concentration of urea exceeds 0.0105 per cent, the concentration of sodium sulfate exceeds 0.255 per cent, or the concentration of sodium chloride exceeds 0.135 per cent.

pH Test. This test is thought to be worthy of further investigation. Unfortunately, the hydrogen-ion concentration of the distilled water used varied markedly and, as yet, the effect of this factor has not been studied. In Figure 1, the average pH values are plotted against the intensity of the violet fluorescence.

One hundred per cent and 92 per cent of the samples which were not considered to contain second-hand material by the fluorescent and chemical tests, respectively, had pH values greater than 6.05. On the other hand, 90 and 97 per cent of the samples considered to contain second-hand material by the fluorescent and chemical tests, respectively, had pH values below 6.05. Thus, at present, a pH value less than 6.05 might be used as confirmatory evidence of second-hand material.

Summary

The chemical, fluorescent, and pH tests to detect the presence of second-hand white cotton filling materials found in articles of bedding and upholstered furniture have been investigated.

The chemical test is based on the determination of the ammonia, urea, sulfate, and chloride content to detect contamination of the material from body excretions. From the results of the experiments on 400 samples of white cotton, the following standards were chosen: ammonia 0.0125 per cent, urea 0.0105 per cent, sodium sulfate 0.255 per cent, and sodium chloride 0.135 per cent. Phosphate determinations were also made on 100 of the above samples, but no standard was chosen, as the results were not significant.

The fluorescent test is a measure of the intensity of the violet fluorescence under ultraviolet light. The 400 samples were divided into 5 groups numbered from 4 to 0, depending upon the intensity of the violet fluorescence exhibited.

The pH test is based on the determination of the hydrogen-ion concentration of the aqueous extract of a sample of white cotton. From the results of the experiments on 100 samples, a pH value of 6.05 was chosen as the tentative standard.

For the detection of second-hand material in a sample of white cotton, the chemical and fluorescent tests were found very satisfactory. From the preliminary investigation which has been made on the pH test, it appears that this test might be of value.

A sample of white cotton is considered to contain second-hand material when the intensity of the violet fluorescence is of the order of 2 or less; the concentration of ammonia exceeds 0.0125 per cent; and either the concentration of urea exceeds 0.0105 per cent, the concentration of sodium sulfate exceeds 0.255 per cent, or the concentration of sodium chloride exceeds 0.135 per cent.

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Correction. In the paper by Steigman, Birnbaum, and Edmonds entitled "Ruthenium Dipyridyl—A New Oxidimetric Indicator" [Ind. Eng. Chem., Anal. Ed., 14, 30 (1942)] reference should have been made to the paper by Smith and Getz [*Ibid.*, 10, 191, 308 (1938)]. The conditions for using the indicator—in 2*M* perchloric acid—were those described by Smith and Getz for titrations with 0.1 *M* ceric nitrate.

Sylvan M. Edmonds

A Supersensitive Schiff's Aldehyde Reagent

Demonstration of a Free Aldehyde Group in Certain Aldoses

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OF RECENT years, cyclic formulas have been generally adopted as best representing many of the known properties of carbohydrates. At the same time, it is generally recognized that certain carbohydrates possess many properties which indicate that in solution a certain proportion of the molecules exist in a straight- or open-chain configuration. In the case of aldoses, this implies the presence of a certain proportion of free aldehyde groups. In the present paper, a very sensitive form of Schiff's reagent is described which can be used for a direct demonstration of aldehydic groups in simple aldoses, and which, conversely, gives a negative reaction with ketoses, glycosides, or polyhydroxy alcohols. Monosaccharide aldoses usually react strongly, and the disaccharide aldoses react rather weakly, so that the test in its present form is of limited value for the latter type of compounds.

Ordinary Schiff's reagent is known to give a positive aldehyde reaction with certain aldoses, but the pink color obtained is usually very weak, owing to the presence of considerably more sulfur dioxide than is necessary to decolorize the fuchsin, and is often obscured by the brown or yellowish impurities which are almost invariably found in samples of commercial basic fuchsin.

Preparation of Reagent

Add 0.500 gram of basic fuchsin to 500 ml. of water, and pass in sulfur dioxide until the weight has increased by 1.0 gram. The solution may retain a considerable portion of its red color, but this will disappear upon standing overnight in a stoppered container, so that the last of the suspended fuchsin dissolves. Make to a volume of 1 liter. The solution will have a brown discoloration due to traces of impurities which are always present in commercial fuchsin. To remove the discoloration, add 1.0 gram of decolorizing carbon, agitate, and filter by gravity, keeping the filter covered with a watch glass to prevent undue contact with the air. If a suitable decolorizing carbon has been used, the filtrate will be completely colorless. Satisfactory brands of fuchsin and carbon are considered under "Reproducibility of the Reagent". A less sensitive form of the colorless reagent, containing 5.0 grams of sulfur dioxide per liter and adapted for the determination of aldehydes in distilled alcoholic liquors, has been previously reported (*8*, *9*).

Determination of Free Aldehyde Groups in Simple Aldoses

Before the reagent is used for testing unknowns, control tests should be run against pure samples of glucose (dextrose) and sucrose. To 0.2-gram portions of the carbohydrates in 7.5-cm. (3-inch) test tubes, add either 1.0 or 2.0 ml. of the reagent. The reagent should be pipetted accurately, and the carbohydrates weighed on a centigram balance or a sensitive decigram balance. Let the tubes stand at room temperature with occasional swirling to dissolve the carbohydrates. After one hour, the tubes containing dextrose show a light but distinct pink color, while those containing sucrose remain completely colorless. If desired, the test may be allowed to run for only 15 or 30 minutes, but in this case positive reactions (pink color) are correspondingly weaker. The colors continue to darken for about 24 hours, but after this time a pink color appears in the sucrose controls.

The regular determination is run in exactly the same fashion. One milliliter of reagent generally gives a more intense pink color with any given aldose than do 2 ml., since there is only half as much sulfurous acid present in proportion to the small amount of free aldehyde present. However, the use of 2 ml. is probably preferable, since it diminishes the weak positive tests obtained with carbohydrates other than simple aldoses, and permits *d*-galactose to dissolve completely. Certain compounds are relatively insoluble in both 1 and 2 ml. of the reagent, but since their reactions are essentially negative, this is of minor importance. If 1 ml. is used, it is desirable to run a test on glucose each time the test is made and to regard pink colors weaker than that obtained with the glucose as "negative" as regards the presence of simple aldoses.

Reactions of Carbohydrates and Glucosides

The results in Table I show that simple aldoses give a distinct positive reaction (pink color) with the reagent. Aldopentoses give a stronger color than aldohexoses. Glucose gives the weakest reaction of any of the simple aldoses tested. The intensities of the pink colors developed are approximately proportional to the concentrations of free aldehyde groups in solutions of the simple aldoses determined polarographically by reduction at a dropping mercury electrode by Cantor and Peniston (*1*). Both the polarographic determinations and the tests with Schiff's reagent indicate that the concentration of free aldehyde is considerably lower in glucose solutions than in equivalent solutions of other common simple aldoses. On this basis, a test with a pure unknown giving a color weaker than that obtained with glucose should be regarded as a negative test for simple aldose.

The weak "false" positive reactions may be variously interpreted. Possibly in the case of certain aldose disaccharides (maltose, melibiose, cellobiose, and lactose) the reactions are due to very low concentrations of the disaccharides in the free-aldehyde condition. They might also be due to the presence of traces of simple aldoses, either primarily present as a slight impurity in the compounds, or produced secondarily by hydrolysis of the compounds with the sulfurous acid of the reagent. Of the sixteen compounds tested (other than simple aldoses) only maltose gave a strong enough false positive to be misleading. It is hoped that there will be opportunity at a later date to study the compounds which react weakly and possibly to develop a reagent which will react to even lower concentrations of free aldehyde than the present one.

Except for the *i*-inositol monohydrate which was manufactured by Pfanstiehl, all the compounds tested were prepared by the Difco Laboratories, Inc., for use in the differentiation of various microorganisms by fermentation reactions, and therefore were of high commercial purity.

TABLE I. INTENSITY OF PINK COLOR PRODUCED BY SUPERSENSITIVE SCHIFF'S ALDEHYDE REAGENT

(After one hour at room temperature, 25° to 30° C.)

Compound	Chemical Type	Intensity of Pink Color: 1 ml. of reagent	2 ml. of reagent
l-Arabinose	Aldopentose	Intense	Very strong
l-Xylose	Aldopentose	Intense	Strong
Rhamnose	Methylaldopentose	Very strong	Light
d-Mannose	Aldohexose	Very strong	Light
d-Galactose	Aldohexose	Strong[a]	Light
d-Glucose	Aldohexose	Light	Faint
Maltose	Disaccharide	Faint	Faint
Melibiose	Disaccharide	Faint[a]	Very faint
Cellobiose	Disaccharide	Faint[a]	Very faint[a]
Lactose	Disaccharide	Faint[a]	Very faint[a]
Melezitose	Trisaccharide	Faint	Very faint
d-Sorbitol	Polyalcohol	Very faint	Colorless
Trehalose	Disaccharide	Very faint	Colorless
Salicin	Glucoside	Very faint[a]	Colorless[a]
Dulcitol	Polyalcohol	Very faint[a]	Colorless[a]
Levulose	Ketohexose	Colorless	Colorless
Sucrose	Disaccharide	Colorless	Colorless
Raffinose	Trisaccharide	Colorless[a]	Colorless[a]
Inulin	Polysaccharide	Colorless[a]	Colorless[a]
Esculin	Glucoside	Colorless[a]	Colorless[a]
d-Mannitol	Polyalcohol	Colorless[a]	Colorless
i-Inositol hydrate	Polyalcohol	Colorless[a]	Colorless[a]

[a] Compound wholly or partly insoluble in the amount of reagent used.

Table II. Relative Intensity of Pink Color Obtained with Basic Fuchsin and Carbon

(Reaction with 0.2 gram of Digestive Ferments Co. glucose dissolved in 1 ml. of reagent at a room temperature of 24° C.)

Reagent No.	Fuchsin	Grams of SO_2	Carbon	Intensity of Pink
1	National Aniline and Chemical Co., 89% dye content, certification No. NF 31	1.3	Norit A	Moderate
2		1.3	Nuchar	Palest
3		1.0	Norit A	Moderate
4		1.0	Nuchar	Moderate
5		1.0	Eimer & Amend	Moderate
6		1.0	Darco S-51	Moderate
7	National Aniline and Chemical Co., 94% dye content, certification No. NF 29	1.0	Norit A	Moderate
8		1.0	Nuchar	Moderate
9	Merck's medicinal, control No. 30,287	3.4	Norit A	Light
10		3.4	Nuchar	Light
11		1.0	Norit A	Strong
12		1.0	Nuchar	Strongest

Reproducibility of Results

In order to demonstrate the reproducibility of the reagent, twelve different lots were prepared, employing different fuchsins and different decolorizing carbons. The results of tests upon pure glucose (Difco Laboratories, Inc., dextrose, Lot No. 316,282), given in Table II, indicate that various combinations of carbon and fuchsin can be used successfully with but minor variations in the amount of color produced. They also indicate that an increase in the amount of sulfur dioxide tends to reduce the sensitivity of the reagent, although the carbon used may be a secondary factor in modifying the sensitivity.

The Norit A was marketed by the Pfanstiehl Chemical Co. The Nuchar was sold by the Eastman Kodak Co., reagent No. 776. The Eimer and Amend carbon was an 8- to 14-mesh activated coconut charcoal. It was used in an attempt to obtain more rapid filtration and reduce the time of exposure to the air, but the rapidity of filtration was not increased, and the amount of carbon had to be raised to 7 grams to obtain a completely colorless reagent.

Both samples of National Aniline and Chemical Co. basic fuchsin had been certified by the Commission on Standardization of Biological Stains, but nevertheless their solutions showed a considerable brown color after treatment with sulfur dioxide. A fuchsin which would not show this discoloration when reduced to the leuco condition in solution would permit the preparation of a very sensitive Schiff's reagent without the necessity for carbon treatment.

Many other varieties of basic fuchsin and carbon can undoubtedly be employed, although it may be necessary to make slight changes in the amount of sulfur dioxide and carbon. Commercial fuchsins not only vary somewhat in the chemical composition of the dye, but also vary from about 80 per cent to 95 per cent in true dye content as put on the market. Thereafter they frequently slowly deteriorate with age, with the formation of a brownish water-soluble material and a black water-insoluble material (*9*). Therefore each new lot of the reagent should be carefully standardized against glucose and sucrose. It is also a good plan to run a blank without any carbohydrate present. Any development of color in the blank, particularly a deeper pink in the topmost layer than in the deeper portions of the mixture, indicates that the sulfur dioxide content of the reagent is too low and that atmospheric oxygen or possibly evaporation is restoring the color of the fuchsin.

When properly prepared, the reagent is extremely stable. The results in Table I were obtained with a reagent prepared from the National Aniline and Chemical Co., basic fuchsin certification No. NF 29, 94 per cent total dye content, and decolorized with Norit A iron-free decolorizing carbon (Pfanstiehl). It had been standing in a nearly full, colorless, glass-stoppered bottle for 13 months, exposed to diffuse daylight and laboratory temperatures of 20° to 35° C.

Discussion

Schiff's aldehyde reagent has seldom been used in testing carbohydrates for aldehydic groupings, although it has occasionally been employed to determine the presence of oxycellulose in cellulose (*3*, *4*). Some standard textbooks even state that the reagent does not react with glucose or other simple aldoses (*6*, *7*, *10*). This is probably due to the fact that as ordinarily prepared the reagent is relatively insensitive. The more sensitive reagent here described should be useful in routine qualitative testing of carbohydrate unknowns in laboratory instruction, as well as in research. It may also be used in a striking lecture demonstration of the presence of free aldehydic groups in solutions of aldoses, by applying the test to glucose on a large scale, with sucrose as a control.

No originality is claimed for the use of carbon as a decolorizing agent for reagents containing leuco basic fuchsin. It has been so used by other workers (*2*, *5*), but seems not to have attracted general attention.

In its present form, the test is satisfactory for simple aldoses, maltose being the only other common carbohydrate giving a strong enough pink color to interfere. The author hopes later to develop the method further to permit the detection of the very small amounts of free aldehyde in solutions of substituted aldoses in a more definite fashion. Some of the compounds recorded as giving a negative (colorless) reaction or a faint false positive test, in Table I, showed an appreciable pink color in the still undissolved solid shortly after addition of the reagent but lost this color when all the solid had dissolved.

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Stable Sodium Thiosulfate and Starch Solutions

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A 0.1 *N* sodium thiosulfate solution that will retain its original titer approximately 5 months is readily prepared by combining 0.05 per cent sodium hydroxide and 0.1 per cent sodium benzoate as preservatives.

A stable starch solution, essentially the preparation of the Association of Official Agricultural Chemists, modified by the addition of sodium benzoate, has the composition: 0.6 per cent soluble starch, 0.1 per cent sodium hydroxide, 0.3 per cent potassium iodide, and 0.1 per cent sodium benzoate. In an iodometric titration 1 cc. of this solution is sensitive in a volume of 200 cc.

Analytical Classes of Cannabinol Compounds in Marihuana Resin

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IN 1896 Wood, Spivey, and Easterfield isolated from cannabis resin a toxic "red oil", seemingly a definite chemical compound, which they named "cannabinol" (*24*). In 1899 they isolated a pure compound from the red oil (as the crystalline acetate) and transferred to it this name, cannabinol (*25*). Their own work, and that of Cahn in 1931 (*11*) showed that the "crude cannabinol" was not just an impure form of pure cannabinol, but rather a mixture of closely related compounds. Other authors, however, especially before 1931, continued to refer to the complex of related compounds as cannabinol (*11*). Blatt, in reviewing the literature in 1938, used "crude cannabinol" intentionally as a collective name for these compounds (*9*).

In this paper all the compounds of the resin, whether they go into the distilled red oil or not, which are closely related to cannabinol, are referred to collectively as "cannabinol compounds".

In 1940 Adams, Hunt, and Clark reported the isolation of another of these related compounds, naming it "cannabidiol" (*2*). Jacob and Todd have isolated still another, calling it "cannabol" (*17*). Pure cannabinol and cannabidiol are stated not to have the specific narcotic effect of marihuana, but Adams and his co-workers have shown that cannabidiol isomerizes to tetrahydrocannabinol (which dehydrogenation converts to cannabinol), and that this compound, and also hexahydrocannabinol, do have "marihuana activity" (*3, 4, 5*).

Extractive Separations

All the work mentioned above was done on the distilled product. In 1913 a German patent issued to F. Hoffmann–La Roche & Co. gave the division of the resin into parts, distinguished from each other in their poisonous and narcotic properties, by means of an organic solvent immiscible with water and aqueous alkali (*15*). Casparis recognized the division of hashish resin into two parts, obtained by shaking the petroleum ether extract with aqueous sodium carbonate solution, but considered that the sodium carbonate solution removed "acid, inert substances", which constituted "up to 50 per cent" of the resin (*13*). Nickolls, on the other hand (though basing his remarks only on the acid Beam test), writes as if the aqueous alkali should be regarded as containing the active principle (*22*). Cahn tried the separation, but his material contained very little of the alkali-soluble compounds (5 per cent or less) and he used the process only as a preliminary to distillation (*11*).

As a matter of fact both parts contain cannabinol compounds, and the separation is of paramount value. However, except for the water-soluble quebrachitol (*6*, which does not require alkali for its extraction) the substances that have been identified in the red oil would all be in the petroleum ether. The colorless cannabinol compounds soluble in aqueous alkali do not distill as such, for they are readily altered by heat to alkali-insolubility. Hence, the extractive separation has little value as a mere preliminary to distillation; and distillation has little or no value for the study of the alkali-soluble cannabinol compounds.

Myttenaere has done considerable work on extractive separations (*19, 20, 21*). Possibly this has been neglected because he calls that part of the petroleum ether extract insoluble in aqueous alkali "the alcohol", as distinguishing it from the alkali-soluble phenols; and yet the known compounds cannabinol and cannabidiol, which have had their formulas established as phenols, both have the property of insolubility in aqueous alkali. All the cannabinol compounds are phenolic; and if some of them are also alcohols they are the ones soluble in aqueous alkali. Nevertheless Myttenaere's work has great value. He separates the alkali-soluble part primarily into two parts, calling them "phenol I" and "phenol II" (*19*), and gives some information about a "phenol III", apparently an oxidation product of "phenol II" (*20, 21*). Further reference is made below to the Myttenaere separation and nomenclature.

Classification of Colorless Cannabinol Compounds

Solutions of fairly fresh resin in ethyl or isopropyl alcohol, or ethyl acetate, are readily decolorized completely by activated charcoal (*23*), and then still contain the greater part of the cannabinol compounds originally present. The writer here presents a classification of the colorless cannabinol compounds into four distinct kinds. There are also yellow or brown colored cannabinol compounds naturally present in the resin, especially when it is old. These are probably oxidation and perhaps decomposition products of the colorless compounds.

The compounds in question give similar reactions in a number of tests, which in this paper are called "cannabinol reactions". These include the Ghamrawy test (with *p*-dimethylaminobenzaldehyde in sulfuric acid, *16*), the Duquenois and Moustapha test (with vanillin, acetaldehyde, and hydrochloric acid, *14*), the mercuric sulfate test (as modified by the writer, originally suggested by Duquenois and Moustapha, *14*), the writer's diazo tests, and others. Phenols commonly react in such tests as these.

The alkaline Beam test (*7*) is of a different kind from those just mentioned. Some cannabinol compounds give this test and some do not. Cannabidiol is the known compound yielding it (*2*); cannabinol and cannabol do not give it (*17*), nor do the physiologically active tetrahydro- and hexahydrocannabinols (*5*).

Cannabidiol constitutes at least 45 to 50 per cent of the purified red oil from Minnesota wild hemp (*6*). In the writer's experience, individual wild hemp plants invariably give the alkaline Beam test, even as tiny seedlings. Moreover, even with separation of the resin into different parts, the depth of the purple color in a suitable form of this test (such as potassium hydroxide in isopropyl alcohol) usually still correlates closely to the depth of color obtained in a diazo test or other cannabinol reaction on the same portion. Adams, Pease, and Clark isolated some cannabinol from Minnesota resin, but it is certainly a very minor constituent in our wild hemp. The cannabinol compounds present must nearly all be such as give the alkaline Beam test.

On the other hand it is easily demonstrated that in the resin from hemp of Manchurian origin (which is occasionally grown in this district) most of the cannabinol material consists of compounds not yielding the alkaline Beam test. With some samples of marihuana, or some plants of Manchurian hemp, the test has been completely negative, even though the cannabinol reactions were very strong. In most plants of the variety, however, both kinds of cannabinol compounds are present; the alkaline Beam, or at least the related and more

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sensitive Bouquet test (*10*), is positive, but, by comparison with the cannabinol reactions very weak. The resin of our Manchurian hemp is probably of similar average composition to that from which cannabinol was first isolated, and to that of the Egyptian *Cannabis indica* from which Jacob and Todd isolated cannabinol, cannabidiol, and cannabol (*17*).

There are at least two distinct "chemical varieties" of hemp, though neither is a pure race in producing only one type of cannabinol compound. Their differences do not depend upon climate, for both grow in Minnesota. Both the Manchurian and the wild hemps are used by marihuana addicts for smoking.

The two kinds of cannabinol compounds, as differentiated by the alkaline Beam test, in the alkali-soluble part of the resin can be separated more or less successfully by Myttenaere's method. However, the writer did not originally depend on this, but on wild hemp for the reactive compounds, and on selected samples or plants of Manchurian hemp for resin containing the nonreactive compounds virtually free of the reactive.

When a petroleum ether solution of the resin from either type of plant is shaken with aqueous alkali or sodium carbonate solution, it is separated into two parts. By extracting with several portions of alkali, which are in turn shaken with a little fresh petroleum ether, the separation is made complete. The part extracted by alkali consists almost entirely (as far as the writer can determine) of cannabinol compounds, even though no such compound has yet been definitely isolated as a chemical individual and named. The petroleum ether retains cannabinol compounds insoluble in alkali (which includes those that have been isolated from red oil), and also the essential oil, the paraffin hydrocarbon, and (if an undecolorized extract is used) nearly all of the chlorophyll.

Designating the cannabinol compounds soluble in aqueous alkali as I, and those remaining in the petroleum ether as II, this analytical distinction by solubilities is independent of the distinction between the classes of cannabinol compounds reactive (RAB) and nonreactive (NRAB) in the alkaline Beam test. Thus there are four kinds of colorless cannabinol compounds: I-RAB, I-NRAB, II-RAB, and II-NRAB. The II-RAB material reacts more quickly and readily than I-RAB in the alkaline Beam test, but by using the test of potassium hydroxide in isopropyl alcohol just as strong a purple is obtained, more gradually, from a similar amount of I-RAB.

A color test which distinguishes I from II in the cannabinol compounds is made with ferric chloride in methyl alcohol. The colors obtained with I compounds—green, blue, red—are suggestive of *o*-diphenols; but the writer has recently found that the *m*-diphenol resorcinol can be made to give a similar test, though less sensitive and with the red color (on addition of ammonium acetate) much more transitory. The II cannabinol compounds give negligible reactions (if any).

The fourfold classification is doubly confirmed. The distinction between I and II compounds by solubilities is confirmed by a color test; and the distinction by the Beam test between reactive and nonreactive compounds is not merely analytical, but relates to the type of cannabinol compounds predominating in the resin of distinct races of hemp.

Change of Compounds from Solubility to Insolubility in Alkali

The writer discovered that the cannabinol compounds soluble in aqueous alkali change readily to insolubility, if heated or even on long standing at room temperature.

The best laboratory conversion found is by refluxing in absolute methyl alcohol for 12 hours or so. A small quantity of colorless compounds soluble in aqueous alkali, say 0.1 gram, is mostly converted to alkali-insoluble by this procedure, with scarcely any development of color. The ferric chloride test is destroyed, but the cannabinol reactions remain almost unchanged. Laboratory manipulations such as concentration of an extract, or evaporation of a solvent and heating of the residue on the steam bath, always convert some of the I compounds to II.

Purified red oil is distilled at 175° to 190° under 2-mm. pressure (*2*). Even without the reduced pressure, which probably assists the change, conversion takes place gradually at much lower temperatures. The writer has not personally distilled red oil, but there is little prospect of finding any of the alkali-soluble cannabinol compounds in it.

Johns of Iowa State College studied marihuana extracts briefly and on a small scale, at the same time as the writer's first conversion experiments, and he also discovered the change. He separated decolorized resin into two parts with petroleum ether and aqueous alkali, and after acidifying reextracted the latter part with petroleum ether. Thus two residues in similar condition were obtained by evaporating the petroleum ether. The alkali-insoluble compounds distilled readily under a good vacuum, but the alkali-soluble compounds gave off a gas, probably water, making it difficult to maintain the vacuum, and then distilled as alkali-insoluble (*18*).

In fresh or recently air-dried marihuana itself the compounds soluble in aqueous alkali predominate, but after it is several years old the alkali-insoluble compounds predominate. Obviously some, at least, of the alkali-insoluble compounds are not produced as such by the plants, but are formed indirectly from the alkali-soluble compounds. In the writer's opinion all the cannabinol compounds insoluble in alkali have this origin. The essential oil (terpene and sesquiterpene) and paraffin hydrocarbon (chiefly *n*-nonacosane) are, of course, insoluble in aqueous alkali from the start.

Simply by extracting fresh marihuana with alcohol and treating with activated charcoal the writer has obtained decolorized resin, 90 per cent of which was soluble in aqueous alkali, and a substantial part of the remaining 10 per cent was paraffin hydrocarbon. Color tests have indicated only traces of the alkali-insoluble cannabinol compounds in decolorized resin from fresh young plants.

However, the statement that the alkali-soluble compounds predominate in fresh resin relates only to the cannabinol compounds in the two parts. In the whole, undecolorized resin the amount of alkali-soluble compounds may not be greater than the total of alkali-insoluble compounds of all kinds. Activated charcoal does remove relatively more alkali-insoluble material, but it is chiefly *n*-nonacosane, chlorophyll, etc.

In the past the importance and amount of the alkali-soluble compounds have been greatly undervalued, for the following reasons:

1. It has not been realized that the alkali-soluble compounds are cannabinol compounds, closely related to the most important known compounds of the insoluble part.
2. It has not been realized that alkali-soluble material may become insoluble during laboratory manipulations and does slowly become insoluble as the marihuana ages.
3. Paraffin hydrocarbon constitutes a fairly large part, in fresh resin even the greater part, of the alkali-insoluble material; whereas the alkali-soluble part consists of cannabinol compounds comparatively free from unrelated compounds.
4. Petroleum ether has generally been used to extract the resin from the crude drug; but some of the alkali-soluble compounds are already combined with alkali in the plant itself, and so are not extracted directly by petroleum ether, without acidification.

Despite the last, and possibly the second, of the foregoing factors, Casparis noted that up to 50 per cent of the resin was soluble in sodium carbonate solution, and Myttenaere gives an analysis of *charas* which was 65 per cent soluble in aqueous alkali (*20*).

The writer has tried to determine the relative proportion of the alkali-soluble and -insoluble cannabinol compounds in the whole resin of fully mature plants, using the diazo color reaction. The alkali-insoluble cannabinol compounds were separated from most of the paraffin hydrocarbon with 90 per cent methyl alcohol, in which the paraffin is but slightly soluble. Colored compounds made the determination somewhat inaccurate, but as well as could be determined, in resin from freshly cut plants late in the fall the alkali-soluble compounds account for close to 90 per cent of the total cannabinol compounds present.

The resin mostly coats the outside of the upper leaves, twigs, and flowers, so that this much alkali-insoluble may easily be formed from alkali-soluble out in the field, under the heat of the summer sun. The available facts indicate that most, and probably all, of the alkali-insoluble cannabinol compounds are formed only indirectly; that the compounds, soluble in alkali and not found in the distilled red oil, are the cannabinol compounds actually formed by the plants.

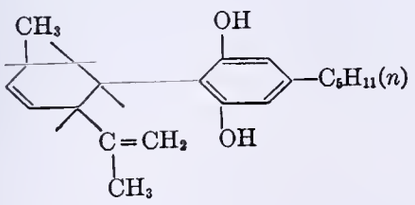
Unless there is some further change on distillation, the extracted II-NRAB must consist largely of cannabinol, and II-RAB mostly of cannabidiol. Then I-NRAB and I-RAB must be largely or even entirely the precursors of cannabinol and cannabidiol, respectively. It is certain that the hemp plants form at least two chemically distinct cannabinol compounds, but whether or not more than two are formed initially has not yet been determined. I-NRAB and I-RAB may each be fundamentally a single compound, with a small part (especially of I-NRAB) combined as a glucoside. (Such a glucoside probably accounts for the original acid Beam test.) However, remembering that the cannabinol compounds of red oil behave in most ways like a single compound and were at first mistaken for a chemical individual, it is possible that both I-NRAB and I-RAB are similarly complex.

Probable Nature of Precursors of Cannabinol and Cannabidiol

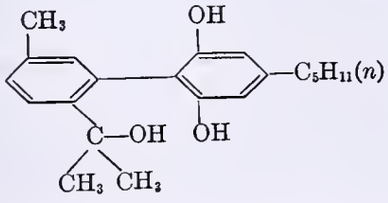
In 1933 Cahn discussed the possibility, suggested by his experiments on synthetic compounds, that a phenolic alcohol may be precursor to cannabinol (*12*). He had observed no formation of water during the distillation of the high-boiling fractions, and concluded therefrom that cannabinol is not formed during this process but had existed in his crude resin. His conclusion was probably correct, but does not follow as to fresh resin. His resin was almost entirely insoluble in alkali (*11*). The change discussed here had already taken place.

Cannabidiol was first formulated as an *o*-diphenol (*2*), but the formula now recognized, (A), has the two phenol groups meta to one another, as in resorcinol (*3*). It is therefore considered much more likely that the precursors of cannabinol and cannabidiol are at the time alcohols and *m*-diphenols, rather than *o*-diphenols. If this is the case, the formula for the precursor of cannabinol is (B). The established formula for cannabinol itself is (C) (*1*).

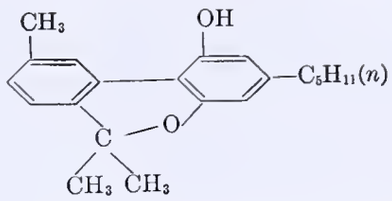
(A)



(B)

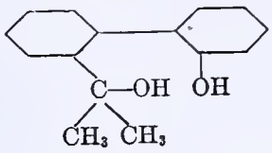


(C)



This accords with Johns' observation on the distillation of compounds soluble in aqueous alkali, and also with Cahn's observation that synthetic phenol-alcohols of type D are partly dehydrated by distillation at 30 mm. (*12*).

(D)



The only point not explained is that cannabidiol, which is certainly a *m*-diphenol, does not give the ferric chloride test. However, this test is linked in some way to solubility in aqueous alkali, and the alkali-insoluble compounds do not have this solubility even though phenol groups are certainly present.

Separations

The resin is best extracted from the marihuana with ethyl or isopropyl alcohol. Unless a quantitative study is desired, it is best to remove the chlorophyll with activated charcoal. With fresh marihuana a colorless solution can thus be obtained. However, in the case of old marihuana the resin itself has turned brown, and the solution cannot be decolorized without the loss of nearly all the resin. Instead of attempting complete decolorization, just enough charcoal should be used to remove the chlorophyll, and the procedure continued with brown resin when necessary.

Dilute the alcoholic extract with two or three times as much water, acidify with hydrochloric acid, and extract with petroleum ether. The concentration of resin in solution can be increased at this step. This is better than trying to obtain a highly concentrated alcoholic extract from the crude drug.

Most of the resin, whether colorless or brown, readily dissolves in the petroleum ether; but a small amount of yellow-brown compounds may not be extracted. Myttenaere distinguished as "phenol III" a small part of the resin relatively insoluble in petroleum ether, but readily soluble in aqueous alkali (*20*). It is probably some of this same material which is more soluble in diluted alcohol than in petroleum ether. Chloroform extracts it readily. In Myttenaere's experiments (*21*) it seemed to be an oxidation product of his "phenol II" here called I-RAB.

Extract the petroleum ether solution with aqueous alkali, using a generous volume of alkali solution, and four or five shake-outs. This easily separates I compounds from II. The writer uses a 2 per cent sodium hydroxide solution, recently boiled to remove oxygen. Sodium sulfite (2 per cent) is also helpful to prevent oxidation. Without these precautions there is likely to be considerable production of the alkaline Beam purple color during the extraction. This color results from a mild oxidation. The purple compound is soluble in aqueous alkali and insoluble in petroleum ether (until acidified), even when formed from II-RAB. Some of the yellow-brown compounds are probably similar oxidation products of the cannabinol compounds.

With old brown resin some material collects at the interface, insoluble in both layers and dark brown in color: compounds that combine with the alkali, but do not thereby become sufficiently soluble in water. This difficulty does not occur with fresh resin.

Remaining in the petroleum ether along with the alkali-insoluble cannabinol compounds are the essential oil and par-

affin hydrocarbons. The cannabinol compounds can be separated, with some difficulty, from the others by extracting with 75 per cent ethyl alcohol, the hydrocarbons remaining in the petroleum ether. This alcoholic extract will contain both II-RAB and II-NRAB, if both were present in the resin. No way has yet been found of separating them by mere extraction.

The aqueous alkali solution contains I-RAB and I-NRAB. By shaking with ethyl ether the I-NRAB goes into the ether, the I-RAB remaining in the alkali. The ether used should be free of peroxides.

This last separation is due to Myttenaere (*19*). I-NRAB corresponds to his "phenol I" and I-RAB to his "phenol II". This is a valid separation but it is difficult to make it absolute. Ether extracts even the I-RAB from carbonate solution, but the separation is fairly good with 2 per cent sodium hydroxide. Possibly there are several different related compounds in both portions, and this may cause some of the difficulty.

Shake the ether extract with dilute hydrochloric acid to free the I-NRAB from its alkali combination. Filter to remove droplets of water and evaporate the solvent without much heating.

If selected marihuana was used, containing only RAB or only NRAB, the step of ether extraction can be omitted. If only NRAB compounds are present they can be extracted like the RAB compounds in the following step.

Acidify the aqueous layer and extract the I-RAB with low-boiling petroleum ether. Filter and evaporate the solvent without much heating. This portion, as well as the mixed II compounds, is, according to Dautrebande, physiologically active (*20*).

Some yellow colored material may remain unextracted by the petroleum ether. It is easily shaken out with chloroform, and is no doubt the same as Myttenaere's "phenol III".

Conversions

Isolates of II-RAB and II-NRAB are best obtained by conversion.

Dissolve I-RAB and I-NRAB separately in absolute methyl alcohol and reflux for about 12 to 20 hours over a small flame. Then dilute the methyl alcohol with water, acidify slightly, and extract with petroleum ether. Shake the petroleum ether solution with aqueous alkali to remove unconverted alkali-soluble compounds, as well as any unrelated compounds extracted by alkali and not converted to insolubility. Wash with a little acidified water, filter, and evaporate the solvent. The writer's experiments have mostly been on a small scale. Conversion of 0.1 to 0.2 gram in 50 cc. of methyl alcohol is almost complete.

By this means II-RAB and II-NRAB can be isolated free from extraneous matter. It seems probable that each is a complex of closely related compounds. This is a controlled conversion that just produces the change from alkali-solubility to alkali-insolubility. Possibly vacuum distillation produces further changes. Presumably, however, II-RAB is mostly cannabidiol and II-NRAB largely cannabinol.

The Color Reactions

Following are specific directions for the color tests as made by the writer, with mention of the results given by the separated classes of cannabinol compounds.

Ghamrawy Test (Modified). Evaporate the solvent from a little of the resin in a casserole. Remove from the steam bath and treat with 1 cc. of Wasicky's reagent, 3 grams of *p*-dimethylaminobenzaldehyde in 100 cc. of 87 per cent sulfuric acid (85 volumes of concentrated sulfuric acid to 15 volumes of water). A color more or less orange is produced, quickly becoming intense scarlet, then gradually intense purplish red. Let stand about 30 minutes. Dilution with water changes the color to pure deep blue, which gradually fades on standing. Dilution may be made with only 3 cc. of water, and the color extracted with 1 cc. or more of benzyl alcohol, or instead of water, 9 or 10 cc. of concentrated ammonium acetate solution (75 grams per 100 cc. of water) may be added to the sulfuric acid solution. The color is changed through blue to green. Shake with 1 to 1.5 cc. of amyl alcohol. The solvent becomes an intense deep green.

The Ghamrawy test is very sensitive. It succeeds with the brown cannabinol compounds in addition to the colorless, and shows little variation with the different classes of colorless compounds.

Diazo Test A. To 1 cc. of ethyl or isopropyl alcohol solution of the resin add 5 cc. of sulfanilic acid solution (1.5 grams of pure sulfanilic acid in 490 cc. of water and 10 cc. of concentrated hydrochloric acid). Note the amount of resin thrown out by the dilution (when the concentration is not otherwise known). Add a small pinch of granulated sodium nitrite and shake. Then add sodium bicarbonate in excess. An intense yellow color is at once produced, quickly becoming orange to orange-red when much cannabinol material is present. (The dye formed is capable of coloring strongly a large volume of solution.) Acidify strongly with (1 to 3) sulfuric acid, adding about 1 cc. in excess. The color becomes bright red to muddy reddish brown. Shake with 1 cc. (or more) of benzyl alcohol. This extracts all or nearly all the color, and becomes deep dark red.

In this test RAB compounds give upon acidification a bright red solution; NRAB compounds give reddish brown insoluble material. In the writer's opinion this is the best test known for tracing the distribution of cannabinol compounds in extractions, determining the completeness of extraction, etc.

Diazo Test B. To 0.7 cc. of stronger sulfanilic acid solution (7 grams of sulfanilic acid in 475 cc. of water and 25 cc. of concentrated hydrochloric acid), in a test tube, add a few grains of sodium nitrite, then an excess of sodium bicarbonate. Pour into this 3 or 4 cc. of an ethyl or isopropyl alcohol solution of the resin. An intense yellow color is instantly produced, which gradually becomes more or less orange. After some standing add 10 cc. of water and pour through a folded filter if full of sediment. The solution is orange. Shake with 1 cc. (or more) of amyl alcohol. Typically, the amyl alcohol becomes orange to bright red, leaving the aqueous layer bright yellow.

The diazo tests succeed with the brown cannabinol compounds, as well as with the colorless. Complete decolorization of the resin is therefore unnecessary, but it is best to remove chlorophyll from the solvent solution with activated charcoal.

Mercuric Sulfate Test. To 3 cc. of a methyl alcohol solution of the resin (decolorized or at least with chlorophyll removed) add 2 cc. of Denigès' reagent (5 grams of mercuric oxide in 20 cc. of sulfuric acid and 100 cc. of water). Either at once, or on standing a short time, both color and precipitation are produced. The color is salmon or pink to scarlet, brick red, or rose-red. The precipitation may begin as a whitish cloudiness, before any color develops, or may not develop until some time after the color. The precipitate formed is of similar color to that produced in the solution, and is wholly or partly crystalline.

In this test the RAB compounds precipitate more readily than the NRAB, and the II compounds develop more color than the I.

I-RAB	Most precipitation; amorphous material and branching thread crystals. Least color; salmon
II-RAB	Precipitation somewhat less but similar to I-RAB; color stronger, tending to scarlet
I-NRAB	Precipitate forms more slowly than with RAB compounds, wholly crystalline, plates. Color pink
II-NRAB	Precipitation as with I-NRAB. Most reactive of the four classes as to color; rose-red

Duquenois and Moustapha Test (Modified). To 1 cc. of alcoholic solution of the resin add 0.5 cc. of 8 per cent vanillin solution (in alcohol), and 0.5 cc. of 2 per cent aldehyde solution (2.8 per cent aldehyde-ammonia in alcohol); then mix with 3 cc. of concentrated hydrochloric acid. An intense purple or violet color soon develops; it may begin either as pink (passing through magenta-red to purple) or as green (passing through blue, or slate and indigo), depending on the kind of resin present. The distinction is more clear if the aldehyde is omitted (vanillin test). The aldehyde makes the test a little more sensitive and uniform. In interpreting a weak result it may be advisable to try a blank with the reagents.

Duquenois and Moustapha state green as the initial color in this test (*14*). This is true of the resin from Manchurian and related varieties, but that from Minnesota wild hemp yields pink as the initial color.

Vanillin Test. To 1.5 cc. of alcoholic solution of the resin (decolorized or at least with the chlorophyll removed) add 0.5 cc. of 8 per cent vanillin in alcohol, then mix in 2 cc. of concentrated hydrochloric acid. About 4 cc. of concentrated hydrochloric acid will develop the maximum color, which then tends to purple or violet, but the initial colors are most significant of the different cannabinol compounds and are best observed with no more than 2 cc. of hydrochloric acid.

The differences are extreme. Blackie states the color produced in his vanillin test as green or blue-green (*8*). Resin from Minnesota wild hemp, however, yields pink to magenta-red (reaction of RAB compounds). In the writer's tests decolorized resin from

fairly fresh Manchurian hemp yielded light green changing to blue, and these colors were stronger and greener with resin from old Manchurian hemp (brown resin). The green or blue with decolorized resin was found to be due to the II-NRAB portion. Yellow-brown cannabinol compounds also yield green. Results on the separated portions have not been entirely consistent, but the purest colorless isolates obtained by the writer (in small quantity) gave initial colors as follows:

I-RAB	Pink	II-RAB	Pinkish lavender
I-NRAB	Pink	II-NRAB	Greenish, then blue

Alkaline Beam Test. Evaporate the solvent from a little of the decolorized resin in a white dish, and treat the residue with a few drops of colorless 2 per cent potassium hydroxide in alcohol. RAB compounds yield a purple color. II-RAB responds much more promptly and readily than I-RAB.

Modified Bouquet Test. To 0.5 gram of marihuana add 0.1 gram of activated charcoal and 6 or 7 cc. of 2 per cent potassium hydroxide in ethyl alcohol. Let stand 5 or 10 minutes, then pour through a filter which will retain the charcoal. With RAB compounds the filtrate is violet. If it is very dark brown, add a little more charcoal and refilter. If light brown or nearly colorless, mix 4 or 5 cc. of filtrate with 1.5 cc. of amyl alcohol and 15 cc. of water in an 18-mm. test tube. The amyl alcohol extracts a purple color if any RAB compounds were present.

This procedure can be used with brown resin to determine if any of the colorless RAB compounds are present. In the writer's experience this is by far the most sensitive variation of the alkaline Beam test.

Peroxide Test. Dilute 2 or 3 cc. of isopropyl (or ethyl) alcohol solution of decolorized resin with 10 cc. of water; add 1 to 3 drops of 3 per cent hydrogen peroxide and 1 drop of 1 to 1000 copper solution (1 to 250 copper sulfate pentahydrate); mix, and add 0.5 cc. of 10 per cent aqueous sodium hydroxide. With RAB compounds a purple color rapidly develops. Amyl alcohol extracts the color.

This is also a variation of the alkaline Beam reaction.

Potassium Hydroxide in Isopropyl Alcohol. To about 3 cc. of isopropyl alcohol solution of decolorized resin add 2 small pellets of potassium hydroxide. The color is produced in other solvents, but isopropyl alcohol, 98 to 99 per cent grade, is much the best for this simple form of the test. With RAB compounds a purple color soon develops, gradually becoming deeper and stronger up to the limit of the reactive compounds present. II-RAB yields the purple color at once, and maximum intensity quickly. With I-RAB the purple develops rather slowly, but in time just as strongly as with the II-RAB. This is the best variation of the alkaline Beam test for obtaining the full reaction with I-RAB.

A yellow color may be produced by the I-NRAB portion. Perhaps this is due to a glucoside of the phenol, or even to some unrelated compound accompanying it throughout the extraction. The I-RAB portion also occasionally yields a yellow before the purple color develops.

Ferric Chloride Test. To 5 cc. of solution of the decolorized resin in absolute methyl alcohol add gradually not over 0.1 cc. at a time, a fresh 1 per cent solution of ferric chloride in absolute methyl alcohol. The reagent in excess produces a green to dark green color. (If sufficient reactive substance is present, a blue or even violet color is produced with the first reagent.) With ordinary marihuana extracts about 0.4 to 0.5 cc. of reagent is usually most satisfactory; seldom is more than 1 cc. required. (*a*) To the green solution (5 to 6 cc.) add 1 cc. of water, and mix. The color is changed to dark blue. (*b*) In another test, to the green solution (5 to 6 cc.) add 1 cc. of ammonium acetate solution (1 per cent in absolute methyl alcohol). The color changes to a beautiful deep purplish red, which soon fades by degrees, the solution finally becoming brownish yellow.

The positive test described is a reaction of the I cannabinol compounds only. Both I-RAB and I-NRAB respond, but the II compounds do not. With solvents other than methanol the reaction either does not occur, or (as seems to be the case in ethyl alcohol solution) the reactive compounds are immediately oxidized by the ferric chloride.

Summary

The term "cannabinol compounds" is used as a collective name for all the compounds of the hemp resin closely related to cannabinol and cannabidiol. These have a number of "cannabinol reactions" in common.

Hemp plants form two kinds of cannabinol compounds, colorless or virtually so; one kind reactive in the alkaline Beam test, the other not (designated as RAB and NRAB). The former always predominate in Minnesota wild hemp, the latter in hemp of Manchurian origin grown in Minnesota. Some plants produce exclusively only one kind or the other.

Some of the cannabinol compounds are extracted by aqueous alkali from petroleum ether solution; some cannot be so extracted. (These compounds are designated as I and II.) This classification is confirmed by a color test with ferric chloride in methyl alcohol, to which only the compounds soluble in aqueous alkali respond.

The two classifications, by the alkaline Beam test and by solubilities, are independent, so that there are four kinds of colorless cannabinol compounds.

The compounds soluble in aqueous alkali readily become changed by heat, or even by long standing at room temperature, to alkali-insoluble compounds. In the writer's opinion all the alkali-insoluble cannabinol compounds have this origin. By decolorizing an alcoholic extract of fresh marihuana from young plants with activated charcoal, resin is obtained with as high as 90 per cent solubility in aqueous alkali. The essential oil and paraffin hydrocarbons are insoluble in aqueous alkali from the start; but referring only to the cannabinol compounds in the whole undecolorized resin, compounds soluble in aqueous alkali greatly predominate in fresh resin, even late in the fall.

Table I. Distinguishing Reactions

Cannabinol Compounds	Alkaline Beam Test	In Aqueous Alkali	Ferric Chloride	Extraction[a]
I-RAB	Purple	Soluble	Positive	AA from PE and from EE
I-NRAB	O or yellow	Soluble	Positive	AA from PE, EE from AA
II-RAB	Purple	Insoluble	Negative	PE from AA
II-NRAB	O	Insoluble	Negative	PE from AA

[a] AA = aqueous alkali, EE = ethyl ether, PE = petroleum ether.

Extractive methods and avoidance of many laboratory manipulations are necessary for study of the alkali-soluble compounds. With distilled red oil the known cannabinol compounds that have been isolated are insoluble in aqueous alkali. Unless some additional change occurs with vacuum distillation, the extracted II-RAB must consist mostly of cannabidiol and II-NRAB largely of cannabinol.

The precursors of cannabinol and cannabidiol are very likely at the same time alcohols and *m*-diphenols.

General directions are given for separations. The alkali-soluble compounds are extracted from petroleum ether by aqueous alkali. I-NRAB can be separated from I-RAB by ethyl ether and aqueous alkali.

The laboratory conversion of alkali-soluble compounds to alkali-insoluble is effected by refluxing for some 12 to 20 hours in absolute methyl alcohol. A small quantity, about 0.1 to 0.2 gram, is almost all converted by this procedure. Isolates II-RAB and II-NRAB are best obtained by separately converting I-RAB and I-NRAB and re-extracting.

Directions are given for performing the color tests, and the effects described. The separated classes show some differences even in their cannabinol reactions, those in the mercuric sulfate and vanillin tests being especially noteworthy.

Table I gives the reactions distinguishing the four kinds of colorless cannabinol compounds.

Acknowledgment

The writer is indebted to Roger Adams and his associates in marihuana research at the University of Illinois for a small amount of pure crystalline cannabidiol. It was verified on this pure substance that its reactions are in all essentials like those described above for the II-RAB portion of the resin, including the responses in the cannabinol reactions, non-extraction by aqueous alkali when shaken with the petroleum

ether solution, and absence of any observable ferric chloride test.

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Amperometric Titrations

Amperometric Determination of Phosphate with Uranyl Acetate

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SINCE phosphate is not reducible at the dropping mercury electrode, its polarographic determination or amperometric titration has to be carried out indirectly. A reagent which forms a precipitate with phosphate and yields a well-defined diffusion current must be used for its determination with the dropping mercury electrode.

A polarographic determination of phosphate by precipitation with an excess of molybdate has been described by Uhl (*21*), and an amperometric titration by means of precipitation with bismuth oxyperchlorate was attempted by Neuberger (*16*). Both methods have the advantage that the precipitation is performed in strongly acid medium in which interference by coprecipitation of alkaline earth metals, etc., does not occur. The precipitation with molybdate has also the very favorable ratio of 1 to 12 between phosphate and molybdate. Both methods, however, involve several disadvantages. The molybdate wave is badly defined and is much affected by changes in the acidity and composition of the solution. The precipitation procedure is complicated and requires heating to boiling, standing overnight, and filtering. Chloride, if present, has to be removed. The titration with bismuth oxyperchlorate as described by Neuberger yields useful results only with relatively concentrated phosphate solutions (lower limit 0.035 *M* in phosphate). The end point does not correspond to the stoichiometric composition of the precipitate and is not easily located. Moreover, all anions except perchlorate must be absent because they form complexes with bismuthyl ions (*17*).

In this paper a simple amperometric titration of phosphate with uranyl acetate is described[1].

Attempts were made to titrate phosphate with lead solutions in weakly acid medium from which alkaline earth phosphates are not precipitated. Although quantitative precipitation could be obtained, the method had serious limitations and cannot be recommended. Uranyl acetate is a well-known reagent for the gravimetric (*7*, *14*) and volumetric (potentiometric) (*1*, *2*, *9*, *13*, *18*) determination of phosphate. According to Chrétien and Kraft (*3*) uranyl acetate forms with solutions of orthophosphates precipitates of the composition UO_2MPO_4 (M = Na, K, NH_4, 1/2 Ca) which are insoluble in acetic acid but freely soluble in mineral acids. Analogous compounds are formed with arsenate (*15*) and vanadate (*14*), which also have been recommended for analytical purposes.

[1] After this paper had been submitted for publication, an important paper on the polarographic determination of phosphate was published by Stern (*20*).

Polarographic Behavior of Uranyl Acetate

The reduction wave of uranyl ion at the dropping mercury electrode was found to be greatly affected by the composition of the medium (*11*). Since the phosphate titration had to be carried out in weakly acid solution, the effect of acetic acid and buffer solutions upon the current voltage curve of uranyl acetate was studied first. These measurements do not involve an exhaustive study of the polarography of uranyl solutions, which is being carried out at present in this laboratory. The experiments described in this paper were carried out with the purpose of finding the potential and concentration range at which uranyl ion gives well-defined diffusion currents in the most suitable titration medium.

Experimental

The manual apparatus, described in previous publications (*10*), was used. The temperature was 25° C., and the solutions were free from air. For the sake of convenience a pool of mercury was usually employed as the anode. Analytically pure chemicals were used. The stock solution was 0.1 *M* in uranyl acetate, and 0.1 to 0.18 *M* in acetic acid, the latter being necessary to prevent hydrolysis. In the literature different statements are found concerning the stability of uranyl acetate solutions. Courtois (*4*) found that both concentrated and dilute solutions were stable in the dark, but that hydrolysis occurred in the presence of light. Although according to Dworzak and Reich-Rohrwig (*6*) a precipitate soon appears in a 0.05 *M* solution, the uranyl-ion concentration is not appreciably diminished, even after several months. Singh and Ahmad (*19*) found uranyl acetate to be 2.13 per cent hydrolyzed in 0.00078 *M* solution at 30° C. In the present work it was found that the solutions were stable only in presence of some free acetic acid. The minimum concentration of acetic acid in 0.1 *M* uranyl acetate solution which yielded quick dissolution of the solid uranyl acetate was 0.1 *M*.

Current-Voltage Curves of Uranyl Acetate in Weakly Acid and in Buffered Solutions. In order to get constant current readings within a reasonably short time it

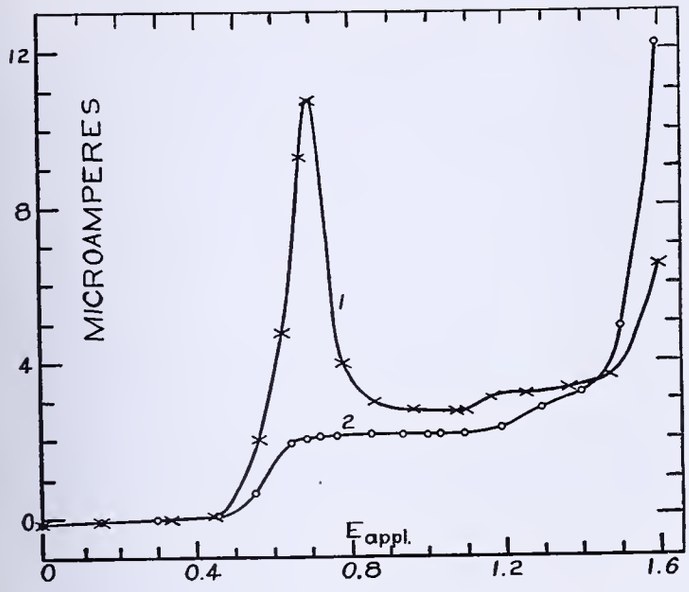

FIGURE 1. POLAROGRAMS OF 0.001 *M* URANYL ACETATE

1. In 0.05 *M* potassium nitrate, 0.04 *M* acetic acid, and 0.005 *M* ammonium acetate. 2. Same in 20% ethanol. Values not corrected for residual current.

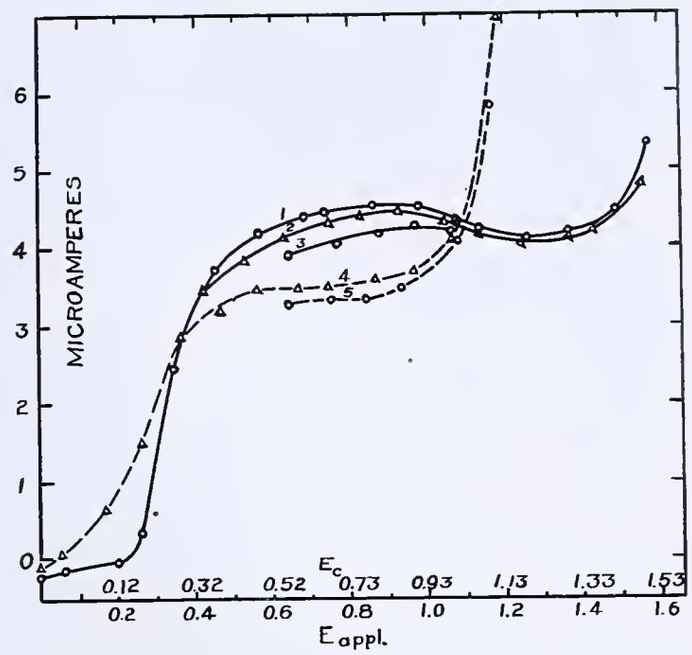

FIGURE 2. POLAROGRAMS OF 0.002 *M* URANYL ACETATE IN 0.002 *M* ACETIC ACID AND 20 PER CENT ETHANOL

1, 2, 3. In 0.1 *M* potassium chloride. 4, 5. In 0.1 *M* potassium chloride and 0.1 *M* potassium nitrate. Curve 1 measured 30 minutes after mixing; curve 2, 30 minutes later; curve 3, 2 hours after curve 2. Curve 4 measured 25 minutes after mixing; curve 5, 25 minutes later. Values not corrected for residual current. Cathode potential refers to saturated calomel electrode.

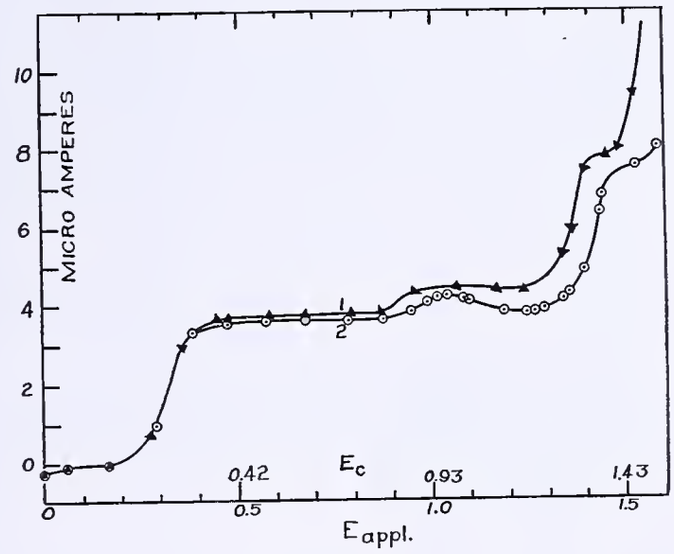

FIGURE 3. POLAROGRAMS OF 0.002 *M* URANYL ACETATE

1. In 0.042 *M* acetic acid, 0.1 *M* potassium chloride, and 20% ethanol. 2. In 0.022 *M* acetic acid, 0.1 *M* potassium chloride, and 20% ethanol. Values not corrected for residual current. Cathode potential refers to saturated calomel electrode.

was found desirable to carry out the titrations in a medium of 20 per cent ethanol. Therefore, most of the current-voltage curves of uranyl acetate were determined in this medium. The presence of alcohol also aids in suppressing or eliminating the maximum of the first uranyl wave, as is evident from Figure 1. The diffusion current of the first wave was constant over an applied voltage range between −0.8 and −1.1 volts.

Solutions which were 0.002 *M* in uranyl acetate, 0.002 *M* in acetic acid, and 0.1 *M* in potassium chloride and 20 per cent in ethanol were not stable. The diffusion current was found to decrease with increasing time of standing. The decrease was noticeable even before a precipitate was visible (Figure 2). This hydrolysis does not interfere in titrations because it takes place slowly, and moreover does not occur in the more strongly acid medium which usually was obtained after the precipitation of phosphate. The first wave of the uranyl ion, which is the only one obtained in the above solution, showed a slight flat maximum and a poorly defined diffusion current. At this uranium concentration the maximum was eliminated when the solution was also made 0.1 *M* in potassium nitrate. However, hydrolysis occurred more quickly in the presence of nitrate (Figure 2).

With increasing acetic acid concentration the wave in 20 per cent ethanol and 0.1 *M* potassium chloride became better defined. At higher acidity the first wave did not show a maximum and the diffusion current was constant between an applied e. m. f. of 0.6 and 0.85 volt. A rise in the current started at about −0.9 volt, the curve showing a peculiar maximum at −1.05 volts. The shape of the curve near the maximum was dependent on the amount of acetic acid present. At the higher acidity no hydrolysis occurred and the current-voltage curves were found unchanged after various periods of standing. Figure 3 shows the current-voltage curves obtained in 0.042 *M* and 0.022 *M* acetic acid. After the peculiar maximum the current became nearly constant, but a new wave started at about −1.25 volts. This wave was better defined in 0.042 *M* than in 0.022 *M* acetic acid. The total height at −1.45 volts was about twice the height of the first wave at −0.7 volt.

In the amperometric titration of phosphate we are mainly interested in the diffusion current of the first wave. In 0.042 *M* acetic acid and 0.1 *M* potassium chloride solution the diffusion current of the first wave was hardly affected by the addition of potassium nitrate or sodium sulfate.

In a medium which was 20 per cent in ethanol, 0.1 *M* in potassium chloride, and 0.02 to 0.05 *M* in acetic acid the first diffusion current was found proportional to the uranyl concentration up to concentrations of 0.0025 *M*. At higher concentrations the currents at various potentials were larger than expected. This, in part at least, is due to the occurrence of a maximum, as is evident from Figure 4. Addition of 0.01 to 0.2 per cent of gelatin suppressed the maximum (Figure 5), but the solutions apparently were not stable in the presence of gelatin. The diffusion current increased gradually on standing. Even if the measurements were made in the presence of

gelatin, the diffusion current increased more than in proportion to the concentration, when the uranyl concentration was greater than 0.0025 *M*. This is clearly brought out by Figure 6. On the left are given the diffusion currents at different concentrations of uranium, measured at an applied voltage of −0.8 volt in a medium which was 20 per cent in ethanol, 0.02 *M* in acetic acid, and 0.1 *M* in potassium chloride, upon successive additions of 0.1 *M* uranyl acetate solution in 0.1 *M* acetic acid. The right-hand side gives the same plot when the solution was also made 0.02 per cent in gelatin. The diffusion current in both cases is proportional to the uranyl concentration up to 0.0025 *M*. When the concentration becomes larger, the diffusion current becomes disproportionately greater. The addition of gelatin in an amperometric titration of phosphate, therefore, is of no advantage. In order to get a straight line after passing the end point the concentration of the excess uranyl acetate should not exceed 0.0025 *M*.

On the basis of this work it is concluded that in a medium of 20 per cent ethanol, 0.1 *M* potassium chloride, and 0.02 to 0.04 *M* acetic acid an applied e. m. f. of 0.7 to 0.8 volt is suitable for the titrations. If potassium nitrate is used in place of potassium chloride, an e. m. f. of 0.9 to 1.1 volts should be applied because of the change of the anode potential (pool of mercury).

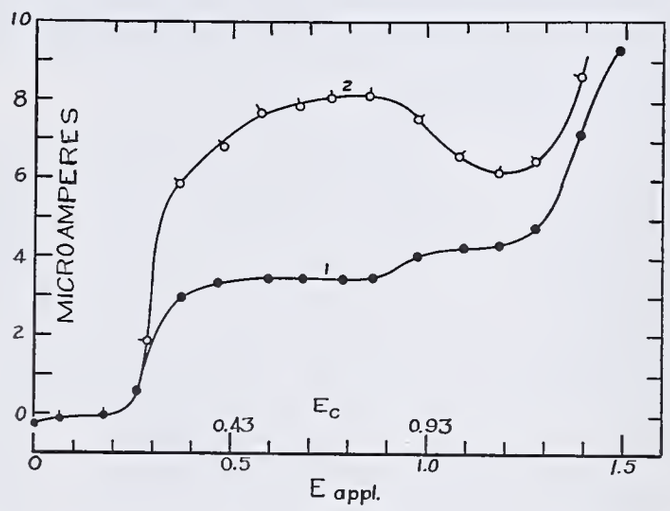

FIGURE 4. POLAROGRAMS OF 0.002 (CURVE 1) AND 0.00373 *M* (CURVE 2) URANYL ACETATE IN 0.1 *M* POTASSIUM CHLORIDE, 0.055 *M* ACETIC ACID, AND 20 PER CENT ETHANOL

Values not corrected for residual current. Cathode potential refers to saturated calomel electrode.

The titration of phosphate has also been investigated in buffers of acetic acid and acetate in a medium of 20 per cent ethanol. Therefore, current-voltage curves of uranyl acetate were also investigated in such buffer solutions. A solution of 0.002 *M* uranyl acetate in 0.2 *M* sodium acetate and 0.022 *M* acetic acid and 0.1 *M* potassium chloride, which had a pH of 5.6, gave a current-voltage curve shown in Figure 7. The curve is similar to the one obtained in 0.002 *M* acetic acid (see Figure 2) except that it was displaced 0.1 volt to more negative potentials. The solution in dilute acetic acid was not stable but on standing deposited a precipitate as a result of hydrolysis. In spite of the much higher pH, the uranyl solution in the buffer was stable and did not form a precipitate on standing. This is explained by complex formation between uranyl and acetate. Dittrich (*5*) found that in more concentrated solutions of sodium acetate the uranium was transported to the anode while in dilute solution it moved to the cathode. The complex formation also accounts for the fact that the wave in the buffer solution occurred at more negative potentials than in dilute acetic acid alone.

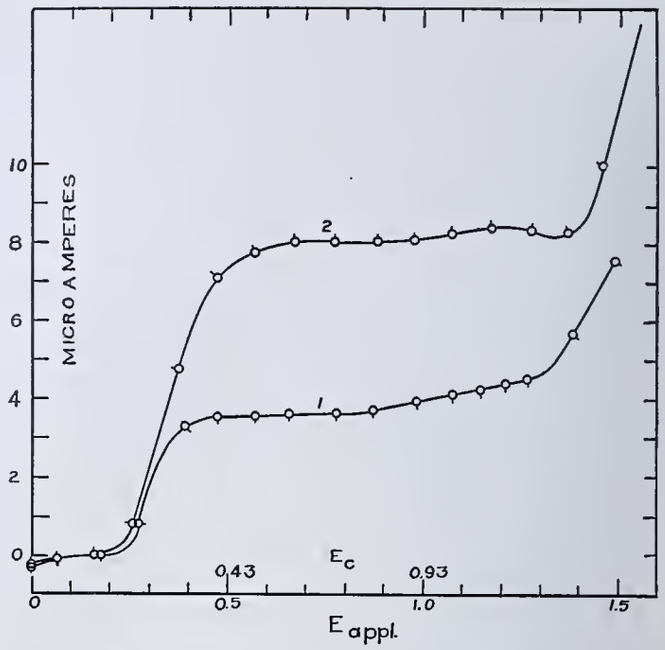

FIGURE 5. POLAROGRAMS OF 0.002 *M* (CURVE 1) AND 0.004 *M* (CURVE 2) URANYL ACETATE IN 0.1 *M* POTASSIUM CHLORIDE AND 20 PER CENT ETHANOL

1 in 0.01% gelatin, 2 in 0.02% gelatin. Values not corrected for residual current. Cathode potential refers to saturated calomel electrode.

Amperometric Titration of Phosphate

After many preliminary experiments, it was decided to carry out the titration in a medium containing 20 per cent ethanol. Alcohol decreases the solubility of the precipitate UO_2MPO_4, and before the end point it considerably reduces the time

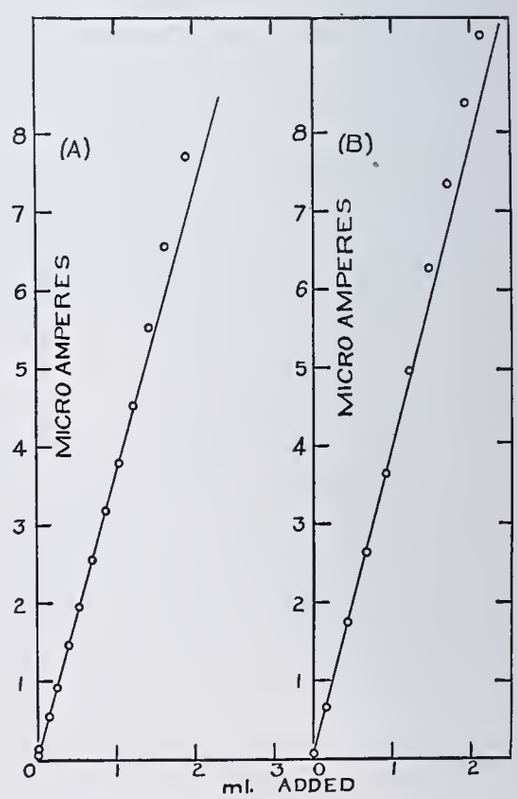

FIGURE 6. DIFFUSION CURRENTS OF INCREASING URANYL CONCENTRATIONS

Successive addition of 0.1 *M* uranyl acetate to 50 ml. of 0.1 *M* potassium chloride and 0.02 *M* acetic acid in 20% ethanol. (*a*) Without gelatin, $E_{appl.}$ 0.8 volt; (*b*) with 0.02% gelatin, $E_{appl.}$ 0.7 volt. Correction applied for dilution.

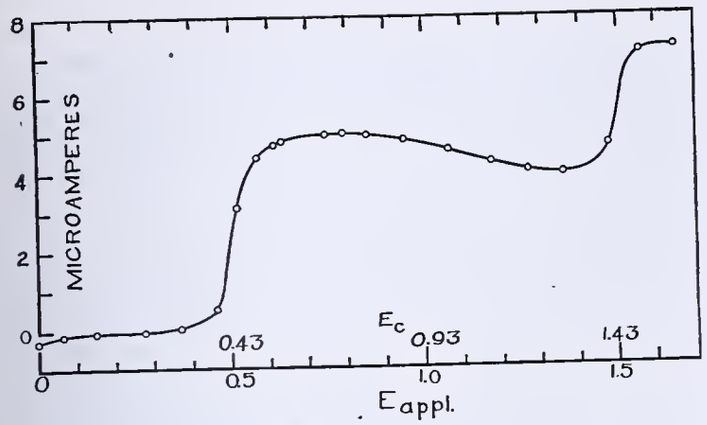

FIGURE 7. POLAROGRAM OF 0.002 *M* URANYL ACETATE IN ACETATE BUFFER pH 5.6 (0.2 *M* SODIUM ACETATE, 0.022 *M* ACETIC ACID), 0.1 *M* IN POTASSIUM CHLORIDE, AND 20 PER CENT IN ETHANOL

Values not corrected for residual current. Cathode potential refers to saturated calomel electrode.

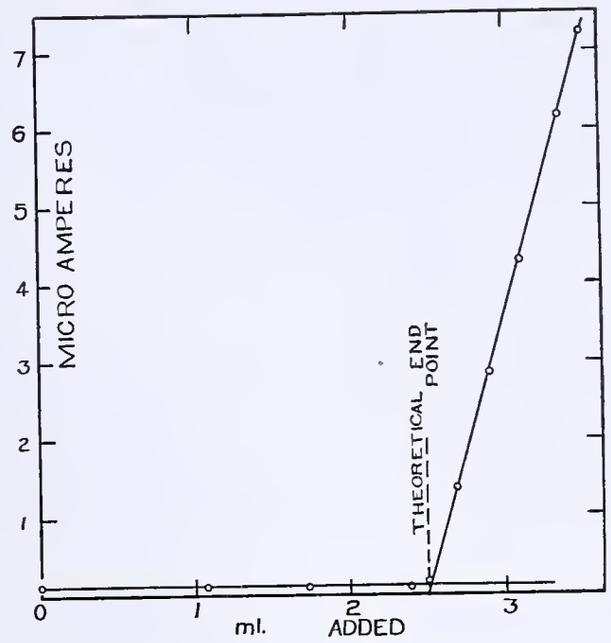

FIGURE 8. TITRATION OF 50 ML. OF 0.005 *M* PHOSPHATE SOLUTION IN 0.1 *M* POTASSIUM CHLORIDE AND 20 PER CENT ETHANOL WITH 0.1 *M* URANYL ACETATE

$E_{appl.}$ 0.8 volt. Correction applied for dilution.

necessary for attaining a constant reading of the current after each addition of uranyl acetate. In the following the authors usually titrated 50 ml. of a solution of monopotassium phosphate in 20 per cent ethanol under the conditions specified in the tables.

A layer of mercury was introduced into the cell to serve as an anode. With the pool of mercury as anode the applied e. m. f. was 0.7 to 0.8 volt when the solution was 0.05 to 0.2 *M* in chloride. If chloride was absent and potassium nitrate was used as the supporting electrolyte, the applied e. m. f. was 1.05 ± 0.05 volt. When an outside saturated calomel electrode is used as an anode, the potential applied to the dropping electrode should be −0.7 ± 0.05 volt. The titrations were carried out at room temperature. Nitrogen (or hydrogen) was passed through the solution until the oxygen had been removed. The uranyl acetate was added in successive portions from a microburet graded in 0.01 ml., nitrogen being passed through for 2 to 3 minutes after each addition. The current readings, after correcting for the dilution, were plotted against the volume of reagent added and the end point was found graphically (*12*).

Results obtained in the titration of monopotassium phosphate solutions are given in Table I. The 0.1 *M* uranyl acetate solution was 0.1 or 0.14 *M* in acetic acid; the more dilute solutions of uranyl acetate were obtained by diluting the 0.1 *M* solution with water; hence, the pH was relatively low during the titrations (3.5). The current values measured in a titration of 0.005 *M* phosphate in 0.1 *M* potassium chloride are plotted in Figure 8. It is seen that the residual current remains small and constant (of the order of 0.1 microampere) until the end point. Hence, it is only necessary to determine the current after two or three additions of reagent before the end point in order to find the practically horizontal precipitation line. Thus it is possible to finish the titrations within half an hour. When the phosphate concentration is greater than 0.004 *M*, the galvanometer can be used simply as a nullpoint instrument. Uranyl acetate is added with exclusion of air until the deflection of the galvanometer suddenly increases (Table I). The accuracy of the titrations is very satisfactory. Concentrations of phosphate greater than 0.0003 *M* can be determined with an accuracy of 1 per cent or better. When the concentration gets smaller, the accuracy decreases because these concentrations are near the lower limit of ordinary polarographic work. Titrations 11 and 12 in Table I show, however, that in 0.00008 *M* phosphate solution an accuracy of 5 per cent could be obtained with two titrations. The addition of ammonium salts, usually recommended in order to obtain well crystalline $UO_2NH_4PO_4$, had no influence upon the titrations.

TABLE I. TITRATION OF MONOPOTASSIUM PHOSPHATE WITH URANYL ACETATE

No.	Molarity of KH_2PO_4	Indifferent Electrolyte Salt	Indifferent Electrolyte Molarity	Concentration of Uranyl Acetate *M*	Uranyl Acetate Used Calculated *Ml.*	Uranyl Acetate Used Found *Ml.*	Error %	
1	0.005	KNO_3	0.2	0.1	2.50	2.48	−0.8	
2	0.005	KCl	0.2	0.1	2.50	2.48	−0.8	
3	0.005	KCl	0.1	0.1	2.50	2.51	+0.4	
4	0.005	KCl	0.1	0.1	2.50	2.495	−0.2	
5	0.005	KCl	0.1					
6	0.005	KNO_3	0.05	0.1	2.50	2.49	−0.4	
7	0.002086	KCl	0.1	0.05	2.086	2.075	−0.5	
8	0.0002	KCl	0.02	0.01	1.00	0.99	−1.0	
9	0.0001	KCl	0.02	0.01	0.50	0.47	−6.0	
10	0.00010093	KNO_3	0.02	0.005	1.009	1.01	0.0	
11	0.00007845	KNO_3	0.02	0.005	0.7845	0.84	+7.1	+4.6
12	0.00007845	KNO_3	0.02	0.005	0.7845	0.80	+2.0	
13	0.00005097	KNO_3	0.02	0.005	0.505	0.56	+11	

TABLE II. EFFECT OF ACETIC ACID CONCENTRATION UPON TITRATION

No.	Molarity of KH_2PO_4	Indifferent Electrolyte Salt	Indifferent Electrolyte Molarity	Molarity of Uranyl Acetate Solution Used $UO_2(C_2H_3O_2)_2$	Molarity of Uranyl Acetate Solution Used $C_2H_4O_2$	Molarity of Acetic Acid At beginning	Molarity of Acetic Acid At end point	Uranyl Acetate Used Calculated *Ml.*	Uranyl Acetate Used Found *Ml.*	Error %
1	0.005	KCl	0.1	0.1	0.18	0.002	0.02	2.50	2.48	−0.8
2	0.005	KCl	0.1	0.1	0.1	0.02	0.0333	2.50	2.46	−1.6
3	0.005	KCl	0.1	0.1	0.18	0.02	0.0371	2.50	2.44	−2.4
4	0.005	KCl	0.1	0.1	0.1	0.04	0.0524	2.50	2.45	−2.0
5	0.005	KCl	0.1							
		KNO_3	0.05	0.1	0.1	0.04	0.0524	2.50	2.44	−2.4
6	0.005	KCl	0.1	0.1	0.18	0.04	0.0562	2.50	2.43	−2.8
7	0.0001009	KNO_3	0.1	0.005	0.008 + 0.001 ammonium acetate	0.04	0.0401 + 0.00005 ammonium acetate	1.01	1.05	+4

The effect of varying amounts of acetic acid added to the phosphate solution was investigated next. According to the literature the precipitated uranyl phosphate, UO_2MPO_4, is not soluble in acetic acid. Nevertheless, the amount of acetic acid present during the titration has an influence on the result, as shown in Table II. In the course of a titration the concentration of acetic acid changes according to

$$KH_2PO_4 + UO_2(C_2H_3O_2)_2 + nC_2H_4O_2 = UO_2KPO_4 + (2 + n)\ C_2H_4O_2$$

In Table II, therefore, the initial concentrations of acetic acid and the concentrations at the end point are given. The table shows that under given conditions a concentration of acetic acid of 0.02 *M* at the end point is about the upper limit for obtaining satisfactory results. If 50 ml. of 0.005 *M* phosphate solution are titrated with 0.1 *M* uranyl acetate in 0.1 *M* acetic acid, the allowed initial concentration of acetic acid in the phosphate solution is 0.006 *M*.

Table III. Effect of Magnesium and Barium in 20 Per Cent Ethanol

(Total volume titrated, 50 ml. of 0.005 *M* KH_2PO_4 solution; concentration of uranyl acetate, 0.1 *M*)

No.	1 *M* $MeCl_2$ Solution Added	Molar Ratio Me:PO_4	1 *M* Acetic Acid Added before Titrating	Indifferent Electrolyte Salt	Indifferent Electrolyte Molarity	Uranyl Acetate Used Calculated	Uranyl Acetate Used Found	Error
	Ml.		*Ml.*			*Ml.*	*Ml.*	%
1	0.1 $MgCl_2$	1:2.5	..	KCl	0.1	2.50	2.49	−0.4
2	1 $MgCl_2$	4:1	..	KCl	0.1	2.50	2.49	−0.4
3	1 $BaCl_2$	4:1	2	KCl	0.1	2.50	2.48	−0.8
4	2 $BaCl_2$	8:1	1	KCl	0.1	2.50	2.48	−0.8

The titration was also performed in buffered solutions. In citrate and in oxalate solutions uranyl ion obviously forms complexes which delay the precipitation of phosphate and interfere with the titration. Correct results were obtained in acetate buffer of pH 4.7 but the precipitation was slow and therefore impracticable. At a pH between 5 and 6 the titration could be carried out in the usual way, but the precipitation remained slow near the end point. In a medium which was 0.2 *M* in sodium acetate and 0.02 to 0.06 *M* in acetic acid and 20 per cent in ethanol, good results were obtained when the phosphate concentration was 0.005 *M* or greater. In more dilute phosphate solutions the precipitation was too slow for practical purposes. In biphthalate buffers with a pH between 5.6 and 3.8 the precipitation was rapid. After the end point, however, the current did not increase linearly with the concentration of uranyl acetate. Still, the end point can be found satisfactorily when the galvanometer is used as a nullpoint instrument and the titration is carried out until the deflection suddenly increases.

Effect of Other Substances on Titration

Organic Acids. Because of the interference of organic anions like citrate, oxalate, much acetate, and probably many others (tartrate, formate, etc.) which form complexes with uranyl ion, it is recommended that organic matters be decomposed by ashing or by treating with sulfuric acid (and nitric acid) similar to the decomposition according to Kjehldahl.

Magnesium and Barium. Magnesium, when present in not too large amounts, did not interfere with the titration. Relatively large amounts of barium also can be present if the precipitation of barium phosphate in the presence of 20 per cent ethanol is prevented by addition of acetic acid (Table III). In experiment 3 with an initial concentration of 0.04 *M* acetic acid in the solution a correct result was obtained, while in corresponding experiments without barium an error of about −2.5 per cent was found (Table II).

Calcium. Calcium in larger concentrations caused low results due to coprecipitation of calcium phosphate with uranyl phosphate. However, when the concentration of calcium is smaller than 0.02 *M* and that of phosphate is 0.005 *M* or less, the error due to coprecipitation becomes negligibly small (Table IV). Therefore, in most practical cases calcium does not interfere.

A more serious error arises when both calcium and sulfate are present in appreciable concentrations. The solubility of calcium sulfate in 20 per cent ethanol is markedly less than in water. When the calcium sulfate precipitates upon addition of ethanol considerable amounts of phosphate are coprecipitated and low results are found (experiments 13 and 14 in Table IV). Addition of acetic acid decreased the coprecipitation only slightly. Even when the precipitation of calcium sulfate took place from about 0.04 to 0.08 *M* hydrochloric acid, there was a marked coprecipitation of phosphate, although it was less than when the precipitation took place in more weakly acid medium (experiments 17 to 24 in Table IV). Before the addition of alcohol, enough hydrochloric acid was added to make the final concentration of chloride in the titration mixture 0.04 to 0.08 *M*. After addition of the ethanol the solution was neutralized with sodium hydroxide, taking the green color of bromocresol green as the end point.

The error due to coprecipitation of phosphate with calcium sulfate depends upon the calcium, sulfate, and phosphate concentrations, and it is not possible to give minimum concentrations of the three separately at which no error occurs. In a mixture of 25 ml. of 0.01 *M* monopotassium phosphate, 1 ml. of 1 *M* calcium chloride, and 0.5 ml. of 1 *M* sodium sulfate diluted to 50 ml., the results were still correct. When 1 ml. instead of 0.5 ml. of sodium sulfate was added, the error was −1.4 per cent and with 2 ml. of sodium sulfate −5.2 per cent. In general, the error caused by the presence of both calcium and sulfate is negligibly small when upon addition of alcohol little or no precipitate is formed. If the amount of precipitated calcium sulfate is appreciable, a separation has to be made. When organic anions which form complexes with uranyl (citrate, etc.) are absent, the easiest method is by precipitation of the phosphate in ammoniacal solution as calcium phosphate. The precipitate, which need not be washed, is dissolved in hydrochloric acid and the solution is neutralized with sodium hydroxide and bromocresol green as indicator. In this way phosphate determinations were carried out in the presence of a hundredfold molar excess of calcium with an accuracy of 0.5 per cent. When organic matter which has to be decomposed is present, the interference of calcium can be eliminated at the same time. From the data just given regarding the amounts of calcium and sulfate which can be present in 0.005 *M* phosphate solution without causing interference, it can be seen that the concentration limit corresponds approximately to a saturated calcium sulfate solution (about 0.015 *M*). However, even when higher concentrations of calcium and sulfate were present, a precipitate was not formed until alcohol was added. When the presence of organic matter makes necessary a decomposition by treating with strong sulfuric acid (and nitric acid) the remaining concentration of calcium sulfate after the treatment with acid does not interfere in the phosphate determination. Such a treatment is usually also used for the dissolution of insoluble phosphates. For the present purposes the removal of calcium in the presence of organic acids can also be accomplished by ashing with an excess of sodium carbonate.

Iron. Iron, both in the ferrous and ferric states, interferes with the titration. The authors found that the simplest way to remove the iron was by precipitation with cupferron.

Table IV. Effect of Calcium in 20 Per Cent Ethanol

(Total volume titrated, 50 ml.; concentration of uranyl acetate, 0.1 *M*)

No.	0.01 *M* KH_2PO_4 Solution Used	1 *M* $CaCl_2$ Solution Used	Molar Ratio Ca:PO_4	1 *M* Acetic Acid Added before Titrating	1 *M* $NaSO_4$ Solution Used	2 *M* HCl Added before Precipitation of $CaSO_4$	Indifferent Electrolyte		Uranyl Acetate Used		
							Salt	Molarity	Calculated	Found	Error
	Ml.	*Ml.*		*Ml.*	*Ml.*	*Ml.*			*Ml.*	*Ml.*	%
1	25	1	4:1	0.05	...	...	KCl	0.1	2.50	2.47	− 1.2
2	25	2	8:1	0.05	...	...	KCl	0.1	2.50	2.46	− 1.6
3	10	1	10:1	0.05	...	...	KCl	0.1	1.00	1.00	0.0
4	25	1	4:1	..	...	...	KCl	0.1	2.50	2.48	− 0.8
5	25	1	4:1	1	...	...	KCl	0.1	2.50	2.45	− 2.0
6	25	1	4:1	1	...	...	KCl	0.1	2.50	2.46	− 1.6
7	25	4	16:1	1	...	...	KCl	0.1	2.50	2.435	− 2.6
8	25	1	4:1	2	...	...	KCl	0.1	2.50	2.43	− 2.8
9	25	2	8:1	2	...	...	KCl	0.1	2.50	2.42	− 3.2
10	10	1	10:1	2	...	...	KCl + 0.02% gelatin	0.1	1.00	1.01	+ 1.0
11	25	1	4:1	0.05	0.5	...	KCl	0.1	2.50	2.50	0.0
12	25	1	4:1	0.05	1	...	KCl	0.1	2.50	2.465	− 1.4
13	25	1	4:1	0.05	2	...	KCl	0.1	2.50	2.37	− 5.2
14	25	1	4:1	0.05	5	...	KCl	0.1	2.50	2.35	− 6.0
15	25	1	4:1	2	5	...	KCl	0.1	2.50	2.40	− 4.0
16	25	1	4:1	2	5	...	KCl + 1 ml. 1 *M* $BaCl_2$	0.1	2.50	2.26	− 9.6
17	25	0.5	2:1	..	5	2	..	..	2.50	2.46	− 1.6
18	25	0.5	2:1	..	5	2	NaAc HAc	0.4[a] 0.04	2.50	2.48	− 0.8
19	25	0.5	2:1	..	5	2	NaAc HAc	0.4[a] 0.04	2.50	2.51	+ 0.4
20	25	1	4:1	..	5	2	..	..	2.50	2.46	− 1.6
21	25	1	4:1	..	5	2	..	..	2.50	2.45	− 2.0
22	25	1	4:1	..	5	4	..	..	2.50	2.44	− 2.4
23	25	2	8:1	0.1	2	2	..	..	2.50	2.30	− 8.0
24	25	5	20:1	..	10	2	NaAc HAc	0.2[b] 0.06	2.50	2.20	−12.0

[a] pH = 5.7. [b] pH = 5.3.

The solution is made 10 per cent in hydrochloric acid and cooled in ice, and the iron is precipitated with about 0.5 *M* cupferron solution. The free phenyl nitrosohydroxylamine and its decomposition products, which are quickly formed in acid medium, yield polarographic waves. Therefore, the iron precipitate with the excess of cupferron and its decomposition products were removed by shaking out with ether. The resulting aqueous phase was freed from ether (by gently heating or bubbling nitrogen through), neutralized to bromocresol green, and titrated after addition of alcohol. As an example, the following analysis is reported.

To a mixture of 25 ml. of 0.01 *M* monopotassium phosphate solution and 1 ml. of 1 *M* ferric chloride solution were added 3 ml. of concentrated hydrochloric acid, and after cooling in ice about 7 ml. of 0.5 *M* cupferron solution. After separation as described above, the aqueous solution was diluted to exactly 100 ml., and 50 ml. were then added to 10 ml. of ethanol and titrated with 0.1 *M* uranyl acetate solution. The error of the titration was −0.8 per cent.

Other Interfering Substances. Interference will occur with all metals which at a pH of about 3.5 precipitate phosphate—e. g., lead, aluminum, and trivalent chromium. Chemical separations might be possible in these cases. Pyrophosphate and other anions, such as arsenate and vanadate, which precipitate with uranyl acetate interfere with the phosphate titration. According to Dworzak and Reich-Rohrwig (*6*), pyrophosphate forms soluble complexes with uranyl: $[UO_2(P_2O_7)_2]^{------}$. Obviously these complexes are not very stable, because when working in a medium of 20 per cent ethanol, pyrophosphate was immediately precipitated with uranyl. It seems possible to eliminate pyrophosphate by precipitation with cadmium according to Hull (*8*).

The precipitation of uranyl by arsenate can also be used for an amperometric titration of arsenate. Preliminary experiments performed by titrating a 0.005 *M* arsenate solution in a medium of 20 per cent ethanol under the same conditions as used in the titration of phosphate gave results accurate to about 1 per cent.

Procedure

On the basis of the various experiments the following general procedure is given.

Reagents. *Uranyl Acetate.* A 0.1 *M* solution is prepared by dissolving 42.422 grams of chemically pure uranyl acetate, $UO_2(C_2H_3O_2).2H_2O$, in a 1-liter volumetric flask by shaking with about 300 ml. of water and 6 to 10 ml. of glacial acetic acid. After complete dissolution the flask is filled up to volume. The dissolution can be accelerated by careful heating. The solution can be standardized against standard phosphate solution. For titrations of low phosphate concentrations the uranyl acetate solution is diluted with water.

Standard Phosphate. Merck's preparation "Sörensen" of monopotassium phosphate is dried at 110° C. for 1 hour and a 0.01 *M* solution is prepared by dissolving 1.3614 grams in 1 liter.

Potassium Chloride. A 1 *M* or 2 *M* solution is prepared by dissolving 74.56 or 149.12 grams of potassium chloride of Merck's purest quality in 1 liter.

Procedure. The dissolved sample is diluted, so that in the final volume (all additions included) the concentration of phosphate is not greater than approximately 0.01 *M*, or if calcium (and sulfate) are present, not greater than 0.005 *M*. The concentration of calcium and sulfate must not be greater than 0.02 *M* and 0.01 *M*, respectively. If a previous neutralization is necessary, a few drops of bromocresol green are added to the solution and sodium hydroxide or hydrochloric acid is added until the indicator has a green color. About 0.5 ml. of 0.1 *M* acetic acid is added and enough potassium chloride to make the chloride concentration approximately 0.1 *M*. The sample is introduced into the titration cell and enough ethanol added to make its concentration 20 per cent. Air is removed by passing nitrogen through the solution for about 15 minutes (the removal is complete when the residual current has become constant), and the titration is carried out at an applied potential of −0.7 to −0.8 volt.

Notes. If a precipitate of barium phosphate is formed by the addition of ethanol, it is dissolved by adding 1 *M* acetic acid dropwise.

In the presence of large amounts of both calcium and sulfate the phosphate is first precipitated in ammoniacal medium and then treated as described above.

Iron if present is removed with cupferron as described above.

In the presence of organic substances like citrate, tartrate, oxalate, etc., which form complexes with uranyl and in the analysis of organic phosphorus, a destruction of the organic matter is necessary. In the latter instance the total content of phosphorus is determined as orthophosphate.

Summary

The polarographic waves of uranyl acetate in weakly acid media were studied.

A procedure has been given for the amperometric titration of phosphate with uranyl acetate at room temperature. The accuracy was 1 per cent or better with 0.01 to 0.0003 *M* con-

centrations of orthophosphate. In 0.0001 *M* phosphate the accuracy was of the order of 4 per cent.

Alkaline earth phosphates can be titrated by the standard procedure. Calcium in large amounts, iron, and organic anions interfere. Methods are described to eliminate the interference.

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Quantitative Determination of 2-Methyl-1,4-naphthoquinone

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THE increased use of 2-methyl-1,4-naphthoquinone as a therapeutic agent necessitated the development of an accurate and convenient method for its quantitative estimation in marketed preparations. The procedure described in this paper has been found reliable for the determination of 2-methyl-1,4-naphthoquinone in quantities as low as 0.05 mg., and has been employed successfully in the assay of oil solutions and of alcoholic extracts containing the drug. In a study of the reactions involved in this determination, the 2,4-dinitrophenylhydrazone of 2-methyl-1,4-naphthoquinone was isolated and characterized, and its absorption spectrum examined in both alkaline and neutral solvents.

Pinder and Singer (*3*) described a colorimetric procedure for the determination of 2-methyl-1,4-naphthoquinone which involves the interaction of that substance with ethyl cyanoacetate. The unstable, purple colored reaction product thus obtained is subsequently hydrolyzed with 6 *N* potassium hydroxide to produce a more stable yellow colored derivative in solution. The yellow solution is then compared with a suitable standard. Results of analysis obtained by the method of Pinder and Singer (*3*) were found, in this laboratory, to be sufficiently accurate, if the naphthoquinone could first be isolated by alcoholic extraction. The method appeared to be inadequate when applied to vegetable oil solutions from which the naphthoquinone could not be quantitatively extracted. Partial saponification of the vegetable oil, which occurred on the addition of potassium hydroxide, produced turbid solutions that required considerable treatment to effect clarification. It was observed also that the presence of oil complicated the formation and subsequent hydrolysis of the intermediate, unstable purple reaction product.

As an alternative, it was considered that the direct spectrophotometric determination of 2-methyl-1,4-naphthoquinone in oil solution, employing the absorption maximum at 3360 Å., might provide a convenient method of assay. However, direct spectrophotometric examination (*1*) was found to be limited in application to those solutions in which the oil used as a vehicle exhibited low absorption in the desired spectral region. The method was of little value when applied to certain commercial products which contained vegetable oil that showed complete absorption at 3360 Å.

Novelli (*2*) described a sensitive color test for 2-methyl-1,4-naphthoquinone and related substances which forms the basis for the method finally adopted in this laboratory for the quantitative estimation of 2-methyl-1,4-naphthoquinone. The procedure detailed below depends on (*a*) formation of the 2,4-dinitrophenylhydrazone of 2-methyl-1,4-naphthoquinone, (*b*) interaction of this dinitrophenylhydrazone with ethanolic ammonia to yield a green to blue-green colored solution, and (*c*) comparison of the intensity of this color with that produced in a control with known quantities of the naphthoquinone.

The 2,4-dinitrophenylhydrazone of 2-methyl-1,4-naphthoquinone in alcoholic ammonia solutions exhibits a bright blue color. The green color described by Novelli (*2*) was found to be a combination of this blue color with the yellow color of the 2,4-dinitrophenylhydrazine present in excess. For quantitative determinations it was found impracticable to eliminate entirely the yellow color of the excess reagent; conse-

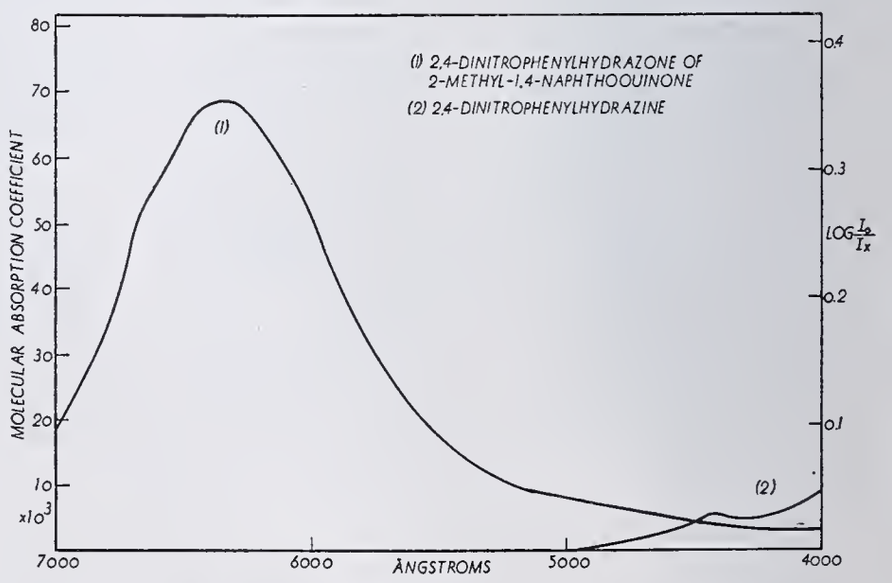

FIGURE 1. LIGHT ABSORPTION OF SOLUTIONS OF THE 2,4-DINITROPHENYLHYDRAZONE OF 2-METHYL-1,4-NAPHTHOQUINONE AND OF 2,4-DINITROPHENYLHYDRAZINE

FIGURE 2. 2,4-DINITROPHENYLHYDRAZONE OF 2-METHYL-1,4 NAPHTHOQUINONE (× 100)

quently the solutions compared colorimetrically were always green to blue-green. Identical shades of green could be obtained in the known and unknown test solutions by controlling the amount of the unknown sample taken for a determination.

Curves representing the light absorption of the 2,4-dinitrophenylhydrazone of 2-methyl-1,4-naphthoquinone and of 2,4-dinitrophenylhydrazine are shown in Figure 1.

The data for these curves were obtained with a Cenco-Sheard spectrophotelometer. The 2,4-dinitrophenylhydrazone was dissolved in a mixture of one part ethanol and one part concentrated ammonium hydroxide (specific gravity 0.90). The solution of 2,4-dinitrophenylhydrazine was prepared by dissolving 2.5 mg. of this substance in 5.0 ml. of 2 *N* hydrochloric acid contained in a 100-ml. volumetric flask, adding 10 ml. of ethanol followed by 10 ml. of a mixture of one part ethanol to one part concentrated ammonium hydroxide, and finally filling to the mark with 95 per cent ethanol.

Molecular absorption coefficients were plotted for curve 1, Figure 1, representing the absorption of the dinitrophenylhydrazone, which has an absorption maximum at 635 mμ. The curve representing the absorption of 2,4-dinitrophenylhydrazine was then plotted to illustrate the relative absorptions of the two components in the solution as used for quantitative determinations. From the curves it may be noted that 2,4-dinitrophenylhydrazine, under the conditions of the determination, possesses no conflicting absorption in the region where the dinitrophenylhydrazone exhibits an absorption maximum. It is suggested that measurements with a spectrophotometer, adjusted for operation at 635 mμ, would increase the accuracy of the method.

The 2,4-dinitrophenylhydrazone of 2-methyl-1,4-naphthoquinone was readily obtained according to the following procedure:

A saturated solution of 2,4-dinitrophenylhydrazine in 2 *N* hydrochloric acid was added to an ethanolic solution of 2-methyl-1,4-naphthoquinone; the solution was warmed at 60° to 70° C. for a few minutes and then slowly cooled. Upon recrystallization from hot chloroform the crystalline product yielded bright orange needles and clusters (Figure 2), which sublimed when heated above 200° C. to form long orange needles, and melted with decomposition at 299° C. Analysis for nitrogen gave the following results:

Calculated for $C_{17}H_{12}O_8N_4$(352.30)	N, 15.92
Found	N, 16.0

The crystalline dinitrophenylhydrazone was very slightly soluble in neutral solvents but dissolved readily on the addition of ammonia or other alkaline reagents with the production of a bright blue color (spectrum, Figure 1). The enhanced solubility and the change in color from orange to blue produced by the addition of bases was attributed to the formation of the aci-salt of the nitro groups in the dinitrophenylhydrazine nucleus, with the consequent production of an *o*- or *p*-quinone configuration coupled to the 2-methyl-1,4-naphthoquinone structure. By careful neutralization of the blue alkaline solutions, orange crystals of the dinitrophenylhydrazone could be recovered.

The *p*-nitrophenylhydrazone of 2-methyl-1,4-naphthoquinone was also prepared and found to exhibit properties similar to those of the 2,4-dinitrophenylhydrazone. However, the instability of *p*-nitrophenylhydrazine as a reagent prevented its use in quantitative determinations. 2,4,6-Trinitrophenylhydrazine was found too insoluble in hydrochloric acid solutions to be used as a reagent.

In Table I a comparison is shown of the results obtained on assay of marketed specimens of 2-methyl-1,4-naphthoquinone dissolved in vegetable oil by direct spectrophotometric measurement (*1*) and by the method described below.

Further controlled experiments indicated that 1 mg. of 2-methyl-1,4-naphthoquinone dissolved in ethanol could be determined with an accuracy of ±3 per cent by this method, employing a visual colorimeter; when the naphthoquinone was dissolved in vegetable oil the accuracy was about ±5 per cent. Chlorobutanol was found to have no effect on the accuracy of the determination.

TABLE I. COMPARISON OF DIRECT SPECTROPHOTOMETRIC AND 2,4-DINITROPHENYLHYDRAZONE COLORIMETRIC METHOD FOR DETERMINATION OF 2-METHYL-1,4-NAPHTHOQUINONE

Specimen No.	2-Methyl-1,4-naphthoquinone Content Found: Dinitrophenylhydrazone method *Mg./ml.*	Direct spectrophotometric method *Mg./ml.*	Difference *Mg./ml.*
A	0.93	0.94	0.01
B	1.90	1.96	0.06
C	0.82	0.77	0.05
D	0.75	0.70	0.05
E	1.00	0.94	0.06
F	2.06	2.02	0.04

Method

REAGENTS. Standard 2-methyl-1,4-naphthoquinone solution. Dissolve 25 mg. of 2-methyl-1,4-naphthoquinone, melting point 105–107° C., in 50 ml. of 95 per cent ethanol. This solution is stable for about one week if stored in a cool, dark place when not in use.

Dinitrophenylhydrazine reagent. Dissolve 50 mg. of reagent quality 2,4-dinitrophenylhydrazine in 20 ml. of 2 *N* hydrochloric acid.

Ethanolic ammonia solution. Mix one part of 95 per cent ethanol with one part of concentrated ammonium hydroxide (specific gravity 0.90).

PROCEDURE. Transfer a sample (oil solution or alcoholic extract) calculated to contain about 0.5 mg. of 2-methyl-1, 4-naphthoquinone to a 50-ml. volumetric flask. Place 1.0 ml. of the standard alcoholic solution of 2-methyl-1,4-naphthoquinone in a

second 50-ml. volumetric flask and adjust the volume in both flasks to 5 ml. with ethanol. If the unknown contains oil, add an equivalent amount of the same kind of oil, free from naphthoquinone, to the flask containing the standard solution and shake both flasks thoroughly. Add 1.0 ml. of the 2,4-dinitrophenylhydrazine reagent to each flask and place both flasks in a water bath maintained at 70° to 75° C. for 15 minutes. If oil solutions are being assayed, the flasks must be shaken vigorously once every 3 minutes to ensure complete reaction; otherwise, only occasional shaking is necessary. At the end of the heating period, cool the flasks to room temperature by immersion in a water bath and add to each flask 5.0 ml. of the ethanolic ammonia solution. Shake the flask thoroughly, fill to the mark with 95 per cent ethanol, and compare the solutions in a colorimeter. When oil is present in the flasks, allow the solutions to stand for 15 minutes in order that the oil may separate, and use the supernatant liquid for the determination.

Note. 2-Methyl-1,4-naphthoquinone is decomposed by prolonged exposure to light; consequently, it is best to carry out the determination in subdued light.

Discussion

Because the excess reagent contributes to the final color, the same volume of dinitrophenylhydrazine reagent solution must be added to the unknown and to the standard when the color comparison is performed visually. In practice, if the developed color of the unknown solution is found to vary more than 10 per cent from the standard, a second determination is made with an adjusted volume of the unknown sample. Consistently accurate results may be obtained in this manner.

Ammonia was found to be the most satisfactory alkaline reagent for the development of the blue color. Strong alkalies, such as sodium or potassium hydroxides in small amounts, led to extensive decomposition of the excess dinitrophenylhydrazine and to the production of colored derivatives, which interfere with color comparison.

It was observed that the addition of concentrated ammonium hydroxide solution produced some decomposition of the excess dinitrophenylhydrazine as a result of high local concentrations of alkali at the point of addition. This difficulty was overcome by the use of less alkaline alcoholic ammonia solution to develop the color. A final concentration of 0.6 *N* ammonium hydroxide was found necessary to effect complete conversion of the alcohol-insoluble 2,4-dinitrophenylhydrazone to the blue alcohol-soluble compound.

Summary

A method for the quantitative estimation of 2-methyl-1,4-naphthoquinone and related substances, applicable to oil solutions or alcoholic extracts, depends upon the interaction of 2,4-dinitrophenylhydrazine with 2-methyl-1,4-naphthoquinone and the subsequent production of a blue to blue-green color by the addition of alcoholic ammonia.

The 2,4-dinitrophenylhydrazone of 2-methyl-1,4-naphthoquinone has been isolated and characterized, and the absorption spectra of the 2,4-dinitrophenylhydrazone of 2-methyl-1,4-naphthoquinone and of 2,4-dinitrophenylhydrazine dissolved in alcoholic ammonia have been determined.

Acknowledgment

The data for the absorption curves reproduced in Figure 1 were obtained through the courtesy of Thorfen R. Hogness, in the laboratories of Spectroscopic Biological Investigations, University of Chicago, with the assistance of Richard Abrams.

The author wishes to express his appreciation to A. E. Sidwell, Jr., of this laboratory, for suggestions and criticisms during the course of this work.

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Colorimetric Determination of Low Concentrations of Sodium Nitrate in Sodium Nitrite

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A METHOD was needed for the determination of sodium nitrate in sodium nitrite samples in concentrations of about 1 per cent or less. In order to obtain fairly precise values, those methods were excluded from consideration which depend upon the determination of nitrates by the difference in the total nitrogen value and the nitrite value, because the errors would all be thrown upon the minor constituent. Of the direct methods which have been reported for nitrate, the colorimetric ones seemed most promising.

These fall into two classes. The first class comprises tests such as those with diphenylamine and with ferrous sulfate which are not specific for nitrates but are also given by nitrites. The use of such tests would require the preliminary removal of nitrites. In order to avoid the necessity for doing this, attention was turned to the second class—namely, tests which have been reported as being specific for nitrates and not subject to interference by nitrites. This claim has been made for two methods in particular; one by Wolf and Heymann (*11*) involves the formation of a color by means of 2,4-diamino-6-hydroxypyrimidine in the presence of sulfuric acid; the other by Pesez (*7*, *8*) depends upon nitrating nitrobenzene to dinitrobenzene by means of the nitrate in sulfuric acid and reacting the product according to Janowski (*2*) with acetone and alkali to produce a color which is a measure of the nitrate present. The authors of both methods claimed that nitrites do not interfere. The present authors have been unable to confirm their claims in this respect; on the contrary, they find that nitrites do interfere. Even recrystallized sodium nitrite gives a variable intensity of color.

The reason for this is of fundamental importance in considering claims for the specificity of any method for nitrates in the presence of nitrites. Whenever it is necessary to carry out such an analysis by treatment of the sample, which still contains nitrite, in the presence of a considerable concentration of strong acid, then the analysis cannot be reliable, because some of the free nitrous acid which is formed will decompose to form nitric acid, presumably according to the well-known reaction:

$$3HNO_2 \longrightarrow HNO_3 + 2NO + H_2O$$

When analyzing samples consisting almost entirely of sodium nitrite, the amount of nitrate which is formed is sufficient to vitiate the analysis.

Since no method was available which could be applied

directly to the presence of nitrites, it became necessary to consider removing the nitrite before applying any method for the determination of the nitrate.

A number of reagents have been recommended for this purpose. Using hydrazine (*4*) sulfamic acid (*1*), urea (*5*), and hydroxylamine (*3*), the authors were unable to prevent the formation of nitric acid. Ammonium chloride was also tried in a manner similar to that described by Nelson, Levine, and Buchanan (*6*) in a slightly alkaline medium (pH about 7.5). The treated solution was then evaporated to dryness, after which it was reacted with silver sulfate in a small volume of added water to remove excess chloride, which would interfere with the nitrobenzene reaction. It was found that tests for nitrate could be obtained with pure sodium nitrite. Since the decomposition of the nitrite is carried out in a slightly alkaline medium, there is little likelihood of the formation of nitrate. It is more likely that some nitrite remains undestroyed. This would interfere with the final color test, if present as such, or if partially converted to nitrate by oxidation upon concentrating the solution. The procedure described by Nelson, Levine, and Buchanan requires a high excess of ammonium chloride for complete destruction of the nitrite. Because of the need for removing excess chloride in the authors' procedure, this was not practical, since they had about 0.1 gram of sodium nitrite and so would require too much ammonium chloride.

Sodium azide was a more satisfactory reagent. It is said to act in the following manner (*10*):

$$HN_3 + HNO_2 \longrightarrow N_2O + N_2 + H_2O$$

Sommer and Pincas (*10*), who recommended it for the destruction of nitrite in order to permit the determination of nitrate, were able to remove nitrite completely and found no conversion to nitrate when the latter was tested for by diphenylamine. The authors confirmed this report, but did not consider diphenylamine a suitable reagent for the quantitative determination of nitrate. One reason is its lack of specificity, since it would respond to contaminants such as ferric iron or other oxidants. Another reason is that the usual technique in applying the diphenylamine test, which involves the formation of a colored ring upon stratifying the sample solution on the diphenylamine reagent, did not seem promising for quantitative purposes. Pesez's method appeared more promising, but the authors found that the use of sodium azide as recommended by Sommer and Pincas did result in the formation of some nitrate from nitrite.

Presumably Pesez's method is more sensitive than the diphenylamine test. In order to use this method, the factors involved in the azide decomposition reaction were studied in order to avoid any formation of nitrate, while at the same time getting complete destruction of nitrite. It was found essential to control the relative concentrations of nitrite, sulfuric acid, and sodium azide. If too much azide is used, there seems to be some destruction of nitrate (possibly by the reducing action of the free hydrazoic acid). If too little is used, some nitrite will escape destruction and will be converted to nitrate during the application of Pesez's method. The concentration of sulfuric acid must be controlled so as to have only a small excess present over the amount which is just necessary to convert the nitrate to nitric acid and the sodium azide to hydrazoic acid. The effect of differences in the time and temperature of the reaction was studied.

After the nitrite has been destroyed, the solution is made alkaline and evaporated to dryness. Pesez's method is then applied to the solid residue. The influence of such variables as time and temperature, volumes of reagents, and moisture, both upon the nitration and upon the development of the color after nitration, was studied.

Precision and Accuracy

By using the method with the technique carefully controlled as described, it has been possible to distinguish without difficulty (in 0.1-gram samples) each of the members of the series—0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, and 1.0 per cent sodium nitrate—from the preceding and the following member of the series. This precision was sufficient for the authors' purposes, but it is possible that the method could be developed to a greater precision. Beyond a concentration of 0.6 per cent the higher intensity of color results in a loss in the sensitivity with which successively higher concentrations may be differentiated.

Method of Analysis

Caution. Because of the poisonous nature of hydrazoic acid it is well to carry out the analysis in a hood.

Reagents. Sodium Azide Solution (A). The sodium azide solution must be made up to be 0.0581 *N*. It is prepared by weighing out and dissolving in water enough solid sodium azide, which has previously been assayed according to the method of Reith and Bouwman (*9*), to make a solution of 3.7765 grams of 100 per cent sodium azide per liter. The azide solution is satisfactory if, using the procedure described, a colorless test solution is obtained with 100 mg. of recrystallized sodium nitrite and a pinkish-lilac color is obtained with 100 mg. of recrystallized sodium nitrite plus 0.1 mg. of sodium nitrate.

Sodium Nitrite Solution (B). C. P. sodium nitrite is recrystallized from water several times to ensure the absence of nitrates. A solution is made up to contain exactly 0.4000 gram per 100 ml.

Sodium Nitrate Solution (C). C. P. sodium nitrate is dissolved in water to give a solution which contains 0.1 mg. of sodium nitrate in 1 ml.

Nitrobenzene. A redistilled product with a freezing point not less than 5.2° C.

Concentrated sulfuric acid, *N* sodium hydroxide solution, 5 *N* sodium hydroxide solution, and *N* sulfuric acid solution.

Preparation of Standard Solutions. Standard solutions are prepared by mixing 25.0-ml. portions of Solution B with Solution C. Each milliliter of Solution C corresponds to 0.1 per cent sodium nitrate. Standards are made up to correspond to 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, and 1.0 per cent sodium nitrate.

Preparation of Sample Solution. The sodium nitrite sample solution is made up to contain exactly 0.1 gram of sodium nitrite in 25 ml. of water. For this purpose the sodium nitrite is assayed by titrating 0.1 *N* sodium sulfanilate in an acid medium with the nitrite, using a potassium iodide–starch paste as a streak indicator for the end point.

Destruction of Nitrite and Development and Matching of Colors. The standard solutions and 25-ml. portions of the sample solution (from a transfer pipet) are placed in 100-ml. beakers. From a transfer pipet 25 ml. of Solution A are added and then 3.5 ml. of *N* sulfuric acid. The solutions are stirred immediately and then occasionally during 15 minutes, at which time all gas evolution should have ceased. The solutions are then made just faintly alkaline with *N* sodium hydroxide solution, spotting on phenolphthalein indicator paper for the end point and using no more than one drop in excess. The amount of sodium hydroxide necessary varies from 0.5 to 0.7 ml. The solutions are then evaporated to a small volume on a hot plate, transferred to a steam bath, and evaporated to dryness two or three times with the aid of some added absolute alcohol to ensure complete absence of moisture from the solid residue.

The residue is powdered with a small stirring rod, the beaker is cooled in ice, and exactly 3 drops of nitrobenzene are added from a capillary dropper. (The same dropper is used for the standards as for the sample in order to be certain of uniformly sized drops.) The nitrobenzene is rubbed up with the residue, and then exactly 0.35 ml. of concentrated sulfuric acid (from a 1-ml. Mohr pipet) is mixed in. The mixture is allowed to stand for 3 minutes in the ice bath with occasional stirring. During this time 5 ml. of 5 *N* sodium hydroxide solution are transferred to a 20-ml. glass-stoppered test tube by means of a transfer pipet or a buret. The reaction mixture in the beaker is then rubbed twice with 5-ml. portions of C. P. acetone. The acetone is transferred to the tube containing the 5 *N* sodium hydroxide solution. (It is not necessary to remove the solid matter from the beaker.) The tube is shaken vigorously with an up and down motion 50 times, then allowed to stand for 3 minutes at room temperature, and finally immersed in an ice bath. After cooling, the colored acetone layer of the sample is compared with those of the standards within 30 minutes. The acetone may, if desired, be removed from the alkali layer. The color seems to fade less rapidly if that is done.

EFFECTS OF VARIATIONS IN CONDITIONS. The solution containing the sample (or standard) and sodium azide was allowed to stand up to 20 minutes before adding the sulfuric acid, with no effect upon the results. The sulfuric acid could be added rapidly rather than dropwise without influencing the values for nitrate. Instead of 3.5 ml. of N sulfuric acid as little as 3.0 ml. and as much as 4.0 ml. were used without adverse effects.

The effect of changing the temperature of the solution from room temperature during the addition of the azide and the sulfuric acid and during the subsequent stirring period was studied. At a temperature of 50° to 60° C. the colors were intensified and it was impossible to obtain colorless blanks. This may have been the result of some loss of hydrazoic acid (boiling point 38° C.) which would cause an incomplete destruction of nitrite, thereby allowing some of the nitrite to become converted to nitrate, which would produce a colored blank. No advantage could be observed in cooling; room temperature seemed to be most advantageous.

Summary

Methods for the determination of low concentrations of nitrates in the presence of nitrites have been examined for which claims have been made that nitrites do not interfere. It has been found impossible to substantiate these claims, since some conversion of nitrite to nitrate always occurs. The removal of nitrites by various reagents was then tested and sodium azide was found most suitable. A procedure was worked out which involves the removal of nitrites by means of sodium azide and the subsequent colorimetric determination of the nitrate by modifications of a reported procedure involving the nitration of nitrobenzene and the development of a color with acetone and alkali. Concentrations of sodium nitrate up to 1 per cent have been determined.

Acknowledgment

Grateful acknowledgment is made to Harold J. Rodenberger for calling attention to the use of sodium azide for the destruction of nitrites.

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Ultraviolet Absorption of Vitamin A in Various Solvents

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SOME disagreement exists regarding the numerical value of the absorption coefficient of vitamin A at the maximum of its absorption curve in the ultraviolet (*2*, *4*, *6*, *7*, *8*, *11*). Since very pure preparations of crystalline vitamin A are now available (*2*), a study of this value was made for solutions in many of the commonly used solvents which might be employed in the assay of vitamin A products. In this paper, vitamin A indicates vitamin A_1.

Experimental Procedure

The absorption values were determined with a photoelectric spectrophotometer recently employed in spectrophotometric studies on chlorophyll (*12*) and linseed oil (*9*). A large Müller-Hilger Universal double monochromator with crystal quartz optics was the dispersion instrument. A hydrogen arc of the Munch type (*10*), constructed of quartz, was the source of radiation for many of these measurements. An incandescent filament lamp with a clear Pyrex bulb was the source of radiation for most of the measurements above 3100 Å. All determinations were made on solutions at 25° C.

For these measurements, all slits were of uniform width, with the exit slit 6 mm. in length. Slit widths were as follows [numbers in brackets indicate the corresponding spectral region isolated, as defined by Hogness, Zscheile, and Sidwell (*5*)]: 0.30 to 0.80 mm. (6 to 16 Å.) at 2240 A.; 0.20 to 0.80 mm. (5.2 to 20.8 A.) at 2400 Å.; 0.08 to 0.15 mm. (4.2 to 7.8 Å.) at 3000 Å.; 0.12 to 0.20 mm. (8.2 to 13.6 Å.) at 3280 Å.; 0.20 mm. (16.4 Å.) at 3500 A.; and 0.08 mm. (8.5 Å.) at 3760 Å. The results were independent of slit width in the ranges employed. For instance, when the slit widths were varied from 0.06 to 0.50 mm., the absorption value at 3280 Å. remained constant. A decrease of only 1 per cent occurred when slits of 0.95 mm. were used.

The absorption spectra reported here are expressed in terms of $E^{1\%}_{1\text{ cm.}}$, defined as follows:

$$E^{1\%}_{1\text{ cm.}} = \frac{\log_{10} \frac{I_0}{I}}{cl}$$

where I_0 = intensity of radiant energy transmitted by solvent-filled cell
I = intensity of radiant energy transmitted by solution-filled cell
c = concentration in grams per 100 ml.
l = thickness of solution layer in centimeters

The cell length was 1 cm. and the concentration was adjusted to keep the $\log_{10} \frac{I_0}{I}$ values between 0.200 and 0.800 in order to obtain high precision (*5*).

"Iso-octane" was distilled over solid sodium hydroxide. Cyclohexane was purified by repeated recrystallization and distillation. Other solvents were purified in accordance with the practices of this laboratory (*13*). All alcohols were treated in the same manner as ethanol. Amber glassware (*3*) was not used in the experiments reported here.

The samples studied were purified in the laboratories of Distillation Products, Inc., by molecular distillation and subsequent crystallization (*2*). Samples B-152 and B-153 were received in vacuum, packed in dry ice. All observations were made 3 to 4 days after receipt of sample. Sample B-210 was received in vacuum at room temperature and stored in dry ice until studied, 6 to 15 days later. Samples were kept in vacuum at dry ice temperatures in the dark as much as possible between the necessary weighings for different solvents. Maximum absorption values of all samples were determined within 10 minutes to 1 hour after the weighings was made.

EFFECT OF INCANDESCENT FILAMENT RADIATION ON ABSORPTION VALUES. Certain tests were made to determine the effect of the radiation employed in the absorption measurements themselves.

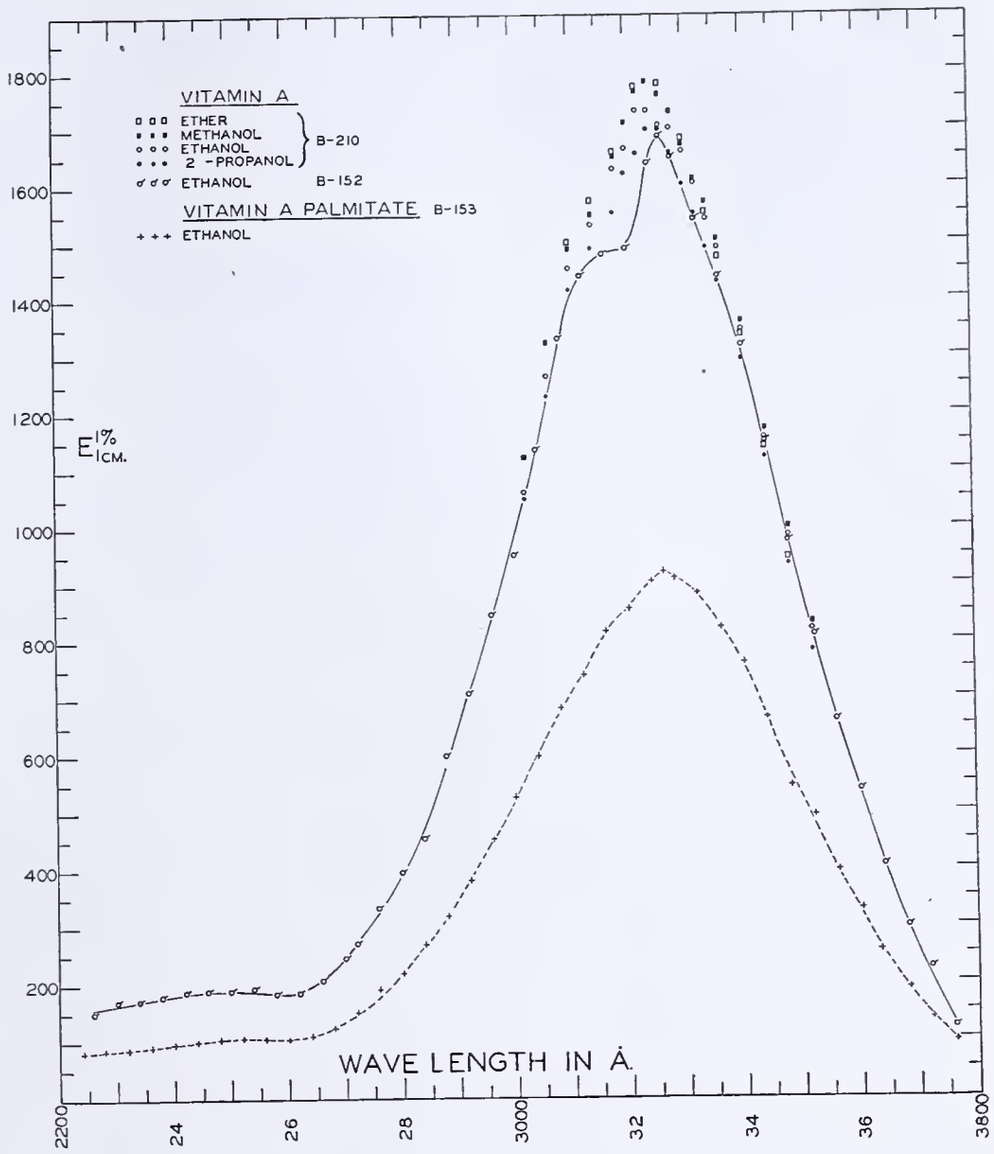

FIGURE 1. ABSORPTION SPECTRA IN ETHER AND ALCOHOL SOLUTIONS

The absorption of a fresh solution of vitamin A (B-210) in ethanol was measured at 3280 Å. An exposure of 6 seconds to a very low intensity of radiation from the exit slit of the monochromator is required for a single determination of $\log_{10} \frac{I_0}{I}$. Three successive determinations at 3280 Å. gave the same result. Then eight determinations were made from 3520 to 3300 Å., followed by three more at 3280 Å. Ten more determinations followed at intervals from 3260 to 3020 A., succeeded by three additional ones at 3280 A. The values obtained at 3280 Å. remained constant to ±0.5 per cent throughout these measurements. Then the same solution in the absorption cell was held directly in front of the 72-watt incandescent filament radiation source for successive periods of 3, 9, and 3 minutes and the absorption at 3280 Å. was determined after each exposure. During this series, the absorption at 3280 Å. remained constant to ±0.2 per cent.

Results

Figure 1 presents the absorption data of vitamin A in diethyl ether and various alcohols and of vitamin A palmitate in ethanol from 2240 to 3760 Å. Figure 2 contains similar data for solutions in several hydrocarbon solvents. Lines are drawn through the points for ethanol and isooctane (2,2,4-trimethylpentane) only.

Tables I, II, and III present the numerical $E^1_{1\text{ cm.}}$ values for all preparations in the solvents studied, at wave lengths 3260 and 3280 Å. Values at 3240 Å. are included in some cases. Data in columns numbered 1 are from the same solution; those in columns numbered 2 are from solutions made from different weighings of the original sample.

TABLE I. ABSORPTION COEFFICIENTS FOR VITAMIN A NO. B-210

(Determined 5 to 6 weeks after preparation)

Solvent	3240 Å. 1	3240 Å. 2	3260 Å. 1	3260 Å. 2	3280 Å. 1	3280 Å. 2
Ether	..	..	1778	1752	1723	1700
Methanol	1782	1734	1760	1734	1730	1696
Ethanol	1730	1712	1715	1712	1700	1682
2-Propanol	1698	1680	1698	1660	1655	1642
Isooctane	..	..	1660	..	1652	..
Cyclohexane	..	..	1605	1590	1605	1570
Hexane	..	..	1688	1662	1665	1660

TABLE II. ABSORPTION COEFFICIENTS FOR VITAMIN A NO. B-152

(Determined 19 weeks after preparation)

Solvent	3260 Å. 1	3260 Å. 2	3280 Å. 1	3280 Å. 2
Ethanol	1685	1695	1660	1640
Isooctane	1610	1630	1590	1600
Hexane	1600	..	1565	..

TABLE III. ABSORPTION COEFFICIENTS FOR VITAMIN A PALMITATE NO. B-153

(Determined 15 weeks after preparation)

Solvent	3260 Å. 1	3260 Å. 2	3280 Å. 1	3280 Å. 2
Ethanol	920	930	910	926
Isooctane	906	...	900	...
Hexane	860	878	862	870

Discussion

For most solvents, a definite inflection appears in the curve in the region of 3150 Å. This is more pronounced for sample B-152 than for B-210. With one exception the maximum of absorption was found at wave lengths shorter

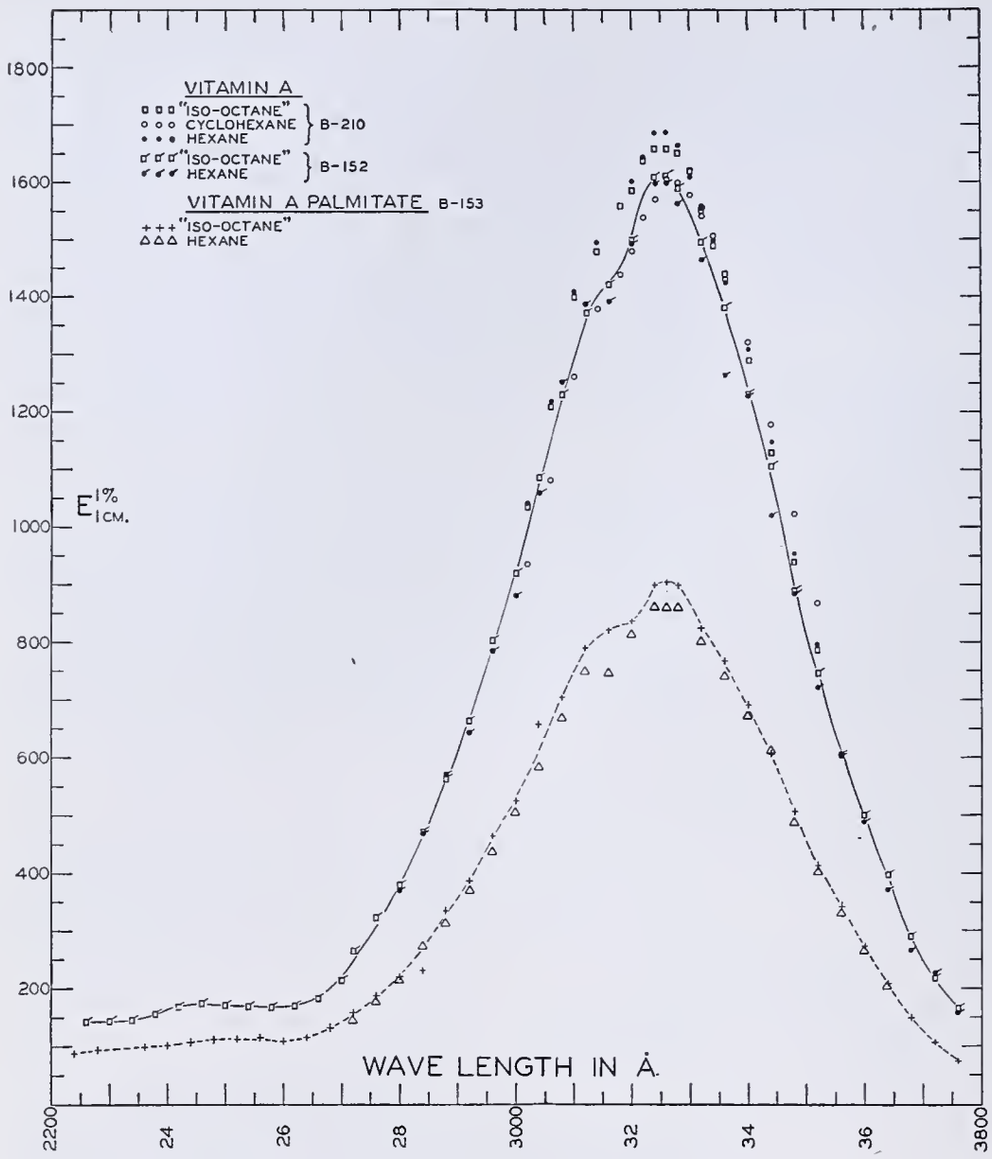

FIGURE 2. ABSORPTION SPECTRA IN HYDROCARBON SOLVENTS

than 3280 A., usually at 3260 Å. (at 3240 A. for vitamin A No. B-210 in alcohols). Baxter and Robeson (*1*) find maximum absorption at 3260 Å. for most preparations. Similar findings were reported by McFarlan, Bates, and Merrill (*7*). These findings are slightly different from the wave length of 3280 Å. usually given for the maximum. The curves of all preparations examined in the region of lower wave lengths have a second maximum (at 2450–2500 Å. for vitamin A and 2500–2550 Å. for the palmitate).

It is noted that the maximum $E^{1}_{1\ cm.}$ value for sample B-210 is higher than that for B-152 in all solvents. Since sample B-210 had been stored for a much shorter total period than B-152 before the spectroscopic determinations were made, more emphasis should be placed on values for the former preparation. It is possible that incomplete evacuation of air from the ampoule could have caused partial oxidation of sample B-152. In general, the maxima are slightly higher in alcoholic solvents than in hydrocarbon solvents.

Baxter and Robeson (*1*) obtained the following maximum $E^{1}_{1\ cm.}$ values for ethanol solutions, usually at 3260 Å., soon after preparation: 1740 for B-210, 1730 for B-152, and 960 for B-153. The maximum values of $E^{1}_{1\ cm.}$ reported here for ethanol solutions of vitamin A (average 1721 for sample B-210 at 3240 Å.) are nearly the same as the average maximum value 1725 (at 328 mμ) reported by Baxter and Robeson (*2*).

It is evident from their results and those reported here that small differences exist among the most carefully prepared samples. The solvent has a definite effect upon the maximum absorption value.

The average maximum $E^{1}_{1\ cm.}$ values for vitamin A palmitate may be compared with those for the vitamin A sample (B-152) which is most comparable to it with respect to age when examined spectroscopically. The ratios of the corresponding values (0.547 for ethanol) agree well with the inverse ratio of the molecular weights

$$\left(\frac{\text{vitamin A } 286}{\text{palmitate } 524} = 0.547\right)$$

This agreement is better at 3260 Å. than at 3280 Å. For solutions in ethanol and hexane, the difference between ratios is only 0.37 per cent at 3260 Å.

Recently Embree (*3*) has discussed reasons for the instability of vitamin A in solution, especially when subjected to ultraviolet radiation, and has emphasized the importance of keeping such solutions in amber glassware. The above experiments on the effect of incandescent radiation indicate that the radiation conditions employed in these measurements did not cause any change in absorption values and that the slit widths employed were sufficiently small to ensure reproducible values. Other tests in the ultraviolet region indicate the absence of significant scattered radiation. Baxter and Robeson (*1*) have been unable to detect deterioration of vitamin A during spectrographic measurements.

Acknowledgment

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use of certain solvents and concerning the study on the effect of radiation during the measurements.

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Determination of Certain Quercetinlike Substances

Using a Klett-Summerson Photoelectric Colorimeter

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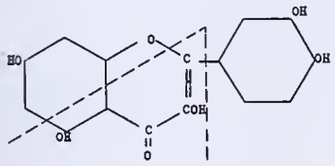
IT WAS observed by one of the authors that boric acid dried with lemon juice gave a brilliant yellow coloration (*3*). Further work led to the conclusion that this coloration was produced by a reaction between the boric acid and a flavone or group of flavones similar in structure to quercetin:



More specifically it has been postulated that the reaction is due to the grouping within the dotted line (*2*, *4*). Included in the materials thus reacting is citrin, believed by Szent-Györgyi to have vitamin activity and tentatively called by him vitamin P (*1*).

The necessity of a flavone-free diet in physiological work with citrin makes practical the test to be used, as its sensitivity will detect amounts as low as 2 or 3 micrograms. The Wilson boric acid test has been discussed and used considerably in a qualitative manner. However, it was the object of this work to develop some method of accurate quantitative measurement of flavones, using this test.

The color-forming substance, insoluble in toluene and chloroform, will dissolve in acetone saturated with boric acid to give a yellow coloration, the intensity of which is a quantitative measure of the amount of flavone present.

For color measurement it was convenient to use a Klett-Summerson photoelectric colorimeter with adaptor for the use of a test tube graduated in 5- and 10-ml. divisions. This eliminates the necessity of matching colors and is an accurate measure of the intensity of color produced. The instrument has a logarithmic scale, thus making possible the production of linear curves on ordinary graph paper.

The intensity of color is deepened considerably by an acid medium, but the strong acids react to give yellow colors with flavones. Acetic acid is too weak to develop the color fully. Anhydrous citric acid has been found suitable, considering strength, acetone solubility, and availability. (Citric acid hydrate may be conveniently rendered anhydrous by allowing it to effloresce completely in air at 30° to 40° C., followed by heating in a thin layer to 100° for 2 hours.) However, on long standing with boric acid, citric acid produces a yellow coloration, so that it was necessary to mix the two materials immediately before use. This was done by mixing equal parts of two filtered solutions prepared as follows:

A. Acetone 100 ml., anhydrous citric acid 10 grams
B. Acetone 100 ml., boric acid to saturate

This mixture is referred to hereafter as borocitric reagent. These separate solutions are apparently stable indefinitely.

It was found that metaboric acid gives a more intense coloration, but the sensitivity of the solution to traces of moisture, as shown by copious precipitates of boric acid, was too great, and the use of metaboric acid was discontinued. The authors recognized the possibility of advantageous use of metaboric acid if its concentration was restricted to the equivalent of boric acid saturation.

As the depth of color is affected greatly by traces of moisture, attempts were made to dehydrate the solvent acetone. As these methods of dehydration gave values which varied slightly, it was considered best to standardize with quercetin and to use one sample of acetone for all reagents. Merck's "Blue Label" acetone was found adequate as to dryness.

Method of Standardization

The standard used for the work with quercetin was prepared by C. E. Sando (Food Research Division, Bureau of Agricultural Chemistry and Engineering, Washington, D. C.) and repurified by Wilson. Weighed amounts of quercetin were dissolved in acetone and diluted to 100 ml. in volumetric flasks. The first sample of 56 mg. was found to give a color far too intense for the scale of the colorimeter. Therefore, further work was done with samples between 10 and 15 mg.

Using the industrial test-tube model of the Klett-Summerson colorimeter, the test tube was filled to the 10-ml. mark with a mixture of equal volumes of solutions A and B, and the zero point set for this reagent, using the blue filter No. 42 accompanying the instrument (value 400 to 465 millimicrons). The acetone-quercetin solution was then dropped into the test tube, using a serological pipet, graduated in 0.01-ml. divisions. After the addition of each 0.1-ml. the tube was removed from the instrument, shaken well to ensure proper mixing, and re-inserted and a reading was taken. The addition of 1 ml., in 0.1-ml. intervals, was found sufficient to determine response curves.

As the quercetin solution is itself yellow, it was necessary also to obtain a curve of colorimeter response to the acetone-quercetin

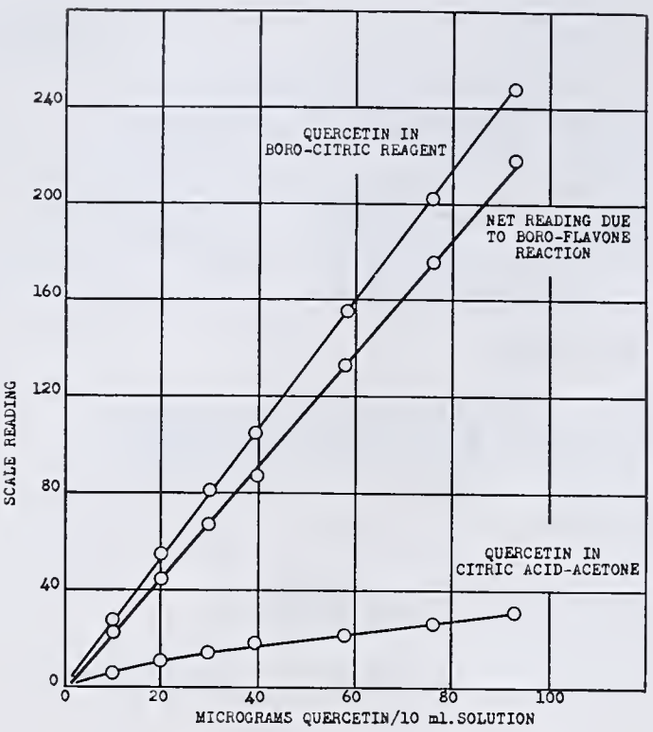


FIGURE 1

TABLE I. CALIBRATION OF COLORIMETER WITH QUERCETIN

(Quercetin strength: 10.2 mg. per 100 ml.)

Ml. of Solution Added to 10 Ml. of Reagent	Micrograms of Quercetin per 10 Ml. of Solution[a]	Scale Reading with: Borocitric reagent	Citric acid–acetone	Net Reading
0.1	10.1	28	6	22
0.2	20.0	55	10	45
0.3	29.7	81	14	67
0.4	39.2	105	18	87
0.6	57.7	155	22	133
0.8	75.6	202	26	176
1.0	92.7	248	31	217

[a] Quercetin solution was added to 10 ml. of reagent, but concentration of quercetin is calculated as micrograms per 10 ml. of total solution.

TABLE II. FLAVONE DETERMINATION IN BIOLOGICAL MATERIALS

Material	Weight Taken Fresh	Weight Taken Dry	Volume of Solution Taken, Ml.	Reading with: Borocitric reagent	Citric acid–acetone	Net Reading	Micrograms of Quercetin Equivalent	Quercetin Equivalent, Mg. per Gram of Fresh Weight
Bean leaf	17.53	1.753	1.0	151	113	38	18.4	0.105
Lemon peel	25.75	6.799	0.1	113	13	100	44.0	1.71
Lemon peel	5.92	1.563	0.5	142	28	114	48.8	1.65
Lemon peel	6.89	1.820	0.5	170	30	140	60.0	1.74
Rabbit liver[a]	...	...	..	..	..	..	..	...

[a] Undetectable.

solution, free of the boroflavone reaction. This was done by mixing it with 5 ml. of solution A and 5 ml. of pure acetone and using the measuring technique described above. Thus a curve could be plotted for the boroflavone reaction and then for the acetone-quercetin solution alone. The net reading of these two curves gave the curve due to the boroflavone reaction with quercetin. From this curve were taken the data needed in determining quercetin equivalents of natural materials.

The reaction is so sensitive to moisture that all precautions must be taken to exclude even traces of moisture from the acetone and from the materials used. As little as 1 per cent of moisture decreases the color response by half. It was necessary to dry the quercetin in a vacuum desiccator for 2 days before use and to use care in mixing the borocitric with the quercetin solution. It was advisable in shaking the test tube to cover its lip with thin tin foil (previously washed in acetone) rather than to run the risk of picking up moisture from the fingers.

The net curves based on these data are shown in Figure 1. As these are straight lines, it is both simple and advisable to replot them for each lot of reagents.

Extraction Procedure

For the determination of the quercetin equivalents of tissues, the following procedure was used:

The tissue was dried in a 60° oven under vacuum, then ground, and a sample was taken, generally from 1 to 5 grams. This was extracted with methyl alcohol in a Soxhlet extraction apparatus, and the extracted liquid was evaporated to dryness on a water bath. Chlorophyll, fats, resins, etc., were next removed from the dry extract by digestion with chloroform. Sometimes, because of the quantity of substances extracted by the methyl alcohol, this chloroform extraction is incomplete, chlorophyll often remaining. In such cases the residue may be redissolved in a small amount of methyl alcohol, a few milliliters of toluene added, the methyl alcohol removed by evaporation, and the chloroform digestion repeated. The resulting residue is dissolved in acetone, filtered, and diluted to 100 ml. in a volumetric flask.

The readings with borocitric reagent shown in Table II were made by taking sufficient of the acetone solution of the extract to give a reading in the desired range (suitably 100 to 250) and adding sufficient reagent to fill the tube to the 10-ml. mark.

Blank determinations were then made, using the same quantities of the extracts and a mixture of equal parts of solution A and acetone.

The net reading is the difference between these two sets of readings, and the quercetin equivalent value was the result of calculating the quantity found on the net curve back to the original material.

Results on some typical plant and animal materials are shown in Table II.

In order to check the accuracy of the method of extraction, 0.82 gram of dried bean leaves (representing 8.2 grams of fresh material) was added to an acetone solution of quercetin containing 1.70 mg. of quercetin. The acetone was evaporated and the fortified leaves were extracted as outlined above. The final residue was taken up in acetone and diluted to 50 ml. Using 1 ml. of this solution, the net reading was 115, equivalent to 50 micrograms of quercetin or 2.50 mg. of quercetin for the 8.2 grams of fresh leaves. According to Table II this quantity of bean leaf contained 0.86 mg. The recovery of added quercetin was 2.50 − 0.86 or 1.64 mg.

Readings with smaller amounts of bean leaf and lemon peel extracts showed a possibility of detection of amounts as small as 2 or 3 micrograms of quercetin with the equipment used. Amounts below this would be difficult to detect.

Rabbit liver, an animal tissue, showed no detectable quercetin, whereas the plant tissues seem to be excellent sources. This may be explained in several ways: the unlikely possibility of a flavone-free diet; the possibility that the flavones are changed in the animal body into materials which do not give the boric acid color reaction; and the possibility that the flavones are conjugated to some form not solubilized by the procedure employed.

Summary

A method for quantitative determination of quercetinlike substances with the use of a Klett-Summerson photoelectric colorimeter has been described. Quercetin equivalent content of bean leaves was found to be 0.105 mg. per gram and of lemon peel, 1.70 mg. per gram. No flavone could be detected in a sample of rabbit liver. Pure quercetin added to dried bean leaves was quantitatively recovered.

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Analytical Determination of *p*-Toluidine in the Presence of Its Isomers

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THERE has long been a need for an analytical method for the determination of *p*-toluidine, particularly when present in small quantities in mixtures of the isomeric toluidines. Commercial *o*-toluidine contains small quantities of both the meta and para isomers, but accurate methods are available for the determination of only the meta isomer (*3*). The present investigation supplies a method for the determination of the para isomer.

Principles of the Method

In earlier work on the stability of diazo compounds (*2*) it was shown that at a temperature of 45° C. the stabilities of *o*- and *m*-toluenediazonium chlorides are identical and very much lower than the stability of the para isomer. For example, *o*- or *m*-toluenediazonium chloride is 99.9 per cent decomposed at the end of 36 minutes, whereas the para isomer requires 21 hours to reach 99.90 per cent decomposition. Thus, if a mixture of the isomeric toluidines is diazotized and allowed to decompose, virtually all of the *o*- and *m*-toluenediazonium chlorides will be decomposed in less than an hour, and all nitrogen which is evolved from the reaction mixture subsequently may be attributed to the decomposition of *p*-toluenediazonium chloride. Since careful determinations have been made of the rates at which the various diazonium compounds decompose (*2*), it is possible to calculate what percentage of the *p*-toluenediazonium chloride originally present will remain at the end of any chosen period of time. It was found, for example, that *p*-toluenediazonium chloride is 62 per cent decomposed after 3 hours at 45° C. Thus, if the nitrogen subsequently evolved from an unknown mixture of isomeric toluenediazonium chlorides after 3 hours of decomposition at 45° C. is gathered and measured, it will represent 38 per cent of the total para isomer which was present in the unknown mixture. By choosing 3 hours for the decomposition time, inaccuracies due to the presence of aniline are avoided, since 2.5 hours are required for benzenediazonium chloride to reach 99.90 per cent decomposition at 45° C. The analytical method described in detail below is an application of these principles.

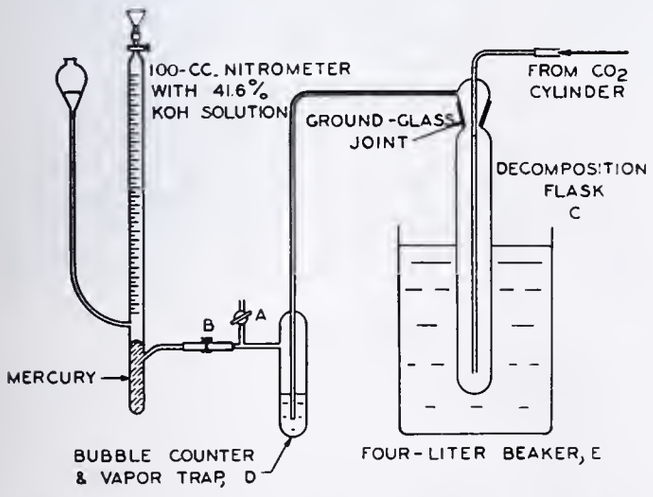

FIGURE 1. DECOMPOSITION AND NITROMETER ASSEMBLY

Apparatus

The earlier work carried out in these laboratories on the stability of diazo compounds involved the use of an automatic recording nitrometer (*1*), which was invaluable in working out the basis for the present analytical method. In the present work, however, a simpler apparatus has been found equally satisfactory. The setup used is shown in Figure 1 and consists of a cylindrical flask fitted with a ground-glass joint, a bubble counter or vapor trap, and a conventional nitrometer. A constant-temperature bath set at 45° ± 0.1° C. is also required but is not shown in Figure 1.

Details of Procedure

A 0.05-mole sample of the mixed toluidines (5.354 grams) is dissolved in 90 ml. of 5 *N* hydrochloric acid. The solution is cooled to 5° C. and *N* sodium nitrite added from a buret, the tip of which extends below the surface of the solution. The solution is stirred mechanically and the sodium nitrite solution added at a rate not greater than 3.5 ml. per minute at the beginning of the reaction and becoming even slower after the addition of 40 to 45 ml. of nitrite (theoretical requirement 50 ml.). It is very important to avoid an appreciable excess of nitrite at any time, as this seriously affects the final results. The temperature must not exceed 8° C.

The progress of the diazotization is observed by dipping a glass stirring rod into the solution and touching the rod to white starch-iodide paper. An immediate development of blue color indicates the presence of excess nitrite and the end point is reached when the immediate blue color which appears can be obtained repeatedly during a period of 5 minutes without further addition of nitrite. At the end of the 5 minutes the solution is transferred to flask *C* and diluted to 250 ml. with distilled water at 5° to 8° C. and the flask is immersed up to the neck and clamped in a constant-temperature bath held at 45° ± 0.1° C. (not shown in Figure 1). The flask is left open to the air and provided with a high-speed electric stirrer. The solution is protected from strong direct daylight and held thus at 45° C. for 180 (±1) minutes. Upon removal it is placed immediately in the ice and water bath (*E*, Figure 1) and cooled as quickly as possible to 0° C.

During the cooling period the assembly shown in Figure 1 is completed. Pinchclamp *B* is closed, stopcock *A* is opened to the air, and carbon dioxide from a pressure cylinder is run through the system in a very rapid stream and allowed to escape into the air at *A*. When the system has been thus flushed for 15 minutes, the rate of flow of the carbon dioxide is reduced to about one bubble per second, the pinchclamp at *B* is opened, and simultaneously the stopcock at *A* is closed. The carbon dioxide is dissolved in 41.6 per cent potassium hydroxide solution which fills the nitrometer. The air and nitrogen trapped in the system are collected over this potassium hydroxide solution. The value of the zero reading is not influenced measurably by the extremely slow evolution of nitrogen at 0° C. When a constant zero reading has been obtained (requiring about 3 minutes), the bulb is leveled, the reading is recorded, the flow of carbon dioxide is almost stopped (one bubble every 5 or 6 seconds), and the ice bath is replaced by a warm water bath. A flame is immediately applied to the water bath and the water is heated to the boil. After 10 or 15 minutes of boiling the flow of carbon dioxide is increased to 2 or 3 bubbles per second and the system flushed until there is no further increase in the volume of gas in the nitrometer. The bulb is then leveled and the volume of gas is read and recorded. The room temperature and barometric pressure are also recorded.

The barometric pressure is corrected for the vapor pressure of potassium hydroxide solution and, knowing the temperature and corrected pressure, the number of milligrams of nitrogen collected is calculated. This value is divided by the weight of the sample in grams and the value obtained is then divided by an empirical factor—namely, 96.06 ($\log_{10}$ = 1.98254). This factor represents the milligrams of nitrogen per gram of sample if the sample had been pure *p*-toluidine, corrected by the extent to which *p*-toluidine is incompletely diazotized under the prescribed

conditions. The figure thus obtained is multiplied by 100 to give the per cent of *p*-toluidine in the sample.

The following typical example will serve to illustrate the method of calculation:

Weight of mixed toluidines diazotized = 5.354 grams
Zero nitrometer reading = 1.30 ml.
Nitrometer reading after complete decomposition = 26.25 ml.
Volume of evolved nitrogen = 26.25 − 1.30 = 24.95 ml.
Room temperature = 25.0° C.
Barometric pressure = 758.0 mm. of mercury
Vapor pressure of 41.6 per cent potassium hydroxide at 25° C. = 7 mm. of mercury (*5*)
Corrected vapor pressure = 751 mm. of mercury
Since the density of nitrogen at standard conditions is 1.25055 (*4*), therefore at 25° C. and 751 mm. pressure 1 ml. of nitrogen = 1.132 mg.

$$\% \text{ } p\text{-toluidine} = \frac{\text{corrected ml. of } N_2 \text{ evolved}}{\text{wt. of sample} \times \text{factor}} \times 100 = \frac{24.95\ (1.132)}{5.354\ (96.06)} \times 100 = 5.49\%$$

Experimental Results

In commercial *o*-toluidine the para content is seldom in excess of 10 per cent. Hence for purposes of testing this method it was considered sufficient to analyze synthetic mixtures of toluidines made up to contain from 0 to 15 per cent of *p*-toluidine. In Table I are given results of analyses of such mixtures of pure *o*- and *p*-toluidines.

The major part of the work in testing the method involved the use of mixtures of the ortho and para isomers. Two experiments were carried out, however, in which the para isomer was mixed with *m*-toluidine; as is indicated in Table II, the method operates as well in the presence of the meta isomer as in the presence of only the ortho isomer.

Table I. Analyses of Synthetic Mixtures of *o*- and *p*-Toluidines

Para Found %	Para Present %
1.31	1.34
1.68	1.44
4.92	4.86
5.13	5.17
13.81	13.80
14.19	14.59

As other impurities may be present in commercial *o*-toluidine, steps were taken to determine whether these impurities would interfere with the successful operation of the analytical method. A common impurity is moisture. Obviously, since the method is carried out in aqueous solution, moisture does not interfere with the reactions involved. However, for accurate work the total diazotizable material present should first be determined. This may be done by means of well-established procedures for determination of aromatic amines.

Table II. Analyses of Synthetic Mixtures of *o*-, *m*-, and *p*-Toluidines

Para Found	Present	
	Para	Meta
%	%	%
4.86	4.74	4.07
5.36	5.18	4.37

Certain high-boiling impurities are sometimes present in commercial material. In order to test the effect of these materials on the analysis, 75 ml. of a commercial *o*-toluidine were distilled until only 2 or 3 ml. of a highly-colored, nonvolatile liquid remained. This remaining impurity was mixed with half of the distillate and the resulting mixture analyzed by the present method. The remaining half of the distillate was likewise analyzed and since, as shown in Table III, there was no difference in the values obtained, it was inferred that the nonvolatile impurity in the commercial sample had no effect on the results of the determination.

Precautions

The most critical aspect of the analytical method is the diazotization. Apparently *o*- and *m*-toluidine diazotize much more readily than *p*-toluidine; however, the resulting diazonium chlorides vary in stability as stated above. Thus, if complete diazotization of the para isomer is to be attempted, considerable decomposition of the other two isomeric diazonium chlorides will result during diazotization, even at very low temperatures, and the lower the temperature the slower the diazotization of the para isomer. Theoretically, the decomposition of *o*- and/or *m*-toluenediazonium chloride during diazotization of the mixture should be of no consequence, since complete decomposition of the ortho and meta isomers is to be carried out before any measurements are made. Practically, however, if the ortho and meta isomers decompose excessively and an excess of nitrous acid is present, side reactions occur which appear to involve *p*-toluenediazonium chloride, decomposed *o*-toluenediazonium chloride, and probably nitrous acid, since very low values are obtained if appreciable excesses of nitrous acid are allowed to accumulate during diazotization.

Table III. Analysis of Commercial *o*-Toluidine in Presence and Absence of High-Boiling Impurities

	Para, %
Pure distillate	0.92, 0.90
Av.	0.91
Pure distillate + residue	0.90, 0.92
Av.	0.91

Consequently, the authors' method prescribes the addition of nitrite so slowly that the troublesome side reactions are virtually eliminated. The prescribed temperature is high enough so that the greater part of the para isomer diazotizes, and yet not enough of the ortho and meta isomers decompose to cause significant errors. The side reactions mentioned can be recognized by the development of a deep coloration during diazotization and/or decomposition. Thus, by use of the diazotization procedure outlined the *p*-toluidine is incompletely diazotized, but if the directions are carefully followed, the reproducibility of the degree of diazotization appears to be well within the limits of accuracy stated.

The accuracy of the analytical method depends not upon complete diazotization but upon diazotization to the same extent every time. It thus becomes necessary for the calculations to include a term which will embrace the extent to which diazotization of the *p*-toluidine is complete. The term 96.06 in the above calculations is the milligrams of nitrogen actually obtained per gram of pure *p*-toluidine when diazotization has been carried out as described herein and has been found to hold over the range of mixtures reported.

Acknowledgments

The authors wish to acknowledge the assistance of Charles D. Compton in the development of the details of this method and to express thanks to A. R. Norton and William Seaman, who have kindly permitted the authors to cite the results of critical tests carried out on the method by members of their staff.

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Electronic Timer

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This paper describes a resistance-capacitance circuit which, when used in conjunction with a thyratron-type tube, provides a time-delay relay instantly adjustable and capable of operating mechanical devices automatically at predetermined time intervals.

IN THE control of laboratory apparatus and in certain types of industrial control problems, it is frequently desirable to operate mechanical devices or electrical circuits in a cyclical manner with predetermined time intervals. Usually it is required that control devices be of such a nature that they will operate automatically and without attention over long periods of time.

An obvious means of accomplishing this end is to employ a motor-driven rotary switch which develops the required timing pulses. Such devices are not usually capable of instant variation over wide ranges of time intervals and frequently require changing gears or other speed-reduction elements. Rotating contacts are not always reliable for continuous operation, especially when relatively large currents are being interrupted. Thermally operated devices are also available but they usually present the same disadvantages as have been attributed to rotary apparatus.

A less commonly used method of timing is one which relies upon the time characteristics of a resistance-capacitance network for control of the time interval. Such a network, when used in conjunction with a tube of the gas-filled or thyratron type, will provide a time-delay relay which is capable of controlling an electromechanical relay having a current-carrying capacity of considerable magnitude.

The apparatus described here is essentially the same as that described by Goldberg (*3*) for a specific use in photography. Similar circuits with other adaptations have been described by Gilson (*2*), Smiley (*5*), and Mucher (*4*). Of course, the fundamental resistance-capacitance network is well known and is thoroughly treated in textbooks on the subject as well as in summaries in handbooks and similar publications (*1*). The principal advantage of the present apparatus is that it provides a repeated time pulse automatically, whereas that used by Goldberg requires resetting after each operation.

When a condenser is allowed to discharge through a resistance shunted across the condenser the time interval required for the condenser voltage to reach a given level is expressed by

$$t = RC \log_\epsilon \frac{E}{e} \qquad (1)$$

where E = voltage to which condenser is initially charged
e = voltage to which condenser falls in time t
R = value of shunt resistance in ohms
C = capacity in farads
ϵ = Napierian logarithmic base

Factor e given in Equation 1 as the potential to which the condenser falls in time t, is also the critical grid potential below which the thyratron will conduct a plate current. To determine its value in the absence of experimental data it is necessary to refer to the characteristics chart of the 885 (or 884) tube.

For any given values of E and e the time interval may be made almost any chosen value, since a large number of combinations of R and C may be obtained from parts which are commercially available. Furthermore, the time interval may be controlled by utilizing variable components for either R or C or both.

Figure 1 shows the diagram of a circuit which will deliver an electrical pulse at definite time intervals, with instant control of the interval by the aid of a calibrated dial.

The diagram indicates the use of type 885 gas triode with a 2.5-volt filament supply. The type 884 gas triode which requires a 6.3-volt filament supply may be substituted for type 885 if so desired. The parts used are all available through the usual radio trade channels. In certain instances manufacturers will supply equivalent parts which may be substituted. Care should be taken to secure apparatus of good design and construction. This is particularly true of the R-C network components where condensers of low leakage value and good stability to atmospheric changes are required if accurate and stable calibration is desired. Hence, care should be taken to see that the desired capacities are

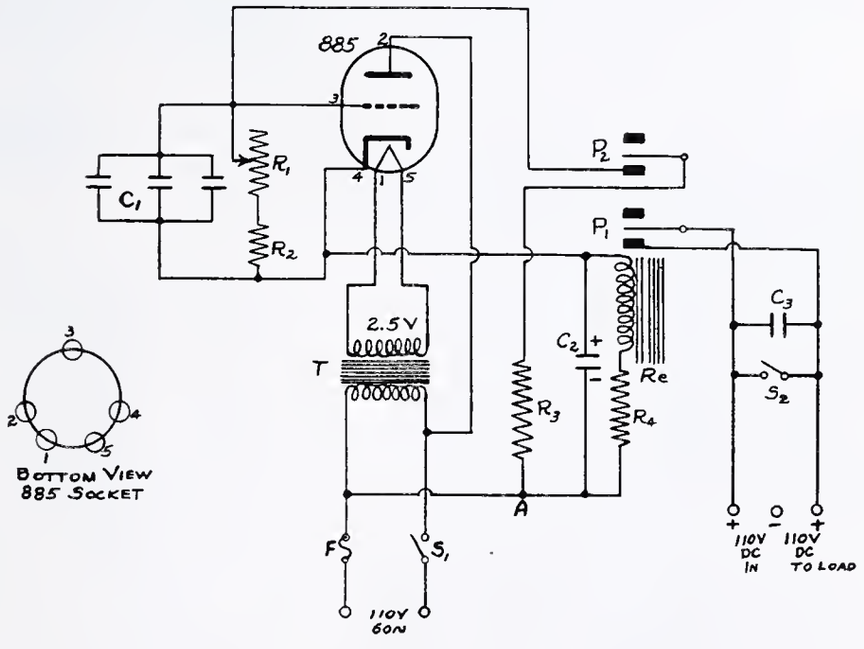

FIGURE 1. WIRING DIAGRAM

C_1.	3 Western Electric telephone type condensers (paper). Actual measured values = 2.23 + 2.18 + 1.10 = 5.51 total μfd.
C_2.	8-μfd. 250 WV electrolytic, Aerovox PBS-8
C_3.	1-μfd. 450 WV paper
R_1.	1-megohm potentiometer, Centralab No. 6 taper
R_2.	50,000-ohm 1-watt carbon
R_3, R_4.	10,000-ohm 1-watt carbons
T.	2.5-volt, 2.5-ampere filament transformer, Stancor P6141
Re.	Double-pole double-throw relay, 2000-ohm coil, Guardian
885.	RCA Type 885 gas-filled triode
F.	2.0-ampere Littelfuse
S_1.	Single-pole single-throw switch
S_2.	Single-pole single-throw switch, nonlocking, for manual control of load

[1] Present address, The Fluor Corporation, Ltd., Los Angeles, Calif.

obtained. Frequently, "rated" or "replacement" values are given for paper condensers which are much greater than the actual effective capacities. Potentiometer R_1 should also be of good construction, so that it will repeat its setting and will not be subject to sudden and unpredictable resistance variations. The relay, *Re*, should be carefully selected, since many of the cheaper varieties are not reliable and may fail to make contact at times because of poor mechanical construction.

The operation of the circuit is simple and may be described by following through a complete cycle.

Let us start at that point in the cycle where a plate current has just started to flow and before the relay has had time to close. The thyratron acts as a half-wave rectifier. Because of the current in the plate circuit the relay coil is energized and there is also a potential drop across the relay coil and R_4 as indicated by the signs of C_2. As soon as the relay contacts have closed, this potential drop acts as an electromotive force in the circuit containing condenser C_1, the terminals of which are connected to the grid and cathode of the thyratron. As a consequence the condenser is charged and the potential of the grid becomes decidedly negative with respect to the cathode. This negative potential of the grid has no effect on the plate current during the time the thyratron is ionized. However, it prevents reionization following the deionization which occurs during that half of each current cycle in which the plate is negative. The rectified current, therefore, ceases and relay contacts P_1 and P_2 open, leaving condenser C_1 charged.

This charge then leaks off through R_1 and R_2 according to Equation 1, and as it does the potential of the grid becomes less negative. After the critical value is reached the tube reionizes as soon as the potential of the plate reaches a peak in the positive direction, thus completing the cycle.

The relay is de-energized most of the time. It receives short pulses at time intervals determined by grid circuit elements C_1, R_1, and R_2. If it is desired to have current flowing in the load circuit during the discharge interval instead of a momentary pulse, the "back contacts" of the relay may be used. The values given for R_1, R_2, R_4, and C_1 provide a time-interval range from 140 to 9 pulses per minute over the range of adjustment of potentiometer R_1. Resistance R_2 determines the minimum time interval available. Changing R_4 will change the time-interval range because of changes produced in the charging voltage, E. If a greater range is desired, a group of resistors may be used with a selector switch as shown by Goldberg (*3*), or other condensers may be switched into the circuit by similar means.

The relay indicated will carry currents up to 5 amperes at 110 volts through its contacts. If the controlled load requires a greater current, other relays may be selected or a second relay of high current-carrying capacity may be operated from points P_1. Either alternating or direct current may be used for load operations. In the particular application shown in Figure 1 the controlled load was actuated by current from a 110-volt direct current source. Condenser C_3 is used to prevent arcing at points P_1. This condenser should be selected to have the best value for the particular reactance and resistance characteristics of the controlled load.

The apparatus described here may be applied to a wide variety of timing operations. For time intervals not available with the components shown in Figure 1 it is only necessary to replace R_1, R_2, and C_1 with components having the desired values which may be calculated from Equation 1.

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Design for Rectifying Column

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FOR rectification of materials boiling in the range −60° to 10° C., there is frequent need for a small low-temperature column which can be readily moved about the laboratory or put away when not in use. Such a column has been described for materials boiling around −50° C. or below, but while simple to operate, it is not easy for the average amateur glass blower to construct. The low-temperature column here described is a simple modification of that described by Simons (*1*), and has been used successfully in this laboratory with liquids boiling at −30° and −5° C.

In Figure 1 is a diagram of the column, which is about 45 cm. (18 inches) in height and fits conveniently into an ordinary quart vacuum flask. *A* is the liquid container with a volume of about 50 cc. Sealed on the bottom is the nipple, *B*, which is wound with a heating coil of asbestos-covered Nichrome wire, B. & S. No. 26. The packed section, *C*, of the column is 9-mm. tubing and contains glass helices supported by a small glass cross bar. *D* is the take-off tube in which is sealed a small thermocouple well, containing a copper-constantan couple. The condenser, *E*, consists of two concentric tubes of 42- and 37-mm. outside diameter, sealed at the top. The 7-mm. tube, *F*, sealed at the top of the condenser, is to permit the escape of air as the material is condensed in *A*. The fractions may be obtained by the use of a three-way stopcock on *D*, or of a chain of traps in series.

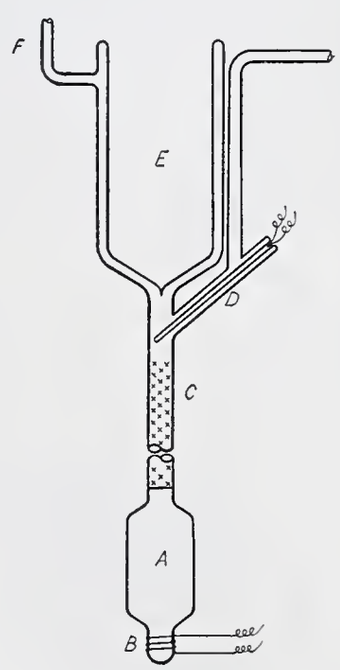

FIGURE 1

The column is very simple to operate. It is placed in a quart-size unsilvered Dewar flask, which is surrounded by a radiation shield of aluminum sheet containing slots cut into it for observation of the pot and reflux. Leaving tube *F* open, the material is condensed in *A* through the take-off tube with the aid of the cooling mixture in *E* and in the Dewar flask.

The Dewar flask is now removed, emptied, and replaced around the column. With *D* closed, *A* is heated if necessary to bring about a reflux. When equilibrium is established, *F* is closed and *D* is opened, and the condensate is received in a series of traps, the last one of which is open to the air. If the take-off is too slow, it may be increased by the use of a very slight vacuum obtained from an ordinary laboratory water-suction pump, applied to the last trap through a stopcock. The amount of take-off can be regulated by adjustments of both the heat supplied to the pot and the vacuum on the receiver. The temperature at the top of the column can be determined with a potentiometer.

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An Improved Soxhlet Extractor

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IN THE course of work on the analysis of commercial soy flours at the laboratory of the Agricultural Marketing Administration, Beltsville, Md., it was necessary to report the oil content values the same day that the samples of soy flour were received. The accepted method for determining the oil content of ground soybeans is to make the extractions in a Soxhlet or Butt extractor with petroleum ether. This method requires from 16 to 20 hours or a 4-hour extraction with regrind after 2 hours. It was much too slow for the needs of the laboratory workers, since results for reports were needed in from 4 to 5 hours.

Among the rapid methods for determining the oil content of oil-bearing seeds are the refractometric method (*5*), the method based on the change in density of a solvent (*1*, *2*), and various shaking procedures (*4*) that employ different types of solvents. All these are not accepted as standard or official for soy flour, but the extraction method is the one adopted by the Soybean Analysis Committee of the Oil Chemists' Society (*3*).

In the author's laboratory, where a battery of 36 Soxhlet extractors is in use, the color of the extract frequently indicates that some extractions proceed faster than others. These differences are probably due to variations in the porosity of the paper thimbles as well as to the manner in which the samples pack themselves within the thimbles. In some cases the retardation of the flow of the solvent is so great that the solvent will flow over the top of the cotton wad in the thimble, rather than through the sides and base of the thimble. Hence, in such cases, very little solvent really percolates through the sample, although if the extraction is allowed to proceed long enough, a complete extraction is obtained in 16 hours.

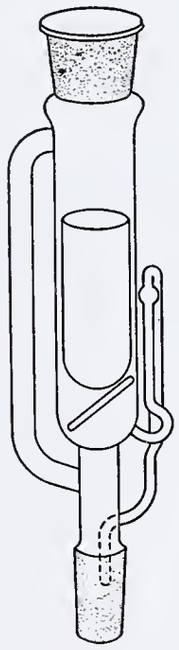

FIGURE 1

It occurred to the writer that having the base of the thimble next to the base of the extractor might retard the flow of solvent through the thimble. Therefore the thimble was elevated by inserting a piece of glass rod at an angle into the extractor and resting the thimble upon the rod (Figure 1). This rod was long enough to raise the thimble 3.75 cm. (1.5 inches) off the base, but the sample (10 grams) within this thimble was still below the overflow siphon of the extractor, so that at intervals the sample was completely immersed in the solvent and channeling was avoided. Another means of achieving the elevation was to make three depressions in the extractor, about 3.25 cm. (1.5 inches) from the base and 120 degrees apart, upon which to rest the paper thimble. The sample was then extracted with Skellysolve F at a rate equivalent to one siphoning about every 3 minutes, whereas with the common method about one minute was required.

The value of the improvement in the Soxhlet extractor can be seen by comparison of the results of extractions by the two methods (Table I). Extraction flasks were changed at the end of 1, 2, and 3 hours, so that each value represents the extraction for each successive hour except in the overnight extraction. All of the soy flours do not extract at the same rate of speed by any method. Extractions varied at the end of the first hour anywhere from 20 to 92 per cent of the total fat content. By use of the elevated thimble method, however, more than 98 per cent of the total fat was extracted during the first hour from all but one of the six samples. The differences between samples are probably due to the nature of each sample as regards its fineness and the distribution of the oil film through the sample. Differences that might be due to variations in porosity of the thimble were eliminated by interchanging the thimbles used in the common extraction in one sample to a position of elevation in the next sample and the elevated one to the common position.

TABLE I. EXTRACTIONS OF OIL FROM SOY FLOUR

(By common method of setting the thimble as compared with improved method of using an elevated thimble)

Fraction	Common		Elevated		Common		Elevated	
	Per cent fat	Per cent of total	Per cent fat	Per cent of total	Per cent fat	Per cent of total	Per cent fat	Per cent of total
	Sample 1				Sample 2			
1st hour	8.36	41.32	20.23	99.30	20.63	91.95	22.40	99.29
2nd hour	4.38	21.66	0.07	0.35	1.65	7.33	0.04	0.18
3rd hour	3.38	16.70	0.01	0.03	0.07	0.32	0.03	0.11
Overnight	4.11	20.32	0.07	0.32	0.09	0.40	0.09	0.42
Total	20.23	100.00	20.38	100.00	22.44	100.00	22.56	100.00
	Sample 3				Sample 4			
1st hour	21.28	95.71	22.02	99.34	10.81	48.66	22.53	99.67
2nd hour	0.79	3.57	0.05	0.20	8.89	40.01	0.00	0.00
3rd hour	0.07	0.31	0.00	0.00	2.52	11.33	0.02	0.09
Overnight	0.09	0.41	0.10	0.46	0.00	0.00	0.05	0.24
Total	22.23	100.00	22.17	100.00	22.22	100.00	22.60	100.00
	Sample 5				Sample 6			
1st hour	13.41	60.75	21.78	98.45	3.81	20.54	14.58	78.06
2nd hour	7.67	34.74	0.19	0.86	2.95	15.91	3.88	20.79
3rd hour	0.77	3.49	0.01	0.05	2.11	11.35	0.03	0.18
Overnight	0.23	1.02	0.14	0.64	9.68	52.20	0.18	0.97
Total	22.08	100.00	22.12	100.00	18.55	100.00	18.67	100.00

Summary

A more rapid method of determining the oil content of soy flour was sought, so that oil content values could be reported the same day samples of soy flour were received by the laboratory of the Agricultural Marketing Administration at Beltsville, Md. To this end an improvement was made in the Soxhlet extractor, the use of which, with petroleum ether, is an accepted method of extraction.

This improvement would no doubt help to shorten the time required to extract other types of materials, such as oil-bearing seeds and plant materials.

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Quantitative Determination of Cellulose in Raw Cotton Fiber

A Simple and Rapid Semimicro Method

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AND
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ALTHOUGH cellulose is the major component of raw cotton fiber, its ratio to noncellulose constituents varies considerably with the growth conditions under which the fiber is produced. For example, analyses have shown that the cellulose of naturally opened bolls may constitute from less than 85 to over 97 per cent of the dry weight of the fiber. It is often desirable to know the cellulose content of cotton fiber. The use of the cellulose content as a criterion of cotton fiber maturity, as suggested by Sakostschikoff (*9*), Gontscharow and Burwasser (*4*), and more recently Conrad *et al.* (*2*), requires a rapid routine method for determination of the cellulose. Furthermore, the evaluation of the quality of cellulose in cotton fiber by means of such measures as copper number, alkali solubility, and fluidity of the cuprammonium solution, makes it necessary that these be expressed on the basis of the cellulose content, if the results are to be truly comparable.

For the routine determination of cellulose, it is desirable to select a method that is as simple and rapid as possible, while still possessing sufficient accuracy for the purpose in hand. Of the many techniques that have been proposed, a large proportion include steps designed to eliminate lignin which so frequently accompanies cellulose. In cotton fiber, lignin may be considered to be approximately, if not completely, absent. This greatly simplifies the procedure by making unnecessary any extensive chlorination treatment.

One of the most practical methods for determining cellulose in the absence of interfering substances is by oxidation and titration. A convenient description of such a procedure, employing potassium dichromate as the oxidant, is given by Launer (*6*); however, for the above-mentioned purpose, it is not necessary to separate the cellulose into its several fractions. In contrast to gravimetric procedures, the tenacious retention of ash constituents is of no consequence in volumetric procedures. However, it is necessary to remove noncellulose organic constituents before oxidation of the cellulose since their oxidation equivalents are similar to that of cellulose.

The chief noncellulose constituents of cotton fibers, aside from the ash, are waxes, pectic substances, nitrogenous substances, and small amounts of residual sugars and pigments. The waxes are a very heterogeneous mixture consisting of alcohols, esters, aliphatic acids, sterols, and hydrocarbons, but as shown by Conrad (*1*) they are rather easily extracted by hot 95 per cent alcohol. The sugars and certain other substances present in small amount are removed by the same solvent. On the other hand, the pectic and nitrogenous substances, while incompletely or not at all removed by the alcoholic extraction, are rather easily removed by boiling with 1 per cent sodium hydroxide solution. Whistler, Martin, and Harris (*10*) concluded that boiling 1 per cent sodium hydroxide destroyed and removed practically all of the pectic substance in the first half hour.

Removal of Nitrogenous Constituents

Since no exactly pertinent data could be found in the literature concerning the rapidity of nitrogen removal by boiling 1 per cent sodium hydroxide, some experiments were undertaken by the writers.

Duplicate samples of finely-cut alcohol-extracted cotton fibers were boiled with approximately 1000 volumes of the solution for 1, 2, and 4 hours. The solution was then filtered off and the residue washed with warm water. The amount of nitrogen was estimated in the residue, using the Kemmerer and Hallett (*5*) micro-Kjeldahl method, but collecting the ammonia in boric acid according to Winkler's (*12*) recommendation. The following results show that the small amounts of nitrogenous constituents remaining in alcohol-extracted cotton are rapidly decomposed by boiling 1 per cent sodium hydroxide and the amount remaining after 2 hours must be insignificant:

Time of Sodium Hydroxide Boil, Hours	Nitrogen, %
0	0.17
1	0.12
2	0.00
4	0.00

Effects of Treatments on the Cellulose

From the above discussion and results it would appear that the noncellulose constituents of cotton fiber may be removed by successive extractions with hot ethyl alcohol and boiling 1 per cent sodium hydroxide. However, attention must be given also to retention of the cellulose. Worner and Mease (*13*) found that continued extraction in a Soxhlet apparatus with hot alcohol and ether had no significant effect on either

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the weight or the properties of cotton fibers after the first 6 hours. On the other hand, extended extraction with boiling 1 per cent sodium hydroxide resulted in progressive loss of the cellulose, at the rate, neglecting the first 6 hours, of 0.34 per cent per hour. The relatively large loss during the first few hours may be assumed to be due to removal of pectic materials. This loss of cellulose was accompanied by a progressive increase in the fluidity (average 0.46 rhe per hour) of the undissolved cellulose, indicating slow progressive hydrolysis of the chain molecules. The work of Davidson (*3*) indicates that the shorter chain fragments dissolve in the alkali. Thus, a compromise must be made in the case of the alkali extraction between complete removal of the noncellulose constituents and the dissolution of the cellulose itself.

Proposed Method

The proposed method allows for a 4-hour extraction of the fiber in a Soxhlet apparatus with hot 95 per cent ethyl alcohol, followed by drying and a 2-hour extraction with boiling 1 per cent sodium hydroxide. Experiments showed that the alcohol extraction, if continued at such a rate that the siphon operated once every 3 minutes, removed approximately 95 per cent of the waxy constituents and it may be assumed that the subsequent alkali extraction removes a considerable portion of the remainder. Since cotton fiber ordinarily contains less than 1 per cent originally, the error from the small residue of these substances must be inconsequential. The 2-hour alkali extraction is chosen as the best compromise for the destruction and removal of pectic and nitrogenous substances with a minimum attendant destruction and dissolution of the cellulose itself. While slight oxidation of the cellulose probably occurs under the conditions employed, its extent could have little effect on the analytical values obtained.

Extract the sample (10 grams or less) of cotton fiber in a large Soxhlet apparatus for 4 hours with hot 95 per cent ethyl alcohol. Remove the fiber from the apparatus, squeeze as dry as possible, and set aside overnight or longer to air-dry. When sufficiently dry, pass through a Wiley (*11*) or similar mill so that the fiber passes a 1-mm. sieve. Store. (When the results are to be used as a basis for reporting copper number, alkali solubility, etc., it is best to expose the fiber for 2 to 3 hours in the vicinity of the balance and weigh out samples for all determinations within a short interval of time.)

Weigh out duplicate 0.1000-gram samples of the finely divided fiber on a small watch glass and transfer with the aid of a camel's-hair brush to a 250-ml. Erlenmeyer flask. With a pipet, add 100 ml. of 1 per cent sodium hydroxide solution (free from carbonates) to the flask and mix with the fiber with a rotary motion. Connect flask to an upright reflux condenser supported over an asbestos wire gauze on a ring stand. Bring to boil and boil for 2 hours. Discontinue heating and filter the mixture with the aid of suction using a 30-ml. fritted Pyrex glass crucible of C porosity, supported in an adapter over a 1-liter filter flask. Wash the mat with 4 approximately 10-ml. portions of hot (60° C.) water. Discard the filtrate and washings.

With the aid of a pointed glass rod transfer as much as possible of the washed fiber mat from the crucible to the original Erlenmeyer flask. Insert into the latter a suitable 2-hole rubber stopper, containing a crucible adapter and glass tube connected to suction line. Insert the crucible in adapter and, with suction turned off, add 15 ml. of 12 molar sulfuric acid. Let stand for a short time (about 2 minutes) to dissolve any cellulose remaining in the crucible and then turn on the suction. Now wash the inside of the crucible twice with 5-ml. portions of 12 molar sulfuric acid, each time interrupting the suction during the addition of the acid, and then turning it on to draw the acid through. Finally wash the crucible with 5 to 10 ml. of distilled water and remove the stopper with adapter from the flask. Accurately pipet 25 ml. of 0.6 *N* potassium dichromate solution into the flask (the total volume should now be 55 to 60 ml.) and boil gently under a reflux condenser for one hour. Remove the flask, stopper, and allow to cool. Add approximately 40 ml. of water and 3 drops of *o*-phenanthroline indicator (1.485 per cent *o*-phenanthroline monohydrate dissolved in 0.025 molar ferrous sulfate solution), and titrate with freshly prepared 0.5 *N* ferrous ammonium sulfate (made up in approximately normal sulfuric acid) until the color changes abruptly from dark green to a deep pink or red.

The difficulty in detecting the color change from a very dark green to a still darker deep pink or red in the titration of the dichromate solution with ferrous ammonium sulfate has been entirely eliminated by titrating above a ground-glass platform beneath which an electric light is fixed.

The ferrous ammonium sulfate is standardized immediately before use by titrating 25-ml. portions of the 0.6 *N* potassium dichromate solution in a flask containing 12 ml. of 95 per cent sulfuric acid, 90 ml. of distilled water, and 3 drops of *o*-phenanthroline indicator.

The weight of pure cellulose in the original sample is computed on the assumptions that it is represented by the empirical formula $C_6H_{10}O_5$, that complete oxidation to carbon dioxide and water occurs, and that 1 ml. of normal potassium dichromate is equivalent to 0.00675 gram of cellulose. Actually Launer (*6*) found an average equivalent value of 0.00677 gram for cellulose from rags, pulp, and paper.

The percentage of cellulose may be expressed in terms of dry weight with the aid of a moisture determination, or the value as found may be used in lieu of a moisture determination as a basis for reporting copper number and other characteristics of cotton cellulose when a knowledge of the moisture content of the fiber is immaterial.

The method here described has many advantages to recommend its adoption. It requires a minimum of equipment, all of which is readily available in any chemical laboratory. The use of small samples results in material savings of reagents and conservation of sample, and permits use of the method where the quantity of available material is limited. Handling of the sample during analysis is reduced to a minimum and the whole sample is titrated rather than an aliquot portion.

Obviously, the method could be adapted readily to a gravimetric determination of the residual cellulose if other determinations on the cellulose sample are required, and it could be used in analyses of fabrics after removal of sizing. The method is not suitable, of course, for cellulosic materials containing more or less lignin.

Comparison with Other Methods

In order to appraise the proposed method, the results obtained with it on a series of ten cottons, selected for a wide range of cellulose content, were compared with those obtained on portions of the same samples by two other well-known methods. The methods chosen for comparison were the Norman and Jenkins method (*7*) which was applied after a 2-hour alkali extraction, and the monoethanolamine method described by Reid, Nelson, and Aronovsky (*8*). However, both of these latter methods were adapted to 0.1000-gram samples by reducing the quantities of reagents but maintaining the same concentrations, ratios of quantities to that of sample, and times for reaction as in the original procedures. In all other ways attempts were made to maintain as nearly as possible the conditions used in the macromethod. Also, in all methods the residual cellulose was determined volumetrically by dissolving and oxidizing the sample in acid dichromate and determining the amount of dichromate used. This same technique was employed for all three procedures, in order that the conditions at this stage of the analyses might be comparable.

In the case of the monoethanolamine method, a 19 × 125-mm. Pyrex test tube, fitted by means of a ground-glass joint to an 8-mm. air condenser 200 mm. high, was substituted for the refluxing apparatus described by Reid, Nelson, and Aronovsky (*8*). It was supported in a glycerol bath, provided with a false bottom, and maintained at 190° ± 3° C.

The samples were prepared for analysis by extraction of 10 grams of raw cotton with 95 per cent ethyl alcohol for 4 hours in a large (50 × 250 mm.) Soxhlet extractor. The cotton was then removed from the extractor, squeezed as dry as possible, and allowed to dry in the air overnight. The material was passed through a Wiley mill, equipped with a 1-mm. sieve, and placed in 120-ml. (4-ounce) bottles for storage. Subsamples, in duplicate, for each of the three cellulose methods and for moisture were weighed out consecutively in such a way as to avoid exposure to serious fluctuations in atmospheric moisture content. The results are shown in Table I.

By reference to Table I, it will be seen that the proposed method gave values intermediate, on the average, between those obtained by the Norman and Jenkins and the monoethanolamine methods. Cellulose content found by the monoethanolamine method is, on the average, higher by 1.33 per cent than that found by the proposed method, while by the Norman and Jenkins method it averages 0.63 per cent lower. Statistically, the mean deviations are in both cases highly significant, indicating that they are real and not accidental.

Table I. Cellulose Found in Alcohol Extracted Cotton Fiber

Sample No.	Cellulose[a] Found: Proposed	Norman and Jenkins	Monoethanolamine	Deviations of Proposed Method from: Norman and Jenkins	Monoethanolamine
	%	%	%	%	%
1124	93.15	91.94	94.35	1.21	−1.20
1137	92.30	92.58	94.01	−0.28	−1.71
1138	93.22	92.88	94.08	0.34	−0.86
1144	90.18	89.76	92.24	0.42	−2.06
1410	84.06	83.37	84.53	0.69	−0.47
1411	90.38	89.20	91.80	1.18	−1.42
1412	83.98	83.40	85.90	0.58	−1.92
1414	87.50	86.13	88.50	1.37	−1.00
1415	93.21	92.14	93.96	1.07	−0.75
2494	83.19	83.48	85.07	−0.29	−1.88
Av.	89.12	88.49	90.44	0.629 ± 0.188	−1.327 ± 0.175

[a] Based on oven-dry weight.

The slightly larger average percentage of cellulose obtained by the proposed technique than by the Norman and Jenkins method is probably to be expected since the latter, designed for removal of lignin in lignin-bearing materials, involves treatment essentially oxidative and additive to that employed in the proposed technique. Whether the difference is due to a greater removal of the noncellulose impurities by the Norman and Jenkins method or to slightly greater loss of the cellulose itself cannot be stated with certainty.

Based on the findings of Worner and Mease (*13*), there should be a loss of 0.68 per cent of cellulose during the 2-hour period of boil with 1 per cent sodium hydroxide. The proposed method gives a yield of cellulose 1.33 per cent less than the ethanolamine method. Taking into account the 0.68 per cent of cellulose that would be lost during the 2-hour boil, there remains a difference of 0.65 per cent of cellulose still unaccounted for. A tendency for the ethanolamine method to give slightly higher yields of crude ash-free cellulose than either the Cross and Bevan or Norman and Jenkins method was noted by Reid, Nelson, and Aronovsky (*8*), using entirely gravimetric techniques. Thus, their results on five agricultural wastes and two spruce wood samples show an average of 54.11 per cent "crude cellulose corrected for ash" by the ethanolamine method, 52.61 per cent by the Cross and Bevan method, and 53.54 per cent by the Norman and Jenkins method. On the other hand, the average yields of "pure cellulose" on these same materials were 39.89, 40.40, and 39.69 per cent, respectively, indicating very little difference in the amount of actual cellulose retained by the three methods. The differences in yield between crude and pure celluloses were due, principally, to pentosans which are incompletely removed by any of the methods. The difference in the present case is therefore in the same direction as observed by Reid, Nelson, and Aronovsky.

Summary and Conclusions

A new, simpler, and more rapid semimicromethod for the quantitative determination of cellulose in raw cotton than heretofore available accomplishes the maximum removal of the accompanying organic noncellulose materials with a minimum of equipment, sample, and damage to the fiber itself.

The method consists of a 4-hour Soxhlet extraction with hot ethyl alcohol followed by drying and a 2-hour extraction with boiling 1 per cent sodium hydroxide. The cellulose is determined by dichromate oxidation of the whole sample and titration of the residual dichromate with ferrous ammonium sulfate.

The waxes, sugars, pectins, nitrogenous, and certain other substances present in small amounts in raw cotton are so thoroughly removed by the treatment that the error due to residues of these substances must be inconsequential.

In comparative analyses of ten different cottons, the average per cent of cellulose was slightly higher by the proposed technique than by a semimicroadaptation of the Norman and Jenkins procedure and lower than that obtained by a similar adaptation of the Reid, Nelson, and Aronovsky ethanolamine procedure.

The method requires no equipment which cannot be made readily, or assembled from apparatus and materials found in the average laboratory.

A special feature of the procedure is the titration of potassium dichromate solutions with ferrous ammonium sulfate using *o*-phenanthroline indicator over a ground-glass plate beneath which is an electric light. This is much more rapid and convenient than the usual titration with an outside indicator and spot plate.

The proposed method is recommended particularly for the determination of the cellulose content of raw cotton and may be used in analyses of desized fabrics. It is not suitable for cellulosic materials which contain lignin.

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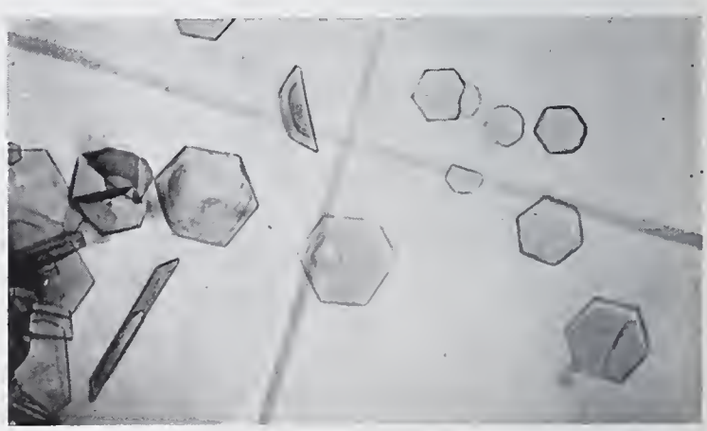

Courtesy, L. M. Willard

Photomicrograph of Potassium Iodide

Reactivity of Substituted Thioureas with Inorganic Ions

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THE examination of the reactions of 60 substituted thioureas with 78 inorganic ions was undertaken to ascertain whether or not any of these reactions might be more sensitive than those given by thiourea, especially for bismuth.

The results presented in this paper show that the substituted thioureas—i. e., compounds containing the reactive group $=N-\overset{\overset{\|}{S}}{C}-N=$, give reactions similar to those of thiourea and that although a number of the reactions are as sensitive, or even slightly more sensitive for bismuth, they offer no advantage to justify their use in place of thiourea.

An attempt was made to correlate the reactivity or nonreactivity of certain ions with the structure of the various substituted thioureas. Because of the similarity of the reactivity no definite conclusions could be formulated. The differences in solubility of the various substituted thioureas undoubtedly play a more important part in their reactivity than does the presence of any particular substituted group. Moreover, no correlation of sensitivity and structure appears to be possible.

The reactions and the sensitivities of the more sensitive ones are given for the substituted thioureas, as well as those for thiourea.

Experimental

The experiments were performed on a spot plate by adding 1 drop of the solution of the reagent to 1 drop of the solution containing the inorganic ion. The reactions were carried out in aqueous, acid, or ammoniacal medium.

Solutions containing 1 mg. per ml. of the following 78 ions were used. (It is recognized that some of the ions are more complex than is indicated by the formulas in this list.): Ag^+, Al^{+++}, $AuCl_4^-$, As^{+++}, AsO_4^{---}, $B_4O_7^{--}$, Ba^{++}, Be^{++}, Bi^{+++}, Br^-, CO_3^{--}, Ca^{++}, CbO_4^{---}, Cd^{++}, Ce^{+++}, Ce^{++++}, Cl^-, Co^{++}, Cr^{+++}, Cs^+, Cu^{++}, Dy^{+++}, Er^{+++}, Eu^{+++}, F^-, Fe^{++}, Fe^{+++}, Ga^{+++}, Gd^{+++}, Ge^{++++} (0.5 mg. per ml.), Hf^{++++} (0.25 mg. per ml.), Hg_2^{++}, Hg^{++}, I^-, In^{+++}, $IrCl_6^{--}$, K^+, La^{+++}, Li^+, Mg^{++}, Mn^{++}, MoO_4^{--}, NO_2^-, NO_3^-, Na^+, Nd^{+++}, Ni^{++}, OsO_5^{--}, HPO_4^{--}, Pb^{++}, $PtCl_6^{--}$, Pr^{+++}, Rb^+, ReO_4^-, $RhCl_5^{--}$, $RuCl_4^-$, S^{--}, SO_4^{--}, Sb^{+++}, Sc^{+++}, SeO_3^{--}, SiO_3^{--}, Sm^{+++}, Sn^{++}, Sn^{++++}, Sr^{++}, TaO_4^{---}, TeO_4^{--}, Ti^{++++}, Th^{++++}, Tl^+, Tm^{+++}, UO_2^{++}, VO^+, WO_4^{--}, Y^{+++}, Yb^{+++}, Zn^{++}, Zr^{++++}.

An alcoholic solution of the reagent containing 10 mg. per ml. was used where the solubility permitted; otherwise a saturated solution was employed.

The reagents were obtained from E. I. du Pont de Nemours & Co., Inc., and the Eastman Kodak Co. and were used without further purification.

The reactions of the following 60 substituted thioureas were observed:

1. Allylthiourea
2. Monolaurylthiourea
3. Dilaurylthiourea
4. *s*-Diethylthiourea
5. Di-*n*-butylthiourea
6. Phenylthiourea
7. Benzylthiourea
8. *o*-Tolylthiourea
9. *m*-Tolylthiourea
10. *p*-Tolylthiourea
11. Xylidylthiourea
12. 2-Carboxyphenylthiourea
13. *o*-Chlorophenylthiourea
14. *p*-Hydroxyphenylthiourea
15. *m*-Nitrophenylthiourea
16. *p*-Fluorophenylthiourea
17. *p*-Ethoxyphenylthiourea
18. *o*-Methoxyphenylthiourea
19. *p*-Methoxyphenylthiourea
20. *p*-Isoamyloxyphenylthiourea
21. *o*-*n*-Butyloxyphenylthiourea
22. 2-Methoxy-5-methylphenylthiourea
23. 2,4-Dimethylphenylthiourea
24. α-Naphthylthiourea
25. Di-α-naphthylthiourea
26. β-Naphthylthiourea
27. *ac.*-Tetrahydro-β-naphthylthiourea
28. *N*-4-Ethoxyphenylpiperidylthiourea
29. Di-*o*-hydroxycyclohexylthiourea
30. Dimethylcyclohexylthiourea
31. Phenylethanolthiourea
32. *N*-(β-hydroxyethyl)-N^1-4-allyloxyphenylthiourea
33. *N*-(β-hydroxyethyl)-N^1-4-isoamyloxyphenylthiourea
34. *N*-Methyl-N^1-4-ethoxyphenylthiourea
35. N-Dimethyl-N^1-4-isopropyloxyphenylthiourea
36. *N*-Dimethyl-N^1-4-ethoxyphenylthiourea
37. *N*-(*n*-Butyl)-N^1-4-ethoxyphenylthiourea
38. *N*-(Di-*n*-butyl)-N^1-4-ethoxyphenylthiourea
39. *N*-Ethyl-N^1-4-isobutyloxyphenylthiourea
40. *N*-(β-Diethylaminoethyl)-*N'*-*n*-butyloxyphenylthiourea
41. *N*-(*p*-chlorophenyl)-N^1-acetylthiourea
42. *s*-Diphenylthiourea
43. *s*-Di-*o*-tolylthiourea
44. *s*-Di-*m*-tolylthiourea
45. *s*-Di-*p*-tolylthiourea
46. *s*-Di(2-methoxy-5-methylphenyl)thiourea
47. Phenyl-*o*-tolylthiourea
48. Diethoxydiphenylthiourea
49. *N*,N^1-di(*p*-*n*-propyloxyphenyl)-thiourea
50. *N*,N^1-di(*p*-bromophenyl)-thiourea
51. *N*,N^1-di(*p*-fluorophenyl)-thiourea
52. *N*,N^1-di(*p*-hydroxyphenyl)-thiourea
53. *N*,N^1-di(*p*-aminophenyl)-thiourea
54. *N*,N^1-di(*p*-acetylamino-phenyl)thiourea
55. *N*,N^1-di(*m*-trifluoromethyl-phenyl)thiourea
56. *m*-Phenylenedithiourea
57. *p*-Phenylenedithiourea
58. Benzidinedithiourea
59. Tolidinedithiourea
60. Poly-*m*-phenylenethiourea

Reactions with Thiourea

Ag^+. Tan precipitate (neutral); black precipitate (ammoniacal)
Bi^{+++}. Bright yellow color (acid)
Ce^{++++}. Decolorized (acid)
Cu^{++}. Brown precipitate (ammoniacal); white precipitate (acid)
Fe^{+++}. Light pink color (acid)
Hg_2^{++}. Gray precipitate (acid)
$IrCl_6^{--}$. Decolorized (acid)
OsO_5^{--}. Brown color (neutral); red color (acid); gray color (ammoniacal)
Pd^{++}. Yellow color (acid and ammoniacal)
$PtCl_6^{--}$. Brown precipitate (ammoniacal)
$RuCl_4^-$. Dark greenish-blue color (acid)
Sb^{+++}. Pale yellow color (acid)
SeO_3^{--}. Red precipitate (acid)
Tl^+. White precipitate (neutral); heavy white precipitate (acid)

Reactions with Substituted Thioureas

The following reactions are listed for each inorganic ion, the numbers referring to the organic compounds listed above. The descriptions of colors and precipitates are probably not complete or exact in all cases, because the reactions are grouped as much as possible for sake of brevity. They are, however, adequate for all practical purposes.

SILVER (Ag^+)

Neutral Medium
Tan to brown precipitates. 2, 3, 9, 11, 13 to 21, 24 to 27, 33, 34, 40 to 52, 55 to 59
White precipitate. 5, 12, 38, 39
Yellow precipitate. 60
Deep purple-brown precipitate. 53, 54

Acid Medium
White precipitate. 2, 3, 5 to 13, 16 to 24, 26, 27, 29, 30, 32 to 39, 41 to 49, 51, 55 to 59
Yellow precipitate. 60

Ammoniacal Medium
All brown to black precipitates except 12, 28, and 38
White precipitate. 38

Gold ($AuCl_4^-$)
Acid Medium
Decolorized. 1 to 5, 7 to 10, 12, 13, 16, 20 to 24, 26 to 39, 41 to 45, 47, 49, 51, 55 to 57
Brown precipitate. 6, 11, 14, 15, 17, 18, 19, 25, 46, 53, 58, 60
Orange color. 40, 48, 50, 52, 54, 59

Bismuth (Bi^{+++})
Acid Medium
Yellow color or precipitate in all cases except 12, 15, and 40

Cerium (Ce^{++++})
Acid Medium
Decolorized. 1 to 11, 13, 14, 17, 18, 20 to 24, 26 to 39, 41 to 47, 51, 52, 53 to 57
Tan color. 12, 19, 60
Pink color. 25, 40
Pale gray color. 48
Blue color. 49
Purple flash, blue color. 53
Yellow color changes to gray precipitate. 58
Pink color changes to gray precipitate. 59

Copper (Cu^{++})
Neutral Medium
White precipitate. 5 to 8, 10, 14, 16 to 24, 26, 27, 32 to 34, 37 to 39, 41, 42, 46, 54, 56, 57, 59
Tan to brown color or precipitate. 9, 44, 50, 51, 53, 60
Green color or precipitate. 12, 13, 25, 30, 40, 45
Unstable red color. 28, 29, 35, 36
Unstable tan color. 43, 47 to 49, 55
Unstable purple color. 52
Green color, changes immediately to blue precipitate. 58
Acid Medium
White precipitate. 5 to 8, 10, 16, 17, 18, 20 to 24, 26, 27, 29, 32, 33, 49, 54, 56 to 59
Pink color or precipitate 3, 9, 13, 25, 43, 44, 47
Brown precipitate. 30, 53
Unstable red color. 11, 28, 35 to 39, 41, 42, 46, 48, 50, 51, 55
Ammoniacal Medium
Gray black precipitate. 20, 21
Brown-black precipitate. 9, 14, 16, 22, 23, 33, 34, 44, 52, 57
Blue color discharged. 1, 7
Brown precipitate. 2, 5, 6, 8, 10, 11, 13, 15, 17, 18, 24, 26, 27, 28, 31, 35 to 43, 45 to 49, 51, 54, 56, 58, 59, 60
Gray-green precipitate. 12, 19, 25, 30, 32
Purple precipitate. 50, 53, 55

Iron (Fe^{+++})
Acid Medium
Pale yellow to tan color. 1, 4, 6, 8, 10, 11, 13, 16, 17, 27, 31, 56, 57, 58, 59
Yellow color, changes immediately to purple. 53

Mercury (Hg_2^{++})
Acid Medium
Gray to black precipitate in all cases except 60
Yellow precipitate. 60

Mercury (Hg^{++})
Neutral Medium
White precipitate. 5, 9, 11, 12, 13, 16, 18, 20 to 23, 25 to 27, 33, 34, 37, 39, 41 to 43, 45, 46, 47, 49, 51, 54, 57
Slightly gray precipitate. 53
Yellow precipitate. 60
Acid Solution
White precipitate. All except 1, 4, 6, 14, 15, 19, 31 35, 36, 52, 53, and 60
Yellow precipitate. 60

Iridium ($IrCl_6^{--}$)
Acid Medium
Decolorized. All except 12, 15, 25, 53, 58, 59, 60
Slightly gray precipitate. 25, 53, 58, 59
Yellow-brown precipitate. 60
Ammoniacal Medium
Unstable blue color. 14

Osmium (OsO_5^{--})
Neutral Medium
Yellow to tan color or precipitate. 2, 5, 7, 8, 10, 17, 21, 23, 24, 28 to 30, 32, 33, 35, 36, 38, 43, 47, 48, 56, 58 to 60
Red to brown color or precipitate. 3, 4, 9, 11, 15, 16, 18, 42, 44 to 46, 54, 55
Pink color or precipitate. 6, 12 to 14, 19, 20, 25, 31, 34, 37, 39 to 41, 49 to 52, 57
Acid Medium
Pink color or precipitate. 5 to 8, 10, 13, 14, 17, 19, 21, 24 to 26, 29, 38, 41 to 43, 45, 47 to 49, 51, 53 to 59
Yellow to tan color or precipitate. 3, 30, 60
Blue color or precipitate. 28, 35, 36
Red to purple color or precipitate. 4, 9, 11, 15, 16, 18, 20, 22, 23, 31 to 34, 37, 39, 40, 44, 46, 52
Ammoniacal Medium
Yellow to tan color or precipitate. 2, 3, 12, 15, 20, 21, 23, 28, 31, 33, 37, 39 to 41, 43, 54, 59
Red to brown color or precipitate. 9, 11, 14, 16, 18, 22, 24 to 26, 32, 34, 42, 44 to 47, 49 to 51, 53, 56 to 58, 60
Purple color or precipitate. 6, 8, 10, 13, 17, 19, 52, 55

Palladium (Pd^{++})
Acid Medium
Orange color or precipitate. In all cases
Ammoniacal Medium
Yellow color or precipitate. 1, 5 to 14, 16 to 21, 23 to 28, 32, 33, 37, 42 to 47, 51 to 53, 55 to 59

Platinum ($PtCl_6^{--}$)
Acid Medium
Yellow color or precipitate. In all cases except 15
Ammoniacal Medium
Slight tan color or precipitate. 1, 6 to 8, 10, 17
Red color. 14

Ruthenium ($RuCl_4^-$)
Acid Medium
Brown color. 2, 4 to 8, 10, 11, 14, 16 to 24, 26 to 29, 31 to 37, 39, 41 to 44, 47, 52, 56, 57
Green blue color. 1

Antimony (Sb^{+++})
Acid Medium
Pale yellow color or precipitate. 2, 3, 5, 9, 11, 18, 20, 21, 23, 24, 27, 28, 30, 33, 34, 37, 39, 43, 44, 46, 49

Selenium (SeO_3^{--})
Acid Medium
Red color or precipitate. 1, 5 to 8, 10, 14, 16, 17, 19, 21, 24, 29, 33, 34, 39
Pink color or precipitate. 2, 4, 23, 27, 28, 31, 32, 36, 37, 43
Yellow color or precipitate. 9, 11, 20, 30, 35, 38, 41, 42, 44, 47, 52, 56, 57

Thallium (Tl^{++})
Ammoniacal Medium
White precipitate. 9, 11, 12, 18, 42, 44, 46, 47
Pink color or precipitate. 21, 33, 39, 55
Yellow to tan color or precipitate. 25, 43, 48, 51, 56, 58

Sensitivity

The sensitivities for the different inorganic ions as observed on the spot plate are reported for thiourea and for the substituted thioureas. Reactions not sensitive to 5 p. p. m. are not listed.

For thiourea: Bi^{+++}, 2 p. p. m.; Pd^{++}, 5 p. p. m.; SeO_3^{--}, 5 p. p. m.
For substituted thioureas:

Bismuth (Bi^{+++})
0.5 p. p. m. 58
1 p. p. m. 3, 20, 27, 30, 37, 39
2 p. p. m. 2, 5, 9 (color fades), 11, 18, 38, 41, 44, 46, 56
3 p. p. m. 4, 10, 13 (color fades), 22 (color fades), 23, 24, 28, 47, 55
5 p. p. m. 1, 7, 8, 21, 60

Copper (Cu^{++})
Acid Medium (color fades in all instances)
1 p. p. m. 11, 28, 30, 46, 47
2 p. p. m. 18, 25, 37, 41, 55
3 p. p. m. 35, 36, 38, 39
Ammoniacal Medium
1 p. p. m. 11, 14 (color fades)
2 p. p. m. 18, 25, 26 (color fades), 46, 52
3 p. p. m. 10, 13 (color fades), 22 (color fades), 23 (color fades, 24 (color fades), 27, 37, 38, 44 (color fades), 45, 47 (color fades), 50, 59
5 p. p. m. 9 (color fades), 16 (color fades), 17, 27 (color fades), 33 (color fades), 39 (color fades), 41, 42 (color fades), 48 (color fades), 51, 60

PALLADIUM (Pd^{++})

2 p. p. m. 9, 11, 18, 46
3 p. p. m. 3, 8, 13 (color fades), 16, 17, 21, 22, 24, 25, 27, 28, 30, 33, 36 to 39, 41, 43, 44, 48 to 50, 54, 55, 58
5 p. p. m. 1, 2, 4 to 7, 10, 12 (color fades), 20 (color fades), 23, 26, 29, 31, 32, 35, 40, 42, 45, 47, 51, 57, 59, 60

Summary

The reactions of 78 inorganic ions with 60 substituted thioureas and the sensitivities of the more sensitive reactions are reported. The reactions are very similar to those observed for thiourea and no definite relationship between reactivity and structure could be formulated. A number of the substituted thioureas give reactions with bismuth and copper that are more sensitive than those of thiourea; however, their low solubility is a disadvantage.

THIS investigation was supported partly by a grant-in-aid from the Carnegie Corporation of New York. It is the third of a projected series based upon studies of organic reagents in inorganic analysis. These studies are being conducted as a cooperative effort in which ten institutions are participating under the direction of John H. Yoe. Those cooperating with the University of Virginia are: Hampden-Sydney, Mary Baldwin, Randolph-Macon (Ashland), Virginia Military Institute, Virginia Polytechnic Institute, Washington and Lee, William and Mary, University of North Carolina, and Tulane University.

Micro-Kjeldahl Nitrogen Determination without Use of Titration Procedure

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THE work described in this paper has for its objective an extension of the Wagner (*1*, *2*) boric acid modification of the Kjeldahl nitrogen determination involving no standard titration solution. The ammonia is evolved in the usual manner, absorbed in dilute boric acid, and evaluated by the determination of the pH of the absorbing solution after dilution to a definite volume.

A recent critical survey of the Kjeldahl method has been made by Zakrzewski and Fuchs (*4*), whose survey includes a complete discussion with a bibliography of research on the method prior to 1937. No further reference to the literature of the subject is therefore necessary.

KJELDAHL METHOD AS MODIFIED BY WAGNER. In the Wagner procedure the digestion of the sample and the distillation of ammonia in the presence of excess alkali are carried out in the usual manner. The distilled ammonia is absorbed in 4 per cent aqueous boric acid solution and is titrated using standard acid with methyl red as indicator. The difficulty involved in the Wagner procedure, especially as applied to the microprocedure, is that of obtaining sufficiently sharp indicator change upon the completion of the neutralization of ammonia.

Present Modification of Wagner Procedure

The first attempt to improve upon the indicator titrational method of Wagner was to substitute a glass electrode pH meter for the methyl red indicator. This proved no more satisfactory than the use of the indicator because of the buffering effect of the boric acid present. This also explains the difficulty met in the use of methyl red as indicator for this titration.

The next attempt involved taking advantage of the buffer capacity of the boric acid by titrating the ammonium hydroxide with 0.01 *N* sulfuric acid to a definite pH, that of the pH represented by the boric acid solution alone. If the total volume of the solution is carefully controlled, and the total volume of boric acid accurately measured, very satisfactory results are obtained, as will be subsequently shown (Table I).

The last method of approach consisted in the determination of the amount of ammonia absorbed by the boric acid by determination of the change in pH of this solution due to the addition of ammonia. This procedure involves no standard titrating solution. Exact volumes of boric acid solution are treated with varying amounts of standard 0.01 *N* ammonium hydroxide and diluted to a definite volume. The pH's of a series of such solutions thus prepared are plotted as a function of the ammonia content. The unknown amounts of ammonia from Kjeldahl digestions and distillations are then determined by the same procedure, reading the amounts of ammonia present as a function of the pH of the solution by reference to the calibration plot previously obtained. By preparing a large volume of boric acid solution for absorption of the

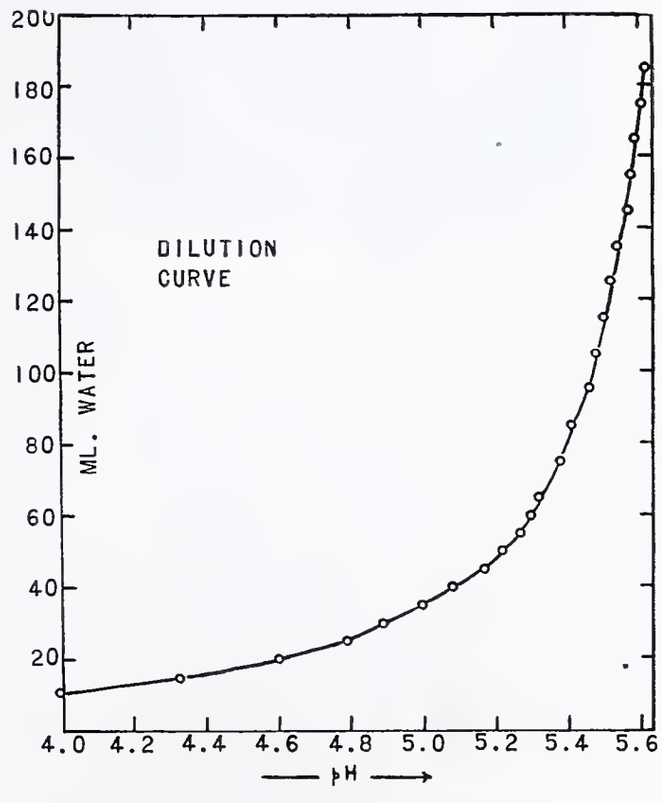

FIGURE 1. VARIATION IN pH WITH DILUTION
10 ml. of 4 per cent H_3BO_3

Table I. pH of Boric Acid Solution as a Function of Added Ammonium Hydroxide

(10.00 ml. of 4 per cent boric acid diluted to 150 ml.)

0.01 N NH_4OH	Total Volume	pH	0.01 N NH_4OH Found		Error, 0.01 N NH_4OH	
			By titration	By pH	By titration	By pH
Ml.	*Ml.*	Found	*Ml.*	*Ml.*	*Ml.*	*Ml.*
2.15	152.2	6.53	2.16	2.20	+0.01	+0.05
2.62	152.6	6.60	2.65	2.57	+0.03	−0.05
3.26	153.3	6.72	3.20	3.35	−0.06	+0.09
3.74	153.7	6.77	3.73	3.72	−0.01	−0.01
4.31	154.3	6.83	4.35	4.25	+0.04	−0.06
4.87	154.9	6.90	4.80	5.00	−0.07	+0.13
5.44	155.4	6.94	5.44	5.42	±0.00	−0.02
5.80	155.8	6.97	5.83	5.85	+0.03	+0.05
			Av. algebraic error		−0.004	+0.023

evolved ammonia one calibration curve can be made to apply to a large number of determinations. A new calibration curve is required for each new lot of boric acid solution. The new procedure is therefore best adapted to use in a routine Kjeldahl process where large numbers of determinations are in demand.

Preparation of Calibration Curves

Since the measurement of pH had to be made at definite volume, it was necessary to determine the most favorable volume to employ—that is, to establish the minimum volume at which the change in pH of the boric acid solution would be negligible upon addition of a small increment of solvent. For this purpose a dilution curve was plotted by adding definite increments of water to 10 ml. of 4 per cent boric acid solution, followed by the determination of the pH. The data thus obtained are shown in Figure 1.

From a study of the data plotted in Figure 1, the dilution of 10 ml. of 4 per cent boric acid solution to 150-ml. volume was selected as suitable. At this volume a further dilution with 10 ml. of water altered the pH by only ±0.01 pH (an amount representing approximately the limiting precision of the usual industrial model direct-reading pH-meter).

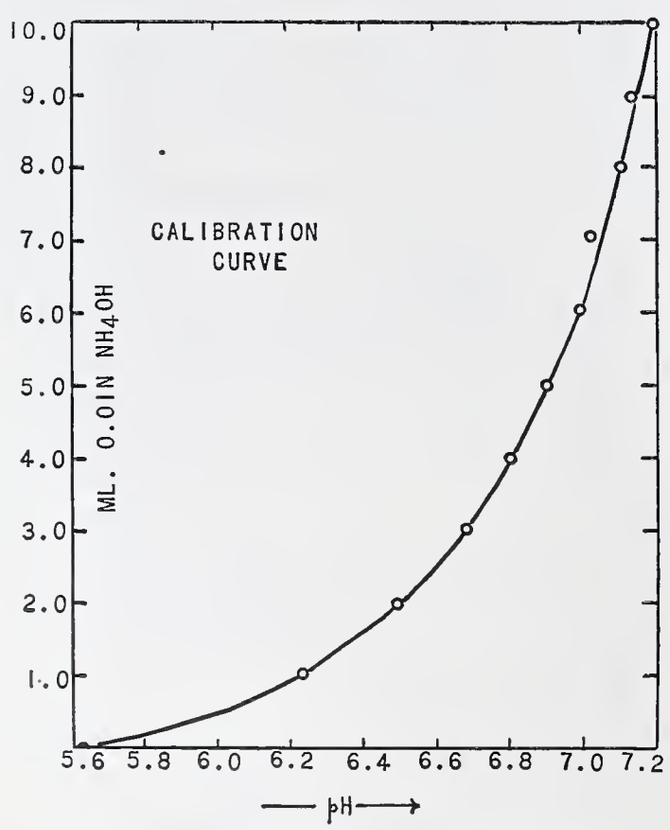

Figure 2. Variation in pH as a Function of Ammonium Hydroxide

A calibration plot was then prepared by the addition of definite amounts of 0.01 N ammonium hydroxide solution to 10.00-ml. portions of 4 per cent boric acid solution diluted to 150 ml. with ammonia-free water. The pH of the solution was determined and in addition the ammonia was titrated using 0.01 N sulfuric acid to the pH represented by the original diluted boric acid solution. The results are shown in Table I.

The data of Table I, columns one and three, are plotted in Figure 2. The data of column five were taken from the plot thus found. The calibration curve of Figure 2 was used for the subsequent estimations reported in this work and checked by direct titration. The estimation of ammonia by determination of the pH is seen by the data of Table I to be somewhat less accurate than that obtained by direct titration to a definite pH.

To check further the accuracy of the new procedure, varying amounts of standard ammonium chloride solution were introduced into the Kjeldahl distillation apparatus and the ammonia evolved was distilled into 10.00 ml. of 4 per cent boric acid. The pH was measured at a volume of 150 ml. and the ammonium hydroxide was titrated, using 0.01 N sulfuric acid, to the original pH of the boric acid alone. The results are shown in Table II.

Entire Kjeldahl determinations were carried out using the modified procedure, with the results given in Table III.

Table II. Determination of Ammonium Hydroxide from Known Amounts of Ammonium Chloride

(0.452 mg. of NH_4Cl per ml. = 0.845 ml. of 0.01 N NH_4Cl per ml. 10.00 ml. of 4 per cent boric acid solution used with final dilution to 150 ml. before determination of pH and titration of NH_4OH)

NH_4Cl Taken	pH	NH_4OH Found and Added			Error	
		Added	By pH	By titration	By pH	By titration
Ml.	Found	*Ml.*	*Ml.*	*Ml.*	*Ml.*	*Ml.*
2.50	6.52	2.12	2.12	2.15	±0.00	+0.03
3.50	6.68	2.96	3.05	3.00	+0.09	+0.04
4.50	6.77	3.80	3.76	3.79	−0.04	−0.01
5.50	6.88	4.65	4.71	4.62	+0.05	−0.03
6.50	6.95	5.49	5.52	5.50	+0.03	+0.01
			Av. algebraic error		+0.026	+0.008

Table III. Analysis of Pure Aminoid-Nitrogen Compounds

Compound	Nitrogen Theoretical	Nitrogen Found
	%	%
$C_6H_5NHCOCH_3$ (acetanilide)	10.36	10.15
		10.55
		10.29
		Av. 10.33
$C_6H_5SO_2NH_2$ (benzene sulfonamide)	8.90	8.81
		8.82
		8.99
		Av. 8.87
$C_6H_4NH_2HSO_3H_2O$ (sulfanilic acid)	7.32	7.50
		7.40
		Av. 7.45

Detailed Procedure for Modified Wagner Microdetermination of Nitrogen

The apparatus used and the technique employed were those described by Niederl (*3*) and involve no modification of the original Pregl equipment. The sample, following digestion to decompose all organic matter in the usual way to convert all nitrogen to ammonium sulfate, is transferred to the standard Pregl distillation microapparatus. A slight excess of 50 per cent nitrogen-free sodium hydroxide is added and the liberated ammonia is distilled into a 125-ml. Erlenmeyer flask containing exactly 10.00 ml. of 4 per cent boric acid solution. When the total volume of distillate has reached 25 ml., the distillation is discontinued. (The amount of boric acid solution is accurately determined using a microburet.)

The solution is transferred to a 250-ml. beaker and diluted to 150 ml. with nitrogen-free boiled conductivity water. The pH of the solution is then measured by means of an industrial model direct-reading Beckman pH meter and the corresponding milli-

liters of 0.01 *N* ammonium hydroxide are obtained from the calibration curve obtained as previously described. From the data thus obtained the per cent of nitrogen in the original sample may be calculated.

The pH of the solution is dependent upon the amount of boric acid present as well as the amount of ammonia and thus it is necessary to measure the amount of boric acid very accurately. In addition, it is necessary to prepare a new calibration curve whenever a fresh stock of boric acid solution is prepared. Special conductivity water need not be prepared if the calibration curve is made using the same water for dilution or if a blank is run.

Conclusions

Two modifications of the Wagner micro-Kjeldahl procedure avoid the troublesome use of methyl red as indicator. The first method consists in a potentiometric titration to the pH represented by the boric acid absorption solution after dilution to a definite volume and estimation of the ammonia content by reference to a calibration curve. This method is more accurate, but involves the use of a standard titration solution.

The second method, while somewhat less accurate, is not dependent upon the preparation and storage of a standard acid and is admirably suited to routine analyses because of the saving of time otherwise required for a titration.

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Refractive Index Measurements at and above the Melting Point of Solids

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PROBABLY the most widely used method for determining the refractive index of crystalline or solid materials involves use of the "Becke line" phenomenon (*1*), for which a microscope and a series of standard liquids of known refractive index are required. For optimum conditions the liquid series used should include numerous duplicates, in order to obtain immersion media in which the solid material is insoluble. Even for the crystallographically simplest of compounds, isotropic in nature, the procedure is somewhat tedious and time-consuming.

Two courses are possible: (1) Crystals, or fragments, of the solid may be immersed in a progressively increasing or decreasing series of the immersion media of known index until a liquid of similar index is found, or (2) the particle may be immersed in a medium of lower refractive index and a second medium of higher index used for dilution until minimum visibility is attained. In this latter method the index of the medium finally attained must either be computed or be measured with a suitable refractometer. For anisotropic substances the procedure is still more complex. The refractive index varies with the direction of transmission and of vibration of the light in the specimen. Two constants must be obtained for uniaxial and three for biaxial crystals. This necessitates use of a polarizing microscope and proper crystallographic orientation of the material for each determination. The complexity of the measurements required may be understood by referring to the procedure recommended by Larsen (*10*). Inasmuch as most crystalline materials may be classified as anisotropic, it is easy to understand the recent statement that the values (refractive indices) for organic solids have not been so well collected as have those for liquids (*8*).

Many organic chemists working in qualitative organic analysis make routine refractive index measurements of liquids as an easily and conveniently determined physical constant to assist in their identification. Because of the complexity of the apparatus, the specialized technique required, and the labor involved, this useful property is rarely applied by such investigators (*2*) to solid materials.

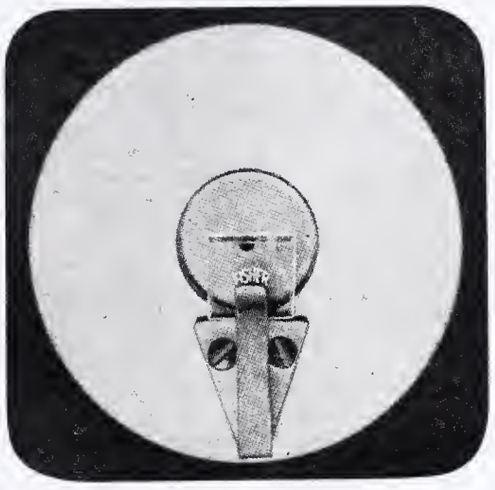

Figure 1. Refractometer Eyepiece

A newly developed attachment for the Fisher refractometer (*5*) now extends the ease of measurements of refractive indices to a much larger group of organic substances. The apparatus permits the simultaneous determination of two common physical constants, melting point and refractive index of the resultant liquid, as well as the approximate estimation of a third, less commonly employed value, the dispersion (*7*). Despite the fact that the method is not applicable to all types of compounds, it is of considerable value to the organic analyst. The substance to be investigated is placed on a heated stage and the temperature raised until the melting point is reached and noted. The heat control may be adjusted to maintain the temperature at the melting point and the refractive index of the resultant true liquid determined. An alternative procedure is to raise the temperature further to some reference point above the melting point and determine the refractive index and dispersion.

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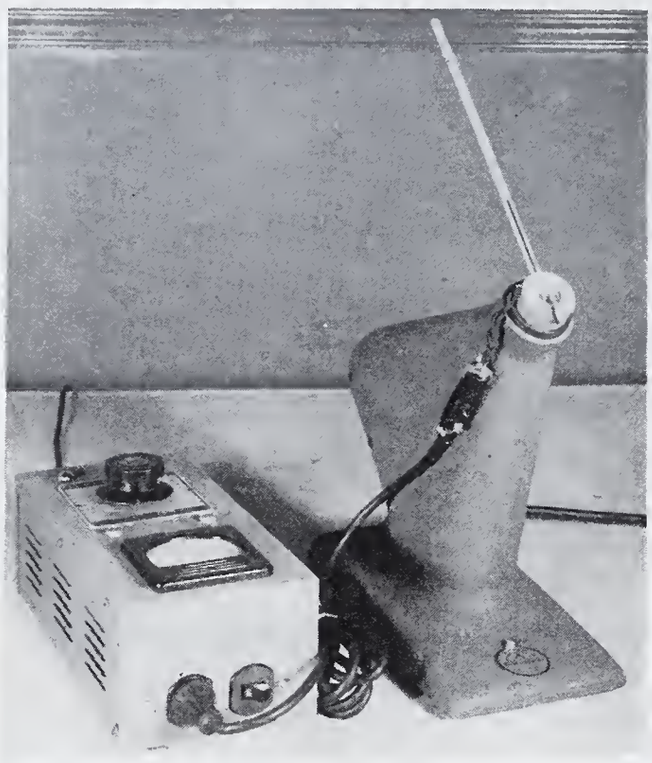

FIGURE 2. LABORATORY SETUP

Apparatus

The instrument employed is a Fisher refractometer, which embodies certain modifications of the principles suggested by Jelley (*6*) and also reported by Edwards and Otto (*3*). An accurately ground and polished glass prism is utilized (Figure 1) for the formation of a "liquid prism" of the substance to be investigated. Simultaneous observation of an illuminated slit and its virtual image, seen through the liquid prism, permits measurement of the vertical displacement of the image from the slit. This distance is dependent upon the refractive index of the liquid investigated. The scale used is calibrated directly in refractive index units from $n = 1.300$ to $n = 1.900$. Measurements are possible to ± 0.002 unit.

For measurements at controlled elevated temperatures a modified eyepiece, consisting of a nickel-plated brass block into which a Nichrome resistance wire heating unit has been sealed, is substituted for the one ordinarily supplied with the instrument. A thermometer, or thermocouple well, is also present. In the instrument used for obtaining the data reported in this paper, a 300° C., red column and magnifying front thermometer graduated in 1° steps was employed. The well is sufficiently deep to permit placing the bulb of the thermometer directly above the peep hole and slightly behind the circular optical glass backing plate.

The temperature was regulated and controlled by plugging the heater cord from the eyepiece into a Varitran (continuously variable auto-transformer) and adjusting the voltage applied between 0 and 60 volts. Fine, stepless, continuous adjustment is thus possible and the instrument may be brought to, and maintained at, any temperature between that of the room and 200° C. For mechanical reasons use of higher temperatures is not recommended.

The complete laboratory setup used for obtaining the data reported herein is shown in Figure 2.

Procedure

The glass plate and prism of the instrument are carefully wiped with a soft cloth, lens paper, or Kleenex, or if necessary, are cleaned by wiping with a cloth wetted with water, benzene, carbon tetrachloride, or any other readily volatile solvent. The prism is then placed on the eyepiece in the position governed by the spring clamp, with the beveled edge forming a well of V-shaped cross section, the lower edge bisecting the small hole behind the permanently mounted optical glass circular plate (see Figure 1). A crystal or fragment (or preferably a few milligrams of finely powdered material) is placed in this well, so that upon melting the liquid will be drawn to the apex and form a liquid prism. The size of sample required depends to a very large extent upon the volatility of the specimen. Two milligrams of sample will suffice for compounds exerting low vapor pressures at the melting point. The heater is plugged into the Varitran and the voltage turned up so that the temperature is rapidly brought to within 5° to 10° of the melting point. The Varitran then should be adjusted so that the temperature continues to rise 1° or 2° per minute.

At the melting point the fragments will liquify rapidly and the resultant liquid will flow into the prism. The exact temperature may now be noted and recorded. Upon depressing the refractometer light switch at the rear of the instrument, the refractive index of the liquid may be read directly from the scale, in the plane of the light slit, by peering through the small hole in the eyepiece. If desired, the heater may be adjusted to maintain the temperature at a predetermined point—i. e., 5°, 10°, 15°, etc., above the melting point—and the index measured under these conditions. Because the readings may be obtained practically instantaneously, it is not necessary to attempt to maintain constant temperatures for appreciable lengths of time. However, since the thermometer bulb is embedded in the block close to the heating element, whereas the sample is separated from the block by a glass plate 1 mm. thick, temperature changes should be effected slowly enough to compensate for thermal lag between the thermometer and sample.

It has been found best policy to clean the glass plate and prism immediately after recording the desired data. This may readily be accomplished by wiping while hot. The Varitran may then be turned to zero, and as soon as the thermometer drops below the melting point of the next specimen to be investigated, the fresh sample may be placed on the eyepiece and studied. A curve indicating the thermometer reading at various settings of the Varitran has been found extremely convenient. By its use approximate temperature settings may be readily made.

TABLE I. COMPOUNDS INVESTIGATED

Compound	Melting Point		Refractive Index at Melting Point		Dispersion Measurements		
	Detd.	Handbook (*9*)	Detd.	Handbook (*9*)	Red	Green	Scale No.
α-Naphthylamine	49	50	1.669	1.6703	1.662	1.698	4
Palmitic acid	62	63–4	1.435	1.430 at 90°	White image		0
Stearic acid	69	69–70	1.434	1.4335	White image		0
o-Nitroaniline	70	71–5	1.660	...	1.638	1.695	6
o-Nitrophenol	89	96	1.569	...	1.560	1.573	2
β-Naphthylamine	109	111–12	1.643	1.6493 at 98°	1.637	1.660	3
p-Nitrophenol	110	113	1.602	...	1.590	1.618	3
m-Nitroaniline	111	114	1.595	...	1.583	1.605	3
o-Tolidine	125	129	1.641	...	1.638	1.659	3
dl-Malic acid	129	128–9	1.450	...	White image		0
Pyrogallol	134	134	1.561	...	1.557	1.566	1
Anthranilic acid	144	145	1.578	...	1.570	1.593	3
p-Nitroaniline	147	146–7	1.670	...	1.655	1.715	7
Ammonium thiocyanate	150	150	1.590	...	1.586	1.598	2
Citric acid	152	153	1.460	...	White image		0
Tartaric acid	169	168–70	1.464	...	White image		0
Potassium thiocyanate	174	173	1.558	...	1.555	1.562	1
Succinic acid	190	189	1.405	...	White image		0

Discussion

With reasonable care in the adjustment of the heater, melting points may easily be determined within 1° or 2° of reported values. The principle is, of course, similar to that used in the Fisher-Johns melting point apparatus (*4*) which has been used by organic chemists for some four years.

Since a regular 110-volt, tungsten filament bulb is used as light source, the beam employed is not monochromatic but "white". For substances having low dispersion—i. e., where all wave lengths of light are similarly diffracted—the virtual image seen is a white image as narrow as the slit itself. For substances having appreciable dispersive powers the image obtained is not a line image but rather a multicolored band. The actual width of the band—i. e., distance between the far edges of the red and violet portions—depends upon

Table II. Difference between Readings Taken on Edges of Red and Green Portions of Image

	Dispersion Scale No.
0 (white image obtained)	0
0.00 to 0.01	1
0.01 to 0.02	2
0.02 to 0.03	3
0.03 to 0.04	4
0.04 to 0.05	5
0.05 to 0.06	6
0.06 to 0.07	7
0.07 to 0.08	8
0.08 to 0.09	9

the dispersive power of the sample. The scale of the instrument has been so constructed that reading of the yellow portion of the spectral band formed indicates the refractive index of the medium as generally determined by using an Abbe or Pulfrich refractometer with light of sodium D wave length. This yellow portion is generally sharply defined as the narrow region between the brilliant red and green portions of the image. The dispersive power of the sample investigated may readily be estimated from readings taken at the visible limits of the spectral band formed. With carefully performed observations the precision attainable, irrespective of the dispersion encountered, is ±0.002 unit. The temperature coefficient may also be ascertained by adjusting the heater so that the temperature slowly rises over the range desired and taking periodic readings on the refractometer as the thermometer reaches predetermined values.

Limitations Encountered. Because of the personal safety factor it is recommended that temperatures in excess of 175° C. be employed but rarely. In making a reading the eye must necessarily be brought close to the specimen; for this reason lachrymatory compounds will be difficult to study. Compounds that sublime cannot be studied. Data will be difficult to obtain for compounds exerting high vapor pressures at or slightly above the melting point. Salicylic acid is an excellent example demonstrating this type of difficulty. Nevertheless there are many organic compounds which do not fall in the above classes and which lend themselves admirably to study by the method and apparatus described.

Data. The data obtained in this preliminary investigation were chosen, not with any specific class of compounds in view, but with the intention of determining the constants on a representative group for the melting range recommended. A large enough group was chosen to include substances ranging from high to low refractive index and from zero to appreciable dispersion. In Table I are listed the compounds investigated, the determined and handbook values for melting point (and refractive index where available), and an indication as to the dispersion observed. The classification of the dispersive power is somewhat difficult to decide upon. Since monochromatic radiations were not used, accurate calculations were not possible (dispersion is usually defined as being proportional to the rate of change of the reciprocal of the velocity with wave length). It seemed likely, however, that some simple, definite, semiquantitative indication of the degree of dispersion would be useful. For that reason the compounds investigated have been classified according to an easily determinable arbitrary method. Readings were taken on the lowest edge of the red and the highest edge of the green portion of the band formed. Readings were not taken to the blue or violet because of the difficulty in locating the edge of these regions. The red and green edges are usually clearly discernible. These readings are also included in Table I and the dispersion scale numbers are based upon the relationships indicated in Table II.

Discussion of Data. For those few compounds for which data could be found in the literature the agreement between the author's values and those previously reported are satisfactory. The compounds used in this study were stock chemicals of the purest grade obtainable commercially but were not crystallized or further purified before use. The determined and handbook values for α-naphthylamine as well as for stearic acid agree remarkably. The handbook value for β-naphthylamine is reported as having been obtained at 98° C., about 14° below the melting point of the pure compound. The author's sample melted at 109°, indicating some impurity, and his value is somewhat less than that mentioned above. The difference between the two values (0.006 unit) may easily be laid to the differences in purity of the two samples and to the temperature difference at which the measurements were made. Small amounts of impurities are apt to have a much larger effect upon the melting point of a compound than upon its refractive index. For this reason the latter property may well be the better for identification studies.

Determination of the specific refraction for such compounds would be an interesting study, although density measurements at the reference temperature would be required. For many compounds the melting and solidification points may be accurately determined by noting the temperature at which the image appears and disappears upon slowly raising and lowering the temperature. Supercooling must be considered for certain samples.

The value of refractive index measurements at elevated temperatures for identification purposes will be enhanced greatly after sufficient data have been obtained and reported to permit the compilation of orderly data tables. In the meantime one may make use of the method by alternate determinations on the unknown material and on known compounds. Obviously, because of the scarcity of low-melting inorganic compounds, the method is not apt to find application in this field. It should prove of value in studies of natural and artificial waxes and similar materials, even though these substances are apt to have melting ranges rather than melting points. A compilation of data for this group of substances is now under way.

Summary

Refractive index measurements of organic compounds at and above the melting point have been proposed for identification studies. Such a procedure necessitates the determination of but a single value, rather than the two or three required on crystalline anisotropic material. This value may be determined more easily and rapidly than similar values for even isotropic materials in the crystalline state.

Apparatus for the simultaneous determination of melting points up to 200° C. and refractive indices between 1.300 and 1.900 has been described. The melting points may be obtained with an accuracy of 1° to 2° C., while the index measurements may be made to ±0.002 unit.

Estimations of dispersion may be made with the apparatus described and a dispersion scale is suggested for classification of compounds.

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Colorimetric Microdetermination of Arsenic

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METHODS for the determination of arsenic are legion for two principal reasons: first, because of the importance of the accurate determination of arsenic in food, biologicals, organic material, etc., and second, because the methods in use are not entirely satisfactory. The literature of arsenic determinations has been surveyed by Minot (*20*), Kleinmann and Pangritz (*16*), and How (*13*).

The methods for the microdetermination of arsenic may be placed in seven groups.

1. The Marsh-Berzelius method (*7*, *18*, *21*) depends upon the generation of arsine and its subsequent decomposition by heat with the formation of an arsenic mirror.
2. The Gutzeit method (*1*, *13*, *15*) depends on the generation of arsine, which in turn reacts with papers impregnated with mercuric chloride or bromide.
3. Variations of this method are the action of arsine on silver nitrate or on other silver and gold salts, as in Rose's method (*9*). Thus Truffert (*30*) used the action of arsine on photographic paper coated with silver citrate to ascertain the arsenic content of materials.
4. The bromate method (*1*) depends on the distillation of arsenious chloride and the subsequent estimation of this compound by a bromate titration.
5. The iodometric method of Cassil and Wichmann (*4*) depends on the evolution of arsine in a special generator; the liberated arsine is trapped by mercuric chloride solution which oxidizes it to arsenious acid, which in turn is estimated by titration with iodine.
6. An example of the nephelometric methods used for the determination of arsenic is that of Kleinmann and Pangritz (*16*). A reagent consisting of equal parts of 1 per cent potassium molybdate solution and 2 per cent cocaine solution, and 2 parts of *N* hydrochloric acid gives a turbidity with small quantities of arsenic pentoxide.
7. Molybdenum blue methods are discussed below.

Gutzeit Method

An official method for the determination of arsenic is the Gutzeit method (*1*, *15*). In 1928, Clarke (*6*) said, "Although the results are very good in some instances, general experience has made it plain that not one of the various modifications of the Gutzeit method can be used by the average analyst with the assurance or probability that his results will be accurate unless he attains considerable experience in its use."

Studies of Gutzeit methods by Barnes and Murray (*2*), Neller (*24*), Gross (*12*), and Mühlsteph (*23*) show that relatively large errors often occur. Using disks, as required by the British Pharmacopeia, is no better than using strips, for arsine may pass unchanged through the disks.

The greatest difficulty in the use of the Gutzeit method is the inability to duplicate the brown arsenic stains on the mercuric bromide papers. There is sufficient evidence in the literature to show that arsine is quantitatively liberated by means of a Gutzeit generator (*13*). The greatest advantage of the Gutzeit method is the simplicity of the generator, the ease of generation of arsine, and therefore the ability to separate arsenic from interferences.

Molybdenum Blue Methods

The molybdenum blue method is probably the most sensitive and accurate for the determination of arsenic. This has been emphasized by Snell (*29*) and by Pierson (*25*).

Phosphorus reacts with ammonium molybdate to form a complex phosphomolybdate, which may subsequently be reduced with the formation of a complex molybdenum compound strongly colored blue. Arsenic undergoes an entirely analogous reaction with the formation of an intensely colored blue complex. This reaction of arsenic and its use in methods for the estimation of arsenic have been discussed by a number of investigators. The term "molybdenum blue complex" is applied to the series of complex oxides formed by arsenic and molybdenum, phosphorus and molybdenum, and molybdenum itself.

The principal variations of the molybdenum blue method for arsenic are those of Maechling and Flinn (*19*), Youngburg and Farber (*32*), Zinzadze (*33*), Morris and Calvery (*22*), and Chaney and Magnuson (*5*).

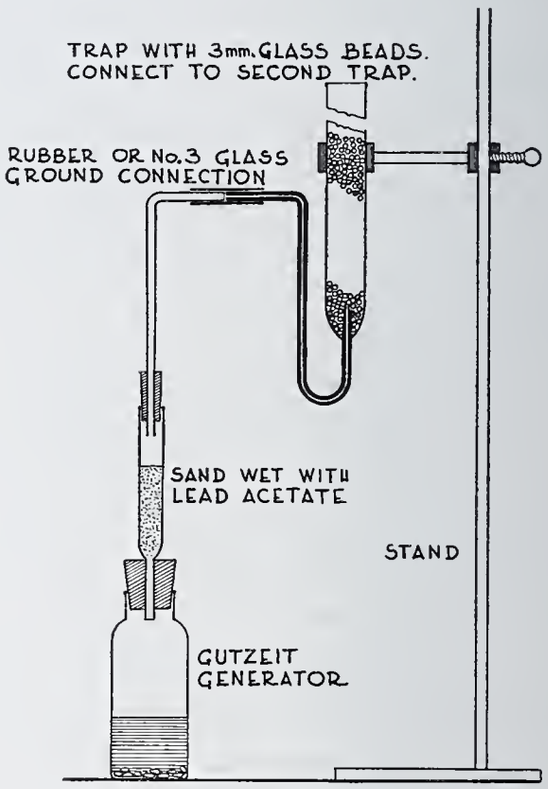

FIGURE 1. APPARATUS FOR ARSINE–MOLYBDENUM BLUE METHOD

The effects of concentration, ionic strength, and interferences on the molybdenum blue reaction were studied by Kuttner and Cohen (*17*) and Schricker and Dawson (*28*).

Arsine–Molybdenum Blue Method

The fact that the Gutzeit method is used so extensively despite its known faults indicates that the methods suggested to replace it do not have sufficient simplicity or adaptability for multiple determinations.

The following method, which is a combination of the official Gutzeit method for the generation of arsine and a simple modification of the molybdenum blue method, has the advantage of simplicity coupled with the ability to be adapted for multiple determinations.

If instead of permitting the arsine to impinge on paper impregnated with mercuric bromide, mercuric chloride, silver nitrate, silver citrate, or some analogous compound, the arsine is absorbed in some trapping solution which oxidizes it to

arsenate, the molybdenum blue method may be used directly for its determination.

The use of bromine water, sodium peroxide, hydrogen peroxide, potassium persulfate, potassium chlorate, potassium bromate, and sodium hypobromite was investigated by the authors. Robinson (*8, 27*) used a normal solution of sodium hypobromite in a Winkler spiral for the trapping of arsine. Deemer and Schricker (*8*) used concentrated nitric acid saturated with bromine but discarded arsine evolution for the trichloride distillation. Cassil and Wichmann (*4*) trapped arsine in mercuric chloride solution, but the authors found that the molybdenum blue method could not be used if mercuric chloride or silver nitrate were the trapping agents. Chaney and Magnuson (*5*) used potassium iodate to oxidize arsenious chloride to arsenate before applying the molybdenum blue test.

The authors found that complete absorption of arsine in one trapping device could be obtained by the use of 3 cc. of a mixture of 3 cc. of half-saturated bromine water and 1 cc. of 0.5 *N* sodium hydroxide solution. The use of bromine water alone necessitated the use of more than one trapping device. Solutions of hydrogen peroxide and sodium peroxide were also inefficient absorbing agents and required a series of bubblers. Potassium persulfate apparently did not oxidize arsine completely to arsenate, although it did oxidize arsenite quantitatively to arsenate.

Apparatus. The apparatus consists of the official Gutzeit generator (*1, 15*) and a trapping device similar to that used for the determination of benzene or toluene in air by the butanone method (*14, 31*). This arrangement is illustrated in Figure 1. If the benzene trap is not available, a simple trap can be made by bending a 17.6-cc. or 20-cc. pipet into the form of the trap shown in the illustration and then filling the bulb portion with beads. The tube containing the mercuric bromide test paper in the usual Gutzeit method is replaced by another tube leading the generated gases to the trapping device.

Preparation of Sample. Make an acid digestion or use the solvent procedure of Wichmann and Clifford as detailed by the Association of Official Agricultural Chemists (*1*) or Jacobs (*15*), or, if necessary, employ combustion methods (*3, 26*). Then prepare an aliquot for an official Gutzeit test as directed in the aforementioned texts. From this point proceed as directed below.

Reagents. Sodium hypobromite solution. Add 0.5 *N* sodium hydroxide solution to half-saturated bromine water in the proportion of 1 cc. of 0.5 *N* sodium hydroxide solution to 3 cc. of bromine water.

Saturated bromine water. Add 2 cc. of liquid bromine to 200 cc. of water in a 250-cc. glass-stoppered bottle, shake well, and allow to stand. Dilute with an equal volume of water before use in the preparation of the sodium hypobromite solution.

Ammonium molybdate solution. Dissolve 25 grams of ammonium molybdate in 300 cc. of water. Dilute 75 cc. of concentrated sulfuric acid to 200 cc. with water and add to the ammonium molybdate solution.

Standard arsenious oxide solution. Dissolve 0.300 gram of arsenic trioxide, As_2O_3, in 25 cc. of 10 per cent sodium hydroxide solution, make slightly acid with sulfuric acid (1 to 6), and dilute with water to 1 liter. Dilute this stock solution, which may be standardized against standard potassium bromate solution, if desired, to an appropriate dilution to make for ease in the removal of aliquots to be used for the standards in the development of the color. Thus, for instance, a 5-cc. aliquot of the stock standard solution diluted to 1 liter yields a solution, 1 cc. of which is equivalent to 1.5 micrograms of arsenic trioxide. This dilute standard should be prepared fresh from the stock standard solution.

Hydrazine sulfate solution. Prepare a saturated solution of hydrazine sulfate, $N_2H_4.H_2SO_4$. Dilute an aliquot of the supernatant liquid 1 to 1 with water.

Sulfuric acid. Prepare a 2 *N* solution of sulfuric acid and standardize against standard alkali in the usual manner.

Procedure. Allow the generation of arsine to proceed as directed in the Gutzeit method and trap the arsine in the bead device to which 3 cc. of sodium hypobromite solution have been added. After generation is completed—that is, after 1 to 1.5 hours—transfer the contents of the trap to a graduated colorimeter tube, Nessler tube, or volumetric flask. Wash the trap with six 2-cc. portions of distilled water, delivering the water to the trapping device with a 2-cc. pipet. Use a rubber bulb aspirator to blow the wash solutions out of the trap into the collection vessel. Press the aspirator bulb gently in this step. Add exactly 5 cc. of 2 *N* sulfuric acid and stir, add 1 cc. of ammonium molybdate reagent, and shake. Add 1 cc. of the half-saturated hydrazine sulfate solution and swirl, make to a volume of 25 cc., and allow to stand for 0.5 hour for full development of the blue color. Compare with standards or a standard treated in a similar way at the same time.

Preparation of Standards. Prepare the standards or standard from the diluted stock standard arsenious oxide solution. Add 3 cc. of sodium hypobromite solution to the aliquot or aliquots selected, dilute to 15 cc. with distilled water, add exactly 5 cc. of 2 *N* sulfuric acid, and stir. Add 1 cc. of the molybdate reagent, stir, add 1 cc. of half-saturated hydrazine sulfate solution, and stir. Make up to the same volume as the test solution. Run a blank on all the reagents as a check.

If a final volume of 25 cc. is to be used in making the comparisons, use exactly 5 cc. of 2 *N* sulfuric acid, in order to have the proper acidity for the development of the molybdenum blue color. If less than this quantity of acid is used, the blank may itself be reduced. If more than this quantity of acid is used, the development of the blue complex will be delayed.

Limitations of Arsine–Molybdenum Blue Method

In general, the procedure for checking this method was the following:

A standard solution of arsenious oxide was prepared as directed above and was standardized against standard potassium bromate solution. An aliquot of the standard arsenious oxide solution was then diluted to an appropriate volume, such as 5 cc. to 1 liter, yielding a solution which contained 1.5 micrograms of arsenic trioxide per cc. Gutzeit generators were prepared as directed by the A. O. A. C. (*1*). To each generator were added an aliquot of the diluted standard arsenious oxide solution and sufficient distilled water to make a total volume of 30 cc. From this point, the method as outlined was followed. Comparisons were made against standards in 25-cc. Nessler tubes. A few comparisons were made with a Duboscq colorimeter. Further comparisons with the Duboscq colorimeter were not made because the plungers often induced the release of gas bubbles.

Completeness of Trapping Arsine. In trapping gases by absorbing solutions in absorbers, incomplete recovery often results (*11, 14*). To overcome this difficulty, multiple absorbers are often used. However, if an absorbing medium is provided in which the gaseous substance undergoes a rapid chemical reaction to form a nonvolatile substance, complete recovery in one absorber is possible. The rate of flow of gas and the type of absorber are also important factors in the degree of recovery.

Using a Gutzeit generator, a scrubbing type of trap, and sodium hypobromite as the absorbing solution, it was possible to trap arsine completely in one absorber. All preliminary experiments were run using two traps in series. No arsenic was found in any of the second traps; hence, the second trap was discarded in making the other tests.

The completeness of trapping of arsine by sodium hypobromite solution was in marked contrast to the incompleteness shown by the use of bromine water, potassium persulfate solution, peroxide solutions, and other oxidizing agents. Indeed, in the case of bromine water, more arsine was found in the second trap, at times, than in the first absorber.

Recovery. Since the trapping of arsine in this method is complete, one would expect the recovery to be 100 per cent. This, however, depends also on the completeness of generation of arsine. The authors found that within the limits of experimental error of this method—that is, the ability to estimate 1.5 micrograms of arsenic trioxide—recovery is 100 per cent. In addition to the use of test solutions with no interferences, known amounts of arsenious oxide of the order of 15 micrograms were added to orange juice which was then subjected to an acid digestion. Orange juice was used because it gave a practically zero blank. Recovery was complete in these instances, also.

Sensitivity. Accurate estimation of arsenious oxide content by this method can be made down to amounts of the order of 1.5 micrograms. At this concentration—that is, 1.5 micrograms in 25 cc. of comparison solution—the color of the test solution is a greenish blue. Lower concentrations of arsenic can be detected, for faint greenish-blue tints are developed, but these cannot be accurately estimated. This in-

dicates that the arsine–molybdenum blue method is far more sensitive than the official Gutzeit method, for which 25 micrograms is considered the optimum concentration.

Amounts of the order of 50 micrograms are too deep to compare accurately by the arsine–molybdenum blue method using visual means. Schricker and Dawson (*28*) state that the limit of applicability of Beer's law for the molybdenum blue reaction is approximately 75 micrograms of arsenic and 30 micrograms of phosphorus. It is not desirable to work with such large amounts.

Interferences (*10*). Phosphorus and silicon form complex molybdates. The use of the Gutzeit generation in the arsine–molybdenum blue method is designed to eliminate these interferences. High concentrations of antimony interfere in the Gutzeit determination. A series of experiments was run to check on the interference of phosphorus, silicon, and antimony. Known amounts of antimony trichloride, disodium hydrogen phosphate, and sodium silicate were added to known amounts of arsenious oxide, of the order of 30 micrograms, in the Gutzeit generator before making a test. No appreciable interference was noted.

Table I. Comparative Determinations

Sample	Official Gutzeit Method			Arsine–Molybdenum Blue Method		
	Aliquot	As_2O_3		Aliquot	As_2O_3	
	Cc.	*Micrograms*	*P. p. m.*	*Cc.*	*Micrograms*[a]	*P. p. m.*
Canned clams	10	7.5	0.75	10	6.0	0.6
	20	12.0	0.60			
			Av. 0.68			
Canned clams	10	5.5	0.55	10	4.5	0.45
	20	8.5	0.43			
			Av. 0.49			
Apple butter	10	12.0	1.2	10	12.0	1.2
	20	22.0	1.1			
			Av. 1.15			

[a] Nearest standard, run in duplicate.

Effect of Concentration of Reagents. The effect of the reagents used in the Gutzeit test itself has been covered in the literature. The concentrations and volumes of the reagents used in the variation of the molybdenum blue method used by the authors must be rigidly observed, if correct results are to be obtained.

Too concentrated solutions of bromine water used for the formation of sodium hypobromite solution prevent the reaction from proceeding properly. The use of solutions of hydrazine sulfate stronger than that recommended—namely, half-saturation—may cause a reduction of the blank. Weaker solutions of hydrazine sulfate may cause no reduction at all or reduction only after long standing. The correct acid concentration is most important. If insufficient acid is present, the blank is reduced by the hydrazine sulfate with the formation of a blue color; on the other hand, too much acid will inhibit the formation of the blue complex in the test solutions.

By keeping the concentrations and volumes of the reagents as specified, the ionic strength of the test and standard comparison solutions is kept practically the same. This tends to make the color comparisons more accurate.

Time of Comparison. The colors are not fully developed until permitted to stand half an hour. At first, greenish tints may predominate over the blue and there may be an apparent nonuniformity of shade. The waiting period provides for color stabilization. The color remains relatively stable for at least one hour after the half-hour period.

Comparison with Official Gutzeit Test

To find the degree of correlation between the arsine–molybdenum blue method and the official Gutzeit test, determinations were made by both methods on products which contained small amounts of arsenic (Table I). No arsenic trioxide was added. One hundred grams each of two samples of canned clams and one of apple butter were prepared for arsenic determinations by means of acid digestions. The wet ash was made up to 100 cc. The official Gutzeit determinations were made by one chemist, while the arsine–molybdenum blue determinations were made by another, each working independently.

Summary

The combined arsine generation and molybdenum blue method for the microdetermination of arsenic is simple and convenient. It is more sensitive than the official Gutzeit test, for accurate determinations of as little as 1.5 micrograms of arsenious oxide can be made.

Its advantages are evident. It makes use of skills and reagents that are well known and combines them into a suitable framework. The apparatus is inexpensive and is available in practically every laboratory. The reagents are simple to prepare and last almost indefinitely. The relative hazard involved in an arsine generation as compared with a hydrochloric acid–arsenious chloride distillation is in the favor of the arsine generation, for while only a minute amount of arsine is ever generated, relatively large quantities of hydrochloric acid are distilled. While the time of generation is comparatively long in comparison with a hydrochloric acid distillation of arsenic trichloride, it is difficult to run multiple arsenious chloride distillations, whereas multiple arsine–molybdenum blue determinations can be run in banks similar to ordinary Gutzeit estimations.

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Orthonitrosophenol as a New Reagent in Colorimetric Analysis

Determination of Cobalt

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ORTHONITROSOPHENOL and some of its metal salts were prepared and described for the first time by Baudisch and co-workers (*3*, *4*). These metal salts are typical organic inner-complex compounds and therefore are usually highly colored. Baudisch mentioned as early as 1912 that the formation of the red-violet, water-soluble copper complex constituted one of the most sensitive qualitative reactions for cupric ions. However, this work was not followed up until recently, when Baudisch discovered new and very simple methods for preparing *o*-nitrosophenol, its homologs, and derivatives (*1*, *2*), based upon the formation of the radical NOH which reacts under suitable conditions with many aromatic compounds to form the corresponding *o*-nitrosophenols. The details of this work will be reported elsewhere.

A study of the metal complexes of about seventy *o*-nitrosophenol compounds revealed certain rules governing their formation and properties, which convinced the author that the *o*-nitrosophenols can be of great value in analytical chemistry.

o-Nitrosophenol forms, in weak acid solutions, strongly colored complex salts with several heavy metal ions. Those of cobalt (grayish brown), palladium (green), and trivalent iron (brown) are distinguished from all the others in that they are easily soluble in petroleum ether and can thus be separated from the original water solution.

The other group consists mainly of divalent copper, and mercury (reddish violet), nickel (red), zinc (red), and divalent iron (green). All these metal salts are soluble in water or certain organic solvents, such as ether, depending upon the conditions under which they are formed, but all are insoluble in petroleum ether.

The color of all the above complexes is stable for at least several hours, and is so intense that most of the ions can be easily detected in concentrations of one part in ten million. Therefore, it seemed worth while to investigate the usefulness of these new compounds for colorimetric analysis.

Preliminary experiments proved that the affinity of *o*-nitrosophenol for the above-mentioned metal ions is very strong. It is sufficient to shake a solution of *o*-nitrosophenol in a solvent, immiscible with water, for a few seconds with a solution of the metal ions in water to form the organic metal complex quantitatively. The color intensity of the solutions thus obtained is completely reproducible and, within the limits of solubility, proportional to the concentrations of the metal.

Unfortunately, because the author had no opportunity to measure the extinction coefficient for the colored solutions, it was impossible to determine exactly to what extent the colored system conforms strictly to Beer's law. However, as is shown in this paper, the system is entirely suitable for use in a photoelectric colorimeter.

Based on these observations, a colorimetric determination of cobalt has been worked out, in which the solution of the cobalt salt in water is shaken with a solution of *o*-nitrosophenol in petroleum ether. After the two liquids have separated, the color intensity of the petroleum ether is measured in a colorimeter.

There are very few limitations to the use of this method. The most important is that acids whose cobalt salts are insoluble at pH 4 must not be present (phosphoric acid, oxalic acid, and others). Of the complex cobalt compounds, only the potassium cobaltic chloride has been investigated and qualitatively it showed the same reactions as the simple cobaltous ions.

According to a private communication from G. H. Ellis, U. S. Plant, Soil and Nutrition Laboratory, Ithaca, N. Y., phosphates present in soil analysis do not interfere in the cobalt determination with *o*-nitrosophenol.

Palladium will not be considered further, since it is seldom present in a cobalt determination. However, since the color of the grayish-brown cobalt compound is quite different from that of the green palladium compound, the determination might be carried out with the help of suitable color filters. The theoretical considerations for the colorimetric analysis of a two-component color system are given by Knudson, Meloche, and Juday (*5*).

The interference of ferric salts can be eliminated in three ways. The surest of these is to precipitate the iron from an acid solution with cupferron, extract the excess cupferron with chloroform or ether, and remove the remaining organic solvent with warm air. A second way is to reduce the trivalent iron quantitatively to the divalent state with isoascorbic acid. However, since difficulties are frequently encountered in this reduction procedure, it has not been worked out in detail. The third possibility consists of the formation of complex ferric compounds, so that the concentration of free ferric ions is negligible. This method is the simplest and in most cases yields satisfactory results. However, in the presence of a large excess of iron, the cobalt value may be somewhat high, probably because the binding of the iron in the complex is not strong enough to exclude entirely its reactions with *o*-nitrosophenol.

In using *o*-nitrosophenol for colorimetric determination, the maintenance of the proper pH is important. The strongest color intensity for a given cobalt concentration is obtained at pH of 3.8 to 4.4. Therefore a pH of about 4.0 must be assured by use of a suitable buffer solution, which at the same time should form a complex compound with trivalent iron without affecting the state of the cobalt ions.

A mixture of sodium citrate and citric acid, made by dissolving 2.1 grams of citric acid in 88.5 ml. of water and adding 11.5 ml. of *N* sodium hydroxide, has been found to be the most suitable buffer solution. The pH of the mixture is 4.0.

A buffer made of tartaric acid and sodium hydroxide may also be used. Phosphate or oxalate buffers are not suitable because they form insoluble cobalt salts. An acetate buffer is not recommended, since its iron complex is not strong enough to prevent reaction of the iron with *o*-nitrosophenol.

The preparation of *o*-nitrosophenol is described by Baudisch (*1*, *2*). Since it can be prepared and is stable only in solution, the proper concentration cannot be given in per cent

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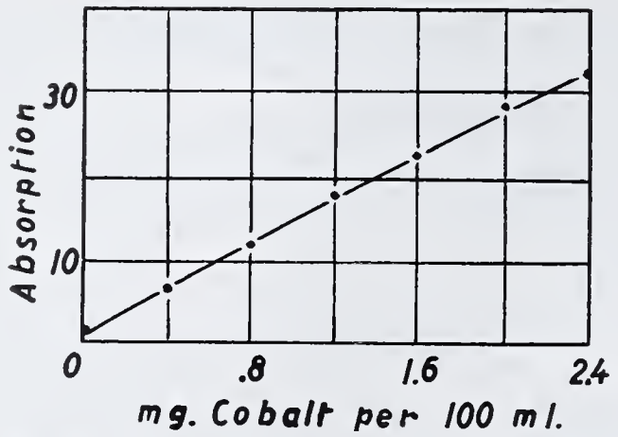

FIGURE 1. CALIBRATION CURVE FOR COBALT DETERMINATION
Absorption = deflection on "absorption" scale of colorimeter

values but must be controlled by the following tests, which should be made at regular intervals:

1. After shaking 1 ml. of the *o*-nitrosophenol solution with 5 ml. of copper sulfate solution (1 per cent) in a test tube, the petroleum ether layer should be absolutely colorless (test for purity).
2. After shaking 2 ml. of the *o*-nitrosophenol solution with 5 ml. of copper sulfate solution (10 mg. of copper sulfate pentahydrate in 1-liter of distilled water) the water solution should be violet while the petroleum ether layer should remain yellowish green (test for sufficient concentration).

The solution of *o*-nitrosophenol in petroleum ether is stable for 2 to 3 weeks if kept in a refrigerator.

Procedure

The solution of the cobalt salt is neutralized, if necessary (spot test with methyl orange or bromophenol blue), and diluted so that it contains not more than 1.5 mg. of cobalt in 100 ml. Ten milliliters of this solution are poured into a small separatory funnel, 5 ml. of the citrate buffer and 2 ml. of *o*-nitrosophenol in petroleum ether are added, and the mixture is shaken vigorously for 15 to 20 seconds. After standing for a short time, the brown petroleum ether layer is separated from the water solution and put into a glass-stoppered flask graduated at 12 ml. This procedure is repeated twice with 2 ml. of *o*-nitrosophenol solution each time. Then the water solution is washed twice with 2 ml. of pure petroleum ether, the washings are added to the brown cobalt extraction, and the whole mixture is diluted with petroleum ether to a volume of 12 ml. This solution is then placed in the 10-ml. cell of the colorimeter and the determination is made in the usual manner.

In some instances, as, for example, in spectrophotometric analysis, when it is necessary to remove the excess *o*-nitrosophenol, the solution of the cobalt complex in petroleum ether is shaken repeatedly with a very dilute solution (0.01 per cent) of copper sulfate until the latter no longer shows the formation of the red-violet copper complex.

Figure 1 shows the data obtained by using a photoelectric colorimeter (Pfaltz & Bauer, New York, N. Y.), following the above procedure. The curve is nearly a straight line. This means that the proposed method is entirely suitable for the colorimetric determination of cobalt, even with polychromatic light.

Furthermore, by means of this curve it is possible to determine the maximum error and the sensitivity of the proposed method. When a storage battery is used with the colorimeter, the fluctuations of the galvanometer are not greater than one-tenth scale division, and it is considered that the readings are made with an accuracy of one-quarter scale division. (This is far from being the limit; for an experienced worker, it is not difficult to estimate one-tenth scale division.) This corresponds to 2 micrograms of cobalt in 10 ml. Taking a reading from 25 to 30 with a corresponding cobalt content of about 200 micrograms in 10 ml. as most suitable, the maximum error in the determination is not greater than 1 per cent.

The sensitivity of the method can be calculated in a similar manner. The lowest limit for which a satisfactory reading can be made is two scale divisions, which corresponds to an absolute cobalt content of 12 micrograms in 10 ml. of petroleum ether. By using a larger cell it is possible to start with 50 ml. instead of 10 ml. of the original cobalt solution. Thus the cobalt in a solution containing as little as 12 micrograms in 50 ml. may be determined.

All the foregoing experiments and calculations were made with the so-called normal sensitivity of the colorimeter. By changing the resistance in the photoelectric circuit the sensitivity of the instrument may be increased ten times. Accordingly a deflection of the galvanometer of two scale divisions would be obtained by one tenth of the above cobalt concentration. However, in consideration of the increased possibility of error, it is safer to take a galvanometer deflection of four scale divisions as the minimum. This means the smallest amount of cobalt which can be determined by the described method is about 5 micrograms or about 8×10^{-8} mole of cobalt in 50 ml.

The values in Figure 1, upon which all these calculations are based, were obtained with a pure cobalt solution. In the presence of other heavy metals which form the water-soluble complex compounds, the method is essentially the same. One has only to consider that part of the *o*-nitrosophenol will be used by ions other than cobalt and therefore the amount of *o*-nitrosophenol has to be increased.

The only interfering ion of practical importance is trivalent iron, which may be eliminated by the use of a citrate buffer solution. The results of the determination of cobalt in the presence of ferric salts are given in Table I. It appears that the amount of ferric salts present, even if 25 times that of cobalt, is of little importance, provided a sufficient amount of buffer solution is present.

TABLE I. DETERMINATION OF COBALT

Cobalt Taken	Buffer Solution	Fe^{+++} Added	Cobalt Found
Mg.	*Ml.*	*Mg.*	*Mg.*
0.120	1	–	0.120
0.120	1	1	0.126
0.120	2	1	0.122
0.120	3	1	0.129
0.120	5	1	0.123
0.120	5	3	0.123

Daylight and strong artificial light should be excluded, because under their influence the ferric ions are reduced in the presence of *o*-nitrosophenol to ferrous ions. The latter form with *o*-nitrosophenol the green water-soluble ferrous complex salts, thus consuming a part of the *o*-nitrosophenol.

Summary

o-Nitrosophenol as a new reagent in colorimetric analysis offers many possibilities for the estimation of small amounts of cobalt, palladium, iron, copper, mercury, and nickel.

In the first application, *o*-nitrosophenol is used for the quantitative estimation of small amounts of cobalt. It is possible to estimate 5 micrograms of cobalt in 50 ml. with an error not exceeding 1 per cent.

The only interfering metals are trivalent iron and palladium. The interference of trivalent iron is eliminated by forming complex iron compounds which do not react with *o*-nitrosophenol. The interference of palladium can be removed by the use of suitable color filters.

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Determination of Divalent Iron

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IN A previous paper (*3*), *o*-nitrosophenol was introduced as a new reagent in quantitative colorimetric analysis. It forms strongly colored inner-complex compounds with a number of metal ions, and the different metal complexes are distinguished by their color and their solubility in different solvents. The most important metals of the water-soluble group are divalent iron, copper, mercury, and nickel. The complexes of these and other metals are red or reddish violet with the exception of divalent iron, which is grass-green colored. No other metal forms a green, water-soluble complex with *o*-nitrosophenol.

Based on this characteristic property, the authors have worked out a quantitative estimation of small amounts of divalent iron. Preliminary experiments (*3*) showed that the reaction between *o*-nitrosophenol and ferrous ions is, in every respect, suitable for a colorimetric determination of this metal. The new method makes use of a reagent dissolved in a water-immiscible organic solvent, a feature not yet common in colorimetric work. The affinity of the divalent iron for *o*-nitrosophenol is so strong that upon shaking the two solutions—i. e., the ferrous salt in water and the organic solvent—the complex green iron salt is formed immediately and quantitatively.

The use of a water-immiscible solvent for the reagent has a very important advantage. Since free *o*-nitrosophenol in an organic solvent is yellowish green, it can be seen directly whether or not the reagent has been used in excess, as should always be the case. If the solution of the free *o*-nitrosophenol becomes colorless after shaking with the ferrous salt solution, it is at once apparent that more reagent should be added.

In using this method the solution of the ferrous salt in water is shaken with a solution of *o*-nitrosophenol in petroleum ether, whereupon the water solution becomes deep green, and after the two liquids have been separated the green water solution is measured in a colorimeter in the usual manner. Petroleum ether is preferred as the solvent for the *o*-nitrosophenol because of the characteristic solubilities of the metal salts of *o*-nitrosophenol (*3*).

These characteristic solubilities account for the fact that by this method only the divalent iron is determined. Ferric ions too react with *o*-nitrosophenol, forming a brown-colored complex compound. However, the reaction between ferric ions and *o*-nitrosophenol is not quantitative and the ferric complex is easily soluble in petroleum ether and thus extracted from the water solution of the green ferrous complex.

The method can be used for the estimation of ferric ions after the latter have been reduced to the divalent state by means of isoascorbic acid. A description of this procedure will be published soon by Baudisch.

The green ferrous complex is stable for at least 24 hours in the absence of very strong oxidizing agents or of very strong light.

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The intensity of the green color formed is always reproducible and is in proportion to the concentration of ferrous ions originally present (curve 1, Figure 1). Unfortunately, the authors did not have the facilities to determine the range in which the colored system conforms strictly to Beer's law.

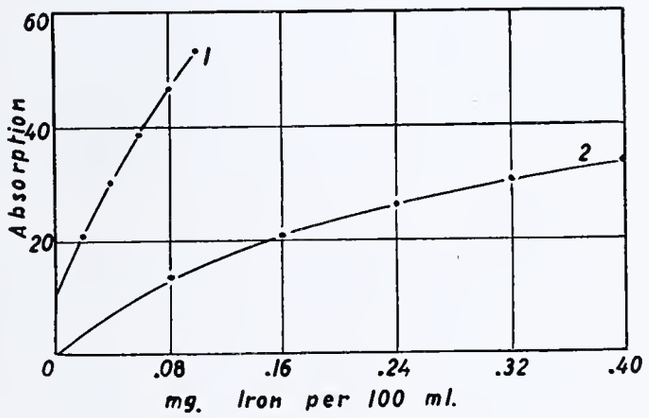

Figure 1. Calibration Curve for Determination of Ferrous Ions

Absorption = deflection on the "absorption" scale of colorimeter
1, obtained with *o*-nitrosophenol; 2, with α,α'-bipyridine

The maintenance of the proper pH is of greatest importance in all colorimetric work with *o*-nitrosophenol. In the case of its water-soluble compounds, a low pH prevents the quantitative formation of the complex, owing to dissociation. If the pH is too high, a mixture of two compounds is formed whose general formula can be written as *N—Me—R* (I) and *N—Me—N* (II), where *N* represents the *o*-nitrosophenol group. These two compounds differ in their color and solubility. I is soluble only in water, while II is soluble in certain organic solvents like ether, but insoluble in petroleum ether.

For the determination of divalent iron the most suitable pH range is from 5.1 to 5.3.

The preparation of *o*-nitrosophenol is described by Baudisch (*1*, *2*). Since it can be prepared and is stable only in solution, the proper concentration of the reagent must be controlled by tests (*3*), which should be made at regular intervals. The solution of *o*-nitrosophenol in petroleum ether is stable for 2 to 3 weeks if kept in a refrigerator.

Procedure

The solution of the ferrous salt is nearly neutralized (spot test with methyl orange or bromophenol blue) and diluted so that it contains not more than 1 microgram of ferrous iron per ml. In a separatory funnel exactly 50 ml. of this solution are mixed with exactly 5 ml. of an acetate buffer solution of pH 5.2. After 5 ml. of *o*-nitrosophenol solution are added, the mixture is shaken vigorously from 15 to 20 seconds and then allowed to separate. The petroleum ether is removed as well as possible by means of a pipet with a rubber bulb and the green water solution is shaken with a second 5-ml. portion of *o*-nitrosophenol solution. After

the two liquids have separated, the petroleum ether should still be yellowish green because of an excess of free *o*-nitrosophenol. The green water solution is filtered through a paper filter directly from the separatory funnel into the 30-mm. cell of the colorimeter. The filtration helps to clear the water solution completely from the last droplets of petroleum ether and prevents the formation of bubbles which might cling to the cell walls and so influence the results.

Curve 1, Figure 1, shows the value obtained with the so-called absorption scale of a photoelectric colorimeter (a modified Lange photoelectric colorimeter manufactured by Pfaltz & Bauer, Inc., New York, N. Y.) using a pure ferrous chloride solution. The light source was a 6-volt incandescent bulb and no light filter was used. Therefore, the values in the curve do not represent the true absorption.

By means of this curve it is possible to determine the maximum error and the sensitivity of the proposed method. When using a storage battery the fluctuations of the galvanometer are not greater than one-tenth scale division, and the readings are made with an accuracy of one-quarter scale division (for an experienced worker it is not difficult to estimate one-tenth scale division), which corresponds to 0.3 microgram of iron in 50 ml. For an iron solution containing about 50 micrograms of iron in 50 ml. this means that the maximum error in the determination is not greater than 0.5 per cent.

The sensitivity of the method can be calculated in a similar manner. The lowest limit for which a satisfactory reading can be made is two scale divisions above the zero point of the curve, corresponding to about 2 micrograms of ferrous ion in 50 ml. of solution.

All the foregoing experiments and calculations were made with the so-called normal sensitivity of the colorimeter but by changing the resistance in the photoelectric circuit, one can increase the sensitivity of the instrument ten times (*3*). The smallest amount of ferrous ion which can be determined by the described method is about 0.5 microgram in 50 ml.—i. e., 1 part in 100 million. (All measurements were made with a regular incandescent bulb. The use of monochromic light of a suitable wave length will increase the sensitivity still more.)

The calculations indicate that the new method with *o*-nitrosophenol is one of the most sensitive for the quantitative determination of ferrous ions. An experimental proof is given in Figure 1 by curve 2 which was obtained with α,α'- bipyridine instead of *o*-nitrosophenol. A comparison of the two curves shows that under the present experimental conditions the new method is at least three times as sensitive as the bipyridine method. (Contrary to the literature, α,α'- bipyridine forms strongly colored red complex compounds with titanium salts. The authors made this observation when S. E. Ashley of the General Electric Company, Pittsfield, Mass., called their attention to the fact that titanium salts react in a similar manner with *o*-phenanthroline.)

The limitations for the new method are few. The most important is the necessity of having the divalent iron present in the ionic state. This means that complex-forming compounds such as phosphoric acid, oxalic acid, and others should not be present. Trivalent iron, cobalt, and palladium do not interfere because their *o*-nitrosophenol salts are soluble in petroleum ether and therefore will be shaken out during the reaction, but as these metals will bind a part of the free *o*-nitrosophenol the amount of the latter has to be increased accordingly. In the presence of trivalent iron strong light must be excluded, because under its influence the trivalent iron will be reduced to a certain extent by *o*-nitrosophenol and thus cause too high results.

Of the other heavy metals known to form water-soluble colored complex compounds with *o*-nitrosophenol, most important are copper, nickel, mercury, and zinc, because their color is so strong that they are detectable in concentrations as low as 10^{-7} to 10^{-8} molar. However, the nitrosophenol complexes of these and other less important metals are all red or reddish violet in color. In their presence, divalent iron can be determined by the use of suitable color filters. The theoretical considerations for the colorimetric analysis of a two-component color system are given by Knudson, Meloche, and Juday (*4*).

Alkali and alkaline earth salts do not interfere with the determination of divalent iron because their *o*-nitrosophenol salts are formed only at a pH above 7.

The described method has been applied to the determination of iron in natural Saratoga mineral waters, which are solutions of bicarbonates of alkalies and alkaline earths, together with an excess of free carbon dioxide. The iron is present only in the divalent state. Because of the bicarbonate content of these waters, the proper pH can be attained by adding only acetic acid. Owing to the high sensitivity of this method, the waters had to be diluted in some cases in the ratio of 1 to 25. Table I shows some of the results obtained.

To demonstrate the accuracy of the new method, the iron content of Lincoln water was determined gravimetrically, using cupferron as precipitating agent (Table I). The difference between the two methods does not exceed 1 per cent.

Table I. Determination of Iron in Natural Mineral Waters

Spring	Colorimetric *Mg./l.*	Gravimetric *Mg./l.*
Lincoln[a]	12.15	12.20
Lincoln[a]	15.45	15.55
Geyser	2.22	...
Coesa	2.33	...
Hathorn No. 2	5.40	...

[a] Two samples taken at several days' interval.

Summary

A new method for the colorimetric estimation of divalent iron is based on the reaction of divalent iron with *o*-nitrosophenol, yielding a green inner-complex salt.

The method is one of the most sensitive colorimetric determinations for this metal, since 0.5 microgram in 50 ml. of solution can be estimated. Trivalent iron, cobalt, and palladium do not interfere. Copper, nickel, and mercury form red or reddish-violet compounds with the reagent. A comparison with a gravimetric determination of iron showed a very close agreement.

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Determination of Fluorine and Other Halogens in Organic Compounds

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A method for the analysis of organic fluoro compounds is presented. The procedure depends upon decomposition of the compound by heating with an alkali metal in an evacuated sealed tube at moderately elevated temperature, and determination of the resulting alkali fluoride by standard methods. The same technique serves for the analysis of chloro, bromo, and iodo compounds and has several advantages over the procedures commonly employed in these determinations. Application of the method to various types of fluoro compounds discloses no compound too stable to be decomposed by the conditions described. A simultaneous determination of fluorine and chlorine in chlorofluoro compounds may be made. The decomposition conditions leave the halide ions in an environment suitable for determination without a preliminary separation. The method is applicable to solids, liquids, and gases, is accurate and rapid, and requires no special apparatus or reagents.

THE increasing research on and use of organic compounds containing fluorine has called attention to the desirability of a simple method for determining this element, especially in the very stable compounds containing more than one fluorine atom on the same carbon atom. The methods at present described in the literature either do not work for the more stable compounds or are described in insufficient detail and without sufficient experimental verification to permit their use. The standard references on organic analysis fail in most cases even to mention the topic (*27*, *32*, *37*).

The determination of fluorine and other halogens in organic compounds resolves itself into two parts: (1) destruction or decomposition of the compound to convert the halogen into an ionizable form, and (2) determination of the halide ion. In the case of organic compounds containing halogens other than fluorine, the halide can usually be obtained by any of several recognized methods—e. g., the Carius method of heating with nitric acid in a closed tube, oxidation by sodium peroxide in a Parr bomb or by fuming sulfuric acid, combustion in oxygen, or hydrogenation in the presence of a suitable catalyst. These methods are of varying degrees of convenience as regards simplicity of apparatus, time required, and skill necessary in the operator.

The separation and determination of chloride, bromide, or iodide ion are well taken care of by standard methods of known accuracy. However, if fluorine is present, difficulties are introduced due to the great stability of the carbon to fluorine bond in aromatic compounds and in highly fluorinated aliphatic compounds. The most widely used method for the recovery of halide ion from organic compounds, the Carius method, cannot be used for fluorine since the hydrogen fluoride or hydrofluoric acid formed attacks the glass of the reaction tube. In speaking of the analysis of certain chlorofluoropropanes, Henne and Renoll (*19*) assert that the chlorine in these substances could be determined only by the Carius method, which required a whole week of continuous heating at 250° to 300° C. to yield quantitative results. All other methods gave an incomplete decomposition. As an indication of the stability of the carbon to fluorine bond, Meyer (*28*) asserts that the other halogens in halofluoro compounds can be determined by the Carius method or by lime fusion in glass without splitting out the fluorine.

The methods suggested in the literature for the decomposition of organic fluorine compounds are classified in Table I. Most of the fluorine compounds for which analyses are reported are of comparatively low fluorine content, commonly having only a single fluorine atom in a molecule of high molecular weight. This choice of compounds makes the methods appear better than they actually are for two reasons: (1) a relatively large error on the basis of fluorine present appears as a small percentage error; and (2) applicability of the method to the more stable compounds containing two or three fluorine atoms on a single carbon atom is not tested. In the present investigation, however, many of the compounds analyzed were chosen because they were supposed to be particularly stable and could not be analyzed by the methods described in the literature.

TABLE I. DECOMPOSITION OF ORGANIC COMPOUNDS CONTAINING FLUORINE

I.	Oxidation Methods	
	A.	Combustion in oxygen (*5*, *25*, *31*, *34*)
	B.	Fusion with sodium peroxide (*12*, *14*, *22*, *35*, *42*)
	C.	Alkaline oxidation (*4*)
II.	Reduction Methods	
	A.	Combustion in hydrogen (*8*, *46*)
	B.	Treatment with sodium in liquid ammonia (*47*)
	C.	Alkali metal fusion (*2*, *23*, *36*, *39*, *43*)
	D.	Treatment with alkali metal in organic solvent (*48*, *49*)
III.	Methods Involving Alkaline Fusion	
	A.	Fusion with calcium oxide (*3*, *10*, *11*, *20*, *24*, *29*, *40*, *41*, *44*)
	B.	Fusion with sodium carbonate (*6*)
IV.	Methods Involving Reaction with Silicon Dioxide	
	A.	Corrosive action on glass (*7*, *17*, *33*)
	B.	Combustion over silicon dioxide using oxygen and hydrogen (*9*, *18*, *19*, *21*)
V.	Hydrolytic Methods (*16*, *26*, *30*, *45*)	

FIGURE 1. FURNACE FOR HALOGEN DETERMINATION

The objective of the research was the development of a simple, rapid, and complete procedure for converting all the halogens present in organic compounds to halide ions in forms and environment permitting their determination with a minimum of further separation. Principal requirements were that the method should serve for the recovery of fluoride ion from stable organic fluoro compounds and should permit the determination of fluorine and other halogens in the same sample.

Principle of Proposed Method

The results of extensive preliminary experiments with the more promising methods described in the literature indicate that the most satisfactory method is based upon fusion with an alkali metal. The compound to be analyzed is heated with metallic sodium or potassium to a moderately elevated temperature in an evacuated sealed tube, yielding alkali halides in addition to the pyrolysis products, chiefly carbon, of the organic residue. The halide ion is then determined by standard procedures. The method described in detail below was evolved after many analyses of stable chlorofluoro compounds under a variety of conditions.

Reagents and Apparatus

A saturated solution of lead chlorofluoride was prepared as described by Scott (*13*). All reagents were of the best obtainable grade and were tested for the presence of the halide ion being determined. It is particularly important to check the sodium and potassium metals for the halide ion being determined, and to use anhydrous ether.

The reaction tubes were made by drawing out the open ends of Pyrex ignition test tubes, 20 × 150 mm., and sealing on 12-cm. lengths of 10-mm. tubing. These reaction tubes may be used repeatedly, since there is little attack on the glass. The ampoules prepared for sampling solids, liquids, and gases are described below. Since a large number of determinations were to be run and the method was to be used for the routine analysis of halogen compounds in this laboratory, a suitable furnace was designed. The furnace together with one of the reaction tubes is shown in Figure 1; an outline drawing is given in Figure 2. It may be constructed at a cost of about three dollars for material and the expenditure of 8 man-hours of labor.

CONSTRUCTION OF FURNACE. The furnace tubes are four iron pipes, 2.5 cm. (1 inch) in diameter and 30 cm. (12 inches) long, supported horizontally by the two 22.5-cm. (9-inch) paint-can lids which constitute the ends of the furnace. The tubes are arranged symmetrically on a circle of 3.25-cm. (1.5-inch) radius, with 2.5 cm. (1 inch) protruding at each end. The ends are threaded to take lock nuts and pipe couplings, into which are screwed the cast-iron closure plugs. Holes are drilled in the plugs to provide for release of pressure. A 9.375-mm. (0.375-inch) pipe through the center and paralleling the furnace tubes serves as a thermocouple well. The five tubes are wrapped as a unit with asbestos paper, and the unit is wound with 525 cm. (17.5 feet) of No. 23 Chromel wire having a resistance of 1.25 ohms per foot. The ends of the resistance wire terminate in a motor plug base set in one end of the furnace near the circumference. The resistance winding is covered with several more layers of asbestos paper held in place with asbestos cord. The jacket of the furnace is a 25-cm. (10-inch) section of galvanized furnace pipe. Sil-O-Cel is used as insulation between the jacket and the asbestos-wrapped unit of five tubes. No insulation was used between the furnace tubes themselves.

The furnace reaches 400° C. on the 110-volt line in 75 minutes, and is held at this temperature by an external resistance of 2 ohms in series with the furnace. The resistance, made by winding approximately 120 cm. (4 feet) of No. 19 Chromel wire on a cone heater, is mounted on a switch box in parallel with a fuse, so that it may be shunted out simply by screwing in the fuse plug. The furnace and external resistance are mounted on a board, the furnace resting in a cradle made of 12.5 × 3 mm. (0.5 × 0.12 inch) strap iron.

Procedure for Analysis

Most of the compounds analyzed were liquids and the procedure was first developed for material in this state.

ANALYSIS OF LIQUIDS. Ampoules for sampling liquids are blown from 3-mm. Pyrex tubing, slightly drawn out. The bulbs are 6 to 8 mm. in diameter, with stems 5 to 6 cm. long; the successive steps in their preparation are shown in Figure 3. A 0.10- to 0.15-gram sample of the liquid compound is drawn into the previously weighed ampoule, and the ampoule is sealed and weighed again. Care is taken to pyrolyze none of the compound during sealing. The ampoule containing the sample is placed in the reaction tube and 5 ml. of ether are added. The sodium or potassium metal is introduced in the form of very small pieces, the amount depending upon the size of sample taken and its halogen content (the optimum amount is usually 0.3 to 0.5 gram, giving several hundred per cent excess), and is weighed roughly or simply estimated. The reaction tube is then connected to an

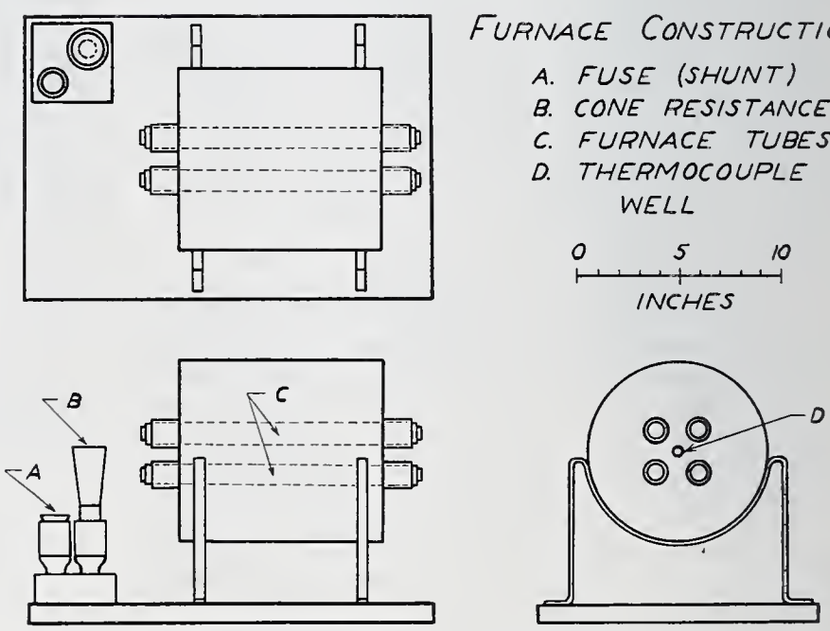

FIGURE 2. FURNACE FOR HALOGEN DETERMINATION

aspirator and all the ether is drawn off. The evacuation of the reaction tube in this manner serves to remove oxygen and water vapor completely from the tube and to give a low pressure. Removal of the oxygen and water vapor maintains the surface of the alkali metal clean, and therefore more reactive, and at the same time prevents attack of the reaction tube. Reduction of the pressure decreases the possibility of explosion of the tube when heated, and increases the vaporization of the compound and alkali metal, thus speeding up the reaction.

The stem of the reaction tube is sealed off with a hand torch while the tube remains connected to the water aspirator. The reaction tube is most conveniently sealed off when supported in a vertical position by means of the rubber tubing from the aspirator. It is obviously advisable to have a trap in the line to prevent water being drawn into the reaction tube in case of a drop in the water pressure. After the reaction tube is sealed, the ampoule is broken by shaking and the reaction tube placed in the furnace at 400° C. After 15 to 30 minutes, depending upon the compound being analyzed, the tube is removed and allowed to cool. It is then opened by breaking off the stem close to the original seal and the excess alkali metal is decomposed by the cautious addition of ethyl alcohol. The contents of the tube are transferred with several portions of wash water to a 100-ml. beaker and the ampoule, including stem, is crushed. The broken glass, carbon, and any silica are then filtered off through a No. 4 Jena glass filtering funnel or crucible or equivalent Pyrex filter. If the filter becomes clogged with carbon, it can be cleaned by immersion overnight in warm chromic acid–sulfuric acid solution.

The filtrate is neutralized with nitric acid and the halide determined in the filtrate by standard methods. In this investigation, chloride, bromide, and iodide were determined gravimetrically by precipitation as the silver salts, and fluoride was determined by precipitation and weighing as lead chlorofluoride, using essentially the precipitation technique of Hawley (*15*). If compounds containing nitrogen are analyzed, the halide procedure used must avoid interference with cyanide ion; the latter does not interfere with the fluoride determination.

If the compound being analyzed contains fluorine and another halogen, both may be determined on the same sample, or a separate sample may be used for each determination, the choice depending upon the relative importance of rapidity and accuracy. To determine both halogens from a single sample, aliquot parts of the fusion filtrate are taken.

Analysis of Low Melting Solids. Solids which have an appreciable vapor pressure at room temperature must be introduced into the reaction tube in sealed ampoules to avoid loss during evacuation of the tube. The ampoules for sampling solids are made from 10-cm. lengths of 6-mm. Pyrex tubing, with a fragile bulb blown in one end (see Figure 3). After the ampoule has been weighed and the sample introduced, it is evacuated and sealed off near the middle with a torch, care being taken to pyrolyze none of the compound. The two sections of the glass are weighed to give by difference the weight of sample contained in the sealed portion. The buoyancy effect of air on the weight of the ampoule is appreciable and a correction is calculated on the basis of the measured inside diameter and length. The correction is given by the formula, $C = r^2\pi ld$, where r is the internal radius of the ampoule in centimeters, l is the length of the sealed ampoule in centimeters, and d is the density of air in grams per cubic centimeter. The correction, C, is simply added to the apparent weight of the sample. This correction assumes that the ampoule is completely evacuated; this, of course, is not true, but the false assumption simplifies calculation and introduces an error of considerably less than 0.1 mg.

The sealed ampoule containing the sample is placed in the reaction tube, fragile end down, and the addition of ether and alkali metal and the evacuation and sealing-off are carried out as for liquids. The fragile end of the ampoule is then easily broken by shaking and the solid intimately mixed with the small pieces of alkali metal by rotating the reaction tube. The rest of the procedure is the same as for liquids, except that in removing the contents of the reaction tube the ampoule is not crushed. It is easily and thoroughly cleaned by adding 5 ml. of distilled water to the reaction tube, bringing the water to boiling, and momentarily removing the tube from the flame to allow the water to cool, thus drawing water into the ampoule. The water is again boiled, forcing it out of the ampoule, and poured into the beaker containing the previous washings of the reaction tube. This process is repeated several times.

Analysis of Gases. Gases are cooled below their boiling points and the resulting liquids poured into ampoules made from 10-cm. lengths of 5-mm. Pyrex tubing. With the lower half of the ampoule immersed in the cooling bath, the open end is drawn out to a fragile tip and sealed. The ampoule is placed in the reaction tube with the fragile tip downward, facilitating release of the sample when desired. Correction is made for the buoyancy

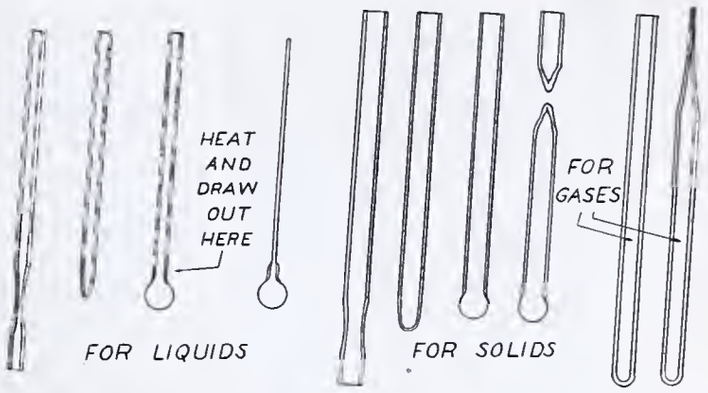

Figure 3. Steps in Construction of Ampoules for Sampling

effect of air. The analysis is carried out as for liquids, and the inside of the ampoule is rinsed as in the analysis of solids. The lowest boiling substance sampled by this method was dichlorodifluoromethane, boiling point −30° C.

Choice of Decomposition Conditions

Many organic halogen compounds were analyzed using decomposition temperatures from 150° to 600° C., varying the time of heating from 15 minutes to 2 hours, and using either sodium or potassium metal. On the basis of these preliminary investigations, the standard procedure adopted for compounds containing only chlorine, bromine, or iodine is fusion with sodium at 400° C. for 15 minutes.

Occasional low results were obtained with organic fluoro compounds with these decomposition conditions. As there is no apparent relation between the type of fluoro compound and the discordant results, the standard procedure adopted for fluoro compounds is decomposition with potassium metal at 400° C. for 30 minutes. It has been observed that potassium metal flows to coat the reaction tube, giving a clean surface of considerable area, whereas sodium does not. The higher cost of potassium amounts to only about one cent per determination.

6-Chlorocoumarin was the only compound containing only chlorine, bromine, or iodine for which conditions other than heating to 400° C. for 15 minutes with metallic sodium had to be applied. The fusion filtrate was always strongly colored, and the colored matter precipitated upon acidification of the fusion filtrate preparatory to precipitation of the chloride as silver chloride. Under the conditions adopted as standard for fluorine compounds, although the temperature was inadvertently run to 450° C., good results were obtained.

Presentation of Data

Table II gives the data obtained in the analysis of 24 organic halogen compounds using the method evolved in this investigation. All results obtained on every compound whose analysis was attempted by means of the proposed method are reported. The mean value and the average deviation for the halogen content of each of the compounds analyzed are given, together with the number of determinations upon which these values were based. Since work was completed on the development of the procedure, a large number of organic halogen compounds have been successfully analyzed by the method.

Since the procedure was developed primarily to fill the need for a rapid and accurate method for determining fluorine in organic compounds being prepared in this laboratory, the majority of compounds analyzed were fluoro compounds. Several compounds containing only chlorine, bromine, or iodine were analyzed because it appeared that the method had certain advantages over the precedures commonly em-

Table II. Analysis of Organic Halogen Compounds by Alkali Metal Fusion

Compounds Analyzed	Source and Purity of Sample	No. of Detns.	Halogen Theory %	Halogen Found %
Fluorobenzene, C_6H_5F	E. K. 2793, rectified, 84.5–84.6° C./754 mm.	6	F 19.77	19.66 ± 0.05
p-Fluoroanisole, $F.C_6H_4.OCH_3$	E. K. 3141, rectified, 156.6–156.7° C./748 mm.	3	F 15.06	15.18 ± 0.04
p-Fluoroaniline, $F.C_6H_4.NH_2$	E. K. 3203, rectified, 187.0–187.1° C./753 mm.	3	F 17.10	17.03 ± 0.04
Dichloromonofluoromethane, $CHCl_2F$	du Pont Freon 21, b. p. 8.9° C. Analyzed as received	6	F 18.46 Cl 68.89	18.72 ± 0.17 68.78 ± 0.19
Dichlorodifluoromethane, CCl_2F_2	du Pont Freon 12, b. p. −29.8° C. Analyzed as received	3	F 31.43 Cl 58.64	31.57 ± 0.11 58.72 ± 0.07
1,2-Dichloro-1,1,2,2-tetrafluoroethane, $CClF_2CClF_2$	du Pont Freon 114, b. p. 3.8° C. Analyzed as received	6	F 44.46 Cl 41.49	44.47 ± 0.34 41.62 ± 0.25
1,1,2,2-Tetrachloro-1,2-difluoroethane, CCl_2FCCl_2F	Research compound, rectified, 88.0–88.5° C./750 mm.	3	F 18.64 Cl 69.57	18.79 ± 0.03 69.87 ± 0.08
1-Chloro-2,2-difluoropropane, $CH_2ClCF_2CH_3$	Research compound, rectified, 55.1–55.4° C./750 mm.	9	F 33.18 Cl 30.96	33.27 ± 0.18 31.10 ± 0.08
o-Chlorofluorobenzene, $Cl.C_6H_4.F$	E. K. 3036, rectified, 134.0° C./750 mm.	3	F 14.55 Cl 27.16	14.73 ± 0.06 27.14 ± 0.12
2-Chlorobutane, $CH_3CHClCH_2CH_3$	E. K. 2461, rectified, 67.8–68.1° C./753 mm.	3	Cl 38.31	38.25 ± 0.02
1,3-Dichloro-2-methylpropane, $CH_2ClCH(CH_3)CH_2Cl$	Research compound, rectified (b. p. 136.0° C.) and recrystallized	3	Cl 55.83	55.73 ± 0.02
m-Chlorotoluene, $Cl.C_6H_4.CH_3$	E. K. 2449, rectified, 161.7–161.9° C./753 mm.	3	Cl 28.01	28.09 ± 0.03
p-Dichlorobenzene, $Cl.C_6H_4.Cl$	E. K. 89, twice sublimed, m. p. 53.0° C.	3	Cl 48.24	48.32 ± 0.13
6-Chlorocoumarin, $C_9H_3O_2Cl$	E. K. 1490, twice recrystallized, m. p. 161.5–161.8° C.	3	Cl 19.64	19.76 ± 0.06
2-Bromobutane, $CH_3CHBrCH_2CH_3$	E. K. 1406, rectified, 90.1–90.3° C./749 mm.	3	Br 58.32	58.31 ± 0.02
Bromobenzene, C_6H_5Br	E. K. 43, rectified, 154.8–155.1° C./752 mm.	3	Br 50.91	50.86 ± 0.10
Bromocyclohexane, $C_6H_{11}Br$	E. K. 1058, b. p. 61–62° C./15 mm. Analyzed as received	3	Br 49.02	48.88 ± 0.02
Iodobenzene, C_6H_5I	E. K. 152, b. p. 70–71° C./12 mm. Analyzed as received	3	I 62.21	62.33 ± 0.02
Research Compounds				
Compound A, $C_3H_3Cl_3F_2$	Rectified, 0.1° C. boiling range	3	F 20.72 Cl 57.99	20.78 ± 0.09 58.10 ± 0.06
Compound B, $C_3H_4Cl_2F_2$	Rectified, 0.2° C. boiling range	3	F 25.51 Cl 47.60	25.70 ± 0.06 47.70 ± 0.05
Compound C, $C_3H_4Cl_2F_2$	Rectified, 0.2° C. boiling range	3	F 25.51 Cl 47.60	25.66 ± 0.04 47.71 ± 0.04
Compound D[a]	Rectified, 0.0° C. boiling range	3	F 60.61	60.90 ± 0.12
Compound E[a]	Rectified, 0.0° C. boiling range	3	F 54.02 Cl 11.20	53.94 ± 0.04 11.20 ± 0.08
Compound F[a]	Recrystallized, 0.5° C. melting range	3	Cl 76.30	76.00 ± 0.06

[a] Empirical formulas and physical constants withheld for reasons of national defense.

ployed for the determination of these halogens in organic compounds. Since the stability of organic halides decreases in the order fluoride, chloride, bromide, and iodide, methods which successfully decompose the more stable of the organic fluorides should be adequate for the decomposition of organic compounds containing the higher atomic weight halogens. For this reason only one iodo and three bromo compounds were analyzed. Table II lists compounds with fluorine content as high as 60 per cent. Research compounds A, B, and C had two fluorine atoms on a single carbon atom, D and E had three fluorine atoms, and F had three chlorine atoms on a single carbon atom. Other examples of stable highly halogenated compounds are listed.

Except for the few compounds noted, all samples were purified before analysis. The term "rectified" is followed by the temperature range over which the fraction used for analysis was taken. Table II gives in the case of compounds A, B, C, D, and E only the boiling range over which the sample was taken for analysis, and only the melting range of compound F.

In general, the best results were obtained with compounds containing only a single halogen rather than both fluorine and chlorine, as was the case with ten of the compounds analyzed. This may be attributed only in part to the fact that when both fluoride and chloride were present, an aliquot of the fusion filtrate was taken for the determination of each halide, rather than the entire fusion filtrate. The precipitated chloride or fluoride from compounds containing both these elements was often discolored. The silver chloride precipitate in particular was frequently light brown in color, apparently from a postprecipitated substance, since the first precipitate formed was white. Results were most often high. The poorest results were obtained with two of the three gases analyzed, dichloromonofluoromethane (Freon 21) and 1,2-dichloro-1,1,2,2-tetrafluoroethane (Freon 114). With these compounds, the negative errors were associated with sample sizes much higher than optimum and it seems likely that the most careful analysis would have given consistently high values. Slightly less than average precision might have been expected with gases, owing to sampling errors, but the principal errors involved in sampling are condensation of atmospheric moisture on the liquefied sample or inside the containing vessel, and pyrolysis of the sample during sealing of the ampoule, both of which would cause low results. In a private communication, A. F. Benning, Jackson Laboratories, E. I. du Pont de Nemours and Co., Inc., has stated that analysis of Freons by decomposition in a Parr bomb and precipitation of the fluoride as lead chlorofluoride gives results about 0.5 per cent high.

Compounds containing only chlorine, bromine, or iodine gave results comparing favorably in precision and accuracy with results obtainable by any method for the determination of organic halogen, and even the least accurate results obtained with fluoro compounds are satisfactory when considered in the light of results obtained by other methods.

Discussion of Method

The chief advantages of the method described are simplicity, rapidity, and accuracy when applied to a wide variety of compounds. No special apparatus or reagents are required, and no separation of the fluoride ion, such as a Willard and Winter distillation (*50*), need be employed before determination of the fluoride. Any furnace giving a temperature in the range 350° to 450° C. is satisfactory. An ordinary Carius furnace was employed in this investigation until the furnace now in use was constructed.

It is possible that the tendency toward high results might be eliminated by a titrimetric determination of the halide ion, although the material which postprecipitates with the silver chloride might obscure the end point in either Fajans' or Mohr's method. The Volhard method might prove satisfactory for determining chloride, and indirectly for determining fluoride by a titration of the chloride in the precipitated lead chlorofluoride (Hawley's method, *15*). Information concerning the latest modification of this method for fluoride has been generously supplied by H. V. Churchill and R. W. Bridges of the Aluminum Company of America (*1*). This method and that of Rowley and Churchill (*38*) for the titration of fluoride in aqueous solution with thorium nitrate are being investigated as substitutes for the gravimetric determination of fluoride as lead chlorofluoride. It was found that the material which contaminated the precipitate could be avoided if the solution was filtered after acidification, but this procedure always gave definitely low results. Other

possibilities for elimination of the difficulty may lie in higher temperatures, longer time of heating, or both, for the decomposition step.

The wide applicability of the method is demonstrated by Table II, good results being obtained with fluoro, chloro, bromo, and iodo compounds; with samples in the liquid, solid, or gaseous state; and with aliphatic, aromatic, alicyclic, and heterocyclic compounds. Fluorine was determined in compounds containing oxygen and nitrogen, and in extremely stable highly halogenated compounds containing both fluorine and chlorine.

For the determination of chlorine, bromine, or iodine in organic compounds, certain advantages over the Carius determination are apparent. The time required for decomposition of the compound is often reduced from hours or days to a few minutes. The danger of explosion is eliminated, since at 400° C., even if no reaction takes place, the pressure inside the reaction tube is only 2 or 3 atmospheres above the external pressure. However, since a reaction uses up the sample, the pressure is much less than this and when the tube is cooled the pressure inside is actually less than atmospheric pressure. Approximately 150 determinations have been made by this technique, with temperatures as high as 600° C., and samples of 0.5 gram and over, and no explosions have resulted. The reaction tubes are introduced into the furnace when it is at reaction temperature and are removed without cooling the furnace, thus effecting a considerable saving of time.

The applicability of this technique to the determination of other elements in organic compounds is being investigated, specifically in the determination of nitrogen, phosphorus, and sulfur. Work on the use of this technique on a micro and semimicro scale is also in progress.

Summary

A method for the analysis of stable organic halogen compounds is based upon decomposition of the compound by heating with an alkali metal in an evacuated sealed tube at moderately elevated temperature, and determination of the resulting alkali halide by standard methods.

Application to a variety of fluoro compounds discloses no compound too stable to be decomposed by the conditions described.

Fluorine and chlorine may be determined simultaneously in chlorofluoro compounds.

The same method serves for the analysis of chloro, bromo, and iodo compounds and offers advantages over procedures commonly employed.

Samples of liquids, solids, or gases are handled with equal ease.

The decomposition conditions leave the halide ions in an environment suitable for determination without a preliminary separation.

The method is accurate and rapid, and requires no special apparatus or reagents.

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Residue Volume Method of Solubility Determination

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A new method of determining solubility by measuring the volume of residues left by mixtures of solute and solvent has proved successful in cases where speed of determination combined with reasonable precision and simplicity is required. The method is particularly useful on commercial mixtures and on substances containing insoluble impurities. The solubility of pure substances may also be determined by this new method, but it is not applicable to volatile solutes or solutes of lower density than the solvent.

THE best solubility data are commonly obtained by the usual methods of phase rule investigation whereby the compositions of the liquid and solid phases in equilibrium are determined and verified by their mutual relations to the equilibria found to exist for systems of slightly altered composition. Solubility relations become vastly more complex and slower to establish as one progresses from two- to three- and then to four-component systems. In systems of three or more components, a variable excess of solid phase beyond that required for saturation in many cases affects the composition of the solution with which it is in equilibrium. This paper deals with the more limited problem of determining the quantity of a soluble solid composition of any number of components in fixed ratios which will enter completely into solution.

It is sometimes possible, of course, to secure the required data by adding known amounts of solute to the system until upon prolonged agitation the solute will no longer disappear, but if insoluble impurities contaminate the material under examination, this method fails. Other factors may also affect this method, as for example, the color and opacity of the solutions or the presence of fluorides or other materials which attack vessels used to carry out the determinations.

Thermal methods, such as the cooling of unsaturated solutions to determine the highest temperature of separation of solid phase, with or without seeding, have obvious limitations.

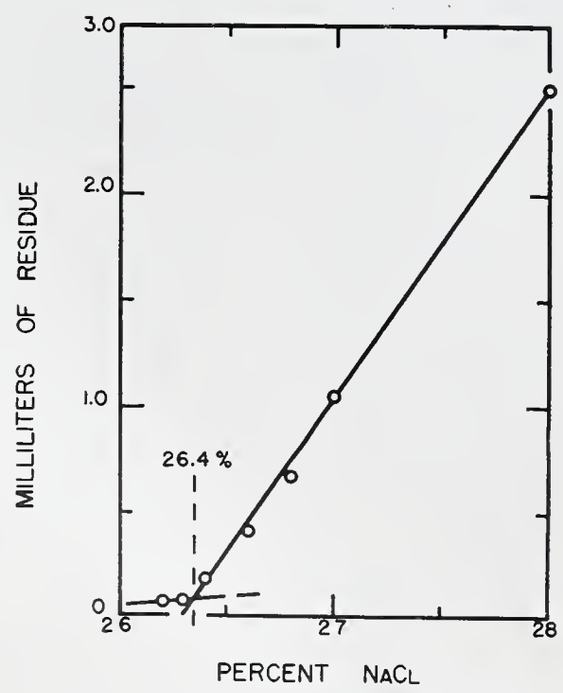

FIGURE 1. SOLUBILITY OF SODIUM CHLORIDE

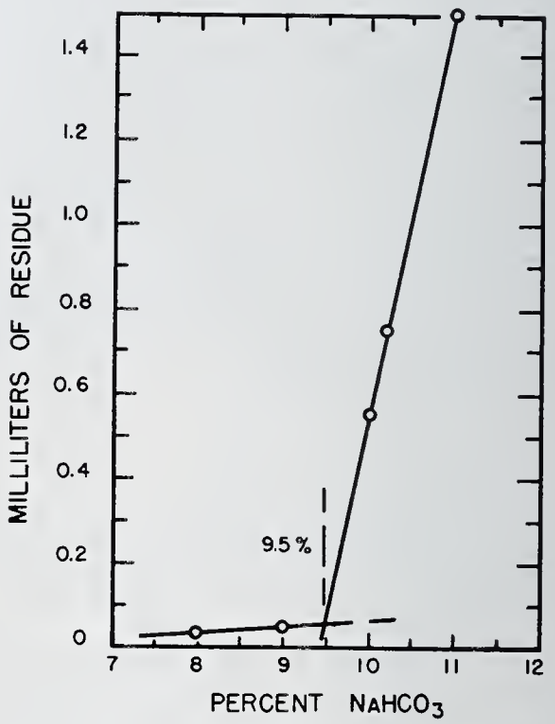

FIGURE 2. SOLUBILITY OF SODIUM BICARBONATE AT 27° C.

The principle of the residue volume method described here rests on the fact that when the solubility limit of a solute in a solvent has been reached, further additions of solute result in a proportional increase in the amount of undissolved residue. The volume of these residues can be measured for a number of solute-solvent ratios and the data obtained plotted as a straight line and extrapolated to zero residue volume as the solubility limit. The method can also be used on materials containing small amounts of insoluble residues, such as are often found in commercial products.

Experimental Procedure

The essential equipment for a solubility determination by the residue volume method consists of an analytical balance, a buret, a thermometer, a centrifuge, and Goetz tubes of 100-ml. capacity with capillaries graduated from 0 to 0.2 ml. in steps of 0.01 ml., and from 0 to 1.0 ml. in steps of 0.05 ml. The Goetz tubes with the small capillaries are used at or below the solubility limit, while the tubes with the larger capillaries are used when the solids present appreciably exceed the solubility limit.

To make a solubility determination, a quantity of the solute is weighed into a Goetz tube and sufficient solvent is added from the buret so that liquid plus solids total 100 grams. It is advantageous to use this total weight both in order to simplify calcu-

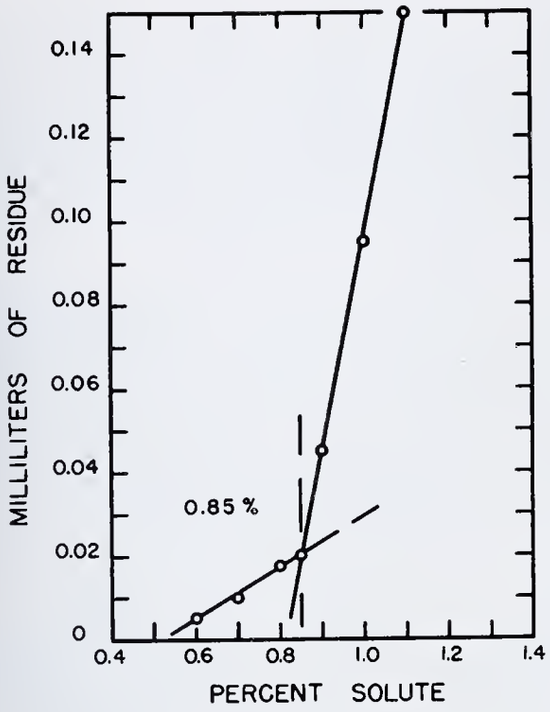

FIGURE 3. DETERMINATION OF SOLUBILITY OF A THREE-COMPONENT MIXTURE

lations and to keep the centrifuge tubes in balance. It is not necessary to know the original volume of the solids added. The tube is stoppered, shaken vigorously for 5 minutes, and then centrifuged for 5 minutes. As soon as it is removed from the centrifuge the temperature of the solution is taken with the thermometer, and the volume of the solids in the capillary is then read and recorded on a graph of residue volume versus per cent dry solids. Six or eight such determinations made at properly distributed solute-solvent ratios above and below the solubility limits usually provide enough points for accurate determination of the solubility of the substance.

When the residue volumes of several different liquid-solid mixtures have been determined and plotted, it will be found that the plotted points may be connected by two straight lines. Below the solubility limit the points will yield a line of very low slope corresponding mainly to traces of impurities. Above the solubility limit a line of very steep slope will be obtained. The intersection of these two lines is taken as the solubility limit.

Experimental Data

Determinations were made on sodium chloride and sodium bicarbonate for the purpose of checking the accuracy of the method. Figures 1 and 2 show the method of plotting the experimental data, and Table I shows the solubilities obtained and compared with published data.

Figure 3 shows the determination of the solubility of a mixture of sodium silicofluoride, niter cake, and a complex sodium phosphate. Figure 4 shows the solubility determination for a mixture of sodium silicofluoride, oxalic acid, niter cake, and a complex sodium phosphate. The commercial salts in both mixtures contained small amounts of insoluble impurities and because of this and the chemical nature of their solutions, which attack glass on standing, the solubilities of these two mixtures would be difficult to obtain by other experimental methods. The residue volume method lends itself admirably to the determination of the solubility of such mixtures.

Discussion

The accuracy of the results obtained is better than was hoped for. It is not difficult to obtain results within the first decimal point of published data, and with a more refined technique greater accuracy could be obtained. The authors have successfully applied the method to numerous mixtures of inorganic substances and to a few containing organic compounds. It should be applicable to certain liquid-liquid determinations as well.

Theoretically, below the solubility limit there should be no residue, but commercial materials often contain traces of insoluble impurities. Below the solubility limit, these traces show up as minute but usually volumetrically measurable quantities varying regularly with the quantity of solute present. Most of the authors' work has been done with materials containing such impurities, and they consider the solubility limit of such materials as the intersection of the two lines obtained, one of low slope for residue volume determinations below the solubility limit and one of very steep slope for determinations made beyond the solubility limit.

Data taken close to the solubility limit tend to be erratic and often fall off the straight line connecting points farther away. This is believed to be caused by the reduced rate of solution obtained when the liquid is almost saturated and very little solid remains to complete the saturation. When there is a greater excess of solute, the liquid is more rapidly saturated because of the relatively large surface area of solute presented to the liquid. Also, as the amount of solute is progressively diminished below the solubility limit, the more dilute solutions obtained completely dissolve the solute much more rapidly. Because of these considerations, the writers place more reliance on data taken a moderate distance from the solubility intersection than very close to it. For any specific case, judgment and a little practice will quickly point out the solute-solvent ratios most suitable for rapid and accurate determination of solubility.

TABLE I. COMPARISON OF SOLUBILITY DATA OBTAINED BY RESIDUE VOLUME METHOD WITH PUBLISHED DATA

Substance	Temperature	Solubility: Residue volume method	Solubility: ICT
	° C.	%	%
Sodium chloride, C. P.	30	26.4	26.49[a]
Sodium bicarbonate, refined	27	9.5	9.56[b]

[a] Calculated from data given as moles per 1000 grams of H_2O.
[b] Interpolated from data given as moles per 1000 grams of H_2O at 15°, 25°, 30°, and 35° C. and calculated to per cent basis.

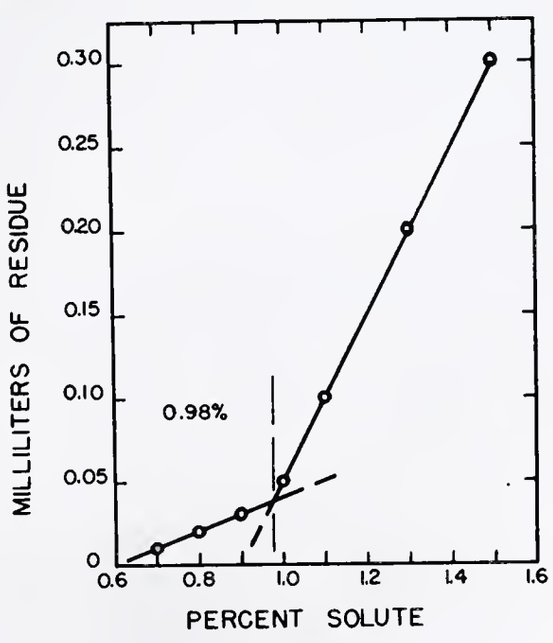

FIGURE 4. DETERMINATION OF SOLUBILITY OF A FOUR-COMPONENT MIXTURE

The particle size of the solute is of considerable importance. The material must be fine enough to give a compact, reproducible residue in the capillary in which it is measured, and to have a rapid rate of solution. However, it must be coarse enough to be readily precipitated into the capillary of a Goetz tube with a moderate centrifugal force. A material screened to pass an 80-mesh sieve is usually satisfactory.

A centrifuge is used to pack the residues in the capillaries of Goetz tubes for volume measurement. While its use is not vitally necessary, the centrifuge facilitates the experimental procedure by greatly reducing the time required to settle out the residues and by yielding well packed uniform columns. It has been the authors' practice to centrifuge for 5 minutes at 2000 r. p. m. with a maximum radius of approximately 18.75 cm. (7.5 inches), using an International centrifuge, Size 1, Type SB. There is no need to centrifuge under these particular conditions, and even considerable variation from them on duplicate determinations has not been observed to affect the results. However, it is best to standardize the time and force used during the experiments for any one solubility determination in order to minimize variations due to the packing of the residues.

The top of the residue column in the capillary is usually not level when removed from the centrifuge. This condition makes accurate reading of the residue volume difficult and may be overcome by holding the tube vertically and gently tapping the capillary until the residue levels itself. Occasionally some liquid is entrapped in the bottom of the capillary, in which case it is best to loosen the residue and recentrifuge. Mechanical difficulties of this sort are minor and can be easily overcome.

Temperature control is difficult. Centrifuging causes a small rise in temperature of the solutions above the air temperature, and it is not easy to control the resulting solution temperatures at any predetermined level. The purposes of the writers have so far been served by starting with the solutions at room temperature and measuring the final temperature of the solutions as they are removed from the centrifuge, regarding the latter as the temperature at which the determination was made.

Conclusions

The residue volume method of determining solubilities was originally designed for commercial mixtures but its scope is considerably broader. The accuracy obtainable is sufficient for the majority of commercial applications, and it can serve usefully for obtaining quickly a preliminary view of solubility limits in many systems. The solubility is found from a number of individual determinations which operate to check one another and to define the precision of the determination as a whole. The fact that no analyses need be made is an important feature from the standpoint of speed, cost, and often the time lost in development of suitable analytical methods. Other features are the ability to give good results on substances containing insoluble impurities and in cases where attack on container vessels interferes. Solubility data obtained may be corrected for insoluble impurities if necessary. Difficulties with supersaturation are not likely to be met.

The method is not applicable to solutes of density lower than the solvent, nor effectively to solutes having very slow rates of solution.

PRESENTED before the Division of Analytical and Micro Chemistry at the 103rd Meeting of the AMERICAN CHEMICAL SOCIETY, Memphis, Tenn.

Determination of Tannin Substances in Boiler Waters

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TANNIN and similar complex organic materials are finding increased use in boiler feed-water treatment. For example, the phlobatannin quebracho will frequently reduce dissolved oxygen concentration, prevent hard scale, retard clogging of feed lines and injectors, and inhibit caustic embrittlement. Both operating experience (*2*, *7*, *9*) and laboratory observation (*4*, *5*, *11–13*) substantiate the value of such substances.

A correlation of the concentration of tannin with the effect it produces is obviously of importance for its efficient and economical use as well as to ensure reproducible results. Analytical procedures must therefore be available for determining dissolved tannin. Color produced by these materials when they are in water solution, loss during ignition of dried solids, and oxygen consumed from permanganate have all been found unreliable and inadequate for estimating concentrations.

Method

The reagent developed in 1912 by Folin and Denis (*6*) to differentiate tyrosine from uric acid was adapted by Mehta (*8*) to estimate lignin extracted from plant tissues. The solution of phosphotungstic and phosphomolybdic acids has been found to be sensitive to small amounts of tannins and similar substances, indicating a suitability for boiler and feed-water analysis. The reagent is reduced by the tannin or lignin to give a blue solution of a color density that varies with the concentration of the reducing agent. Procedures for Nessler tubes and photoelectric cell colorimetry have been developed. The method is so rapid and simple that it may be used as a routine procedure[1].

REAGENTS. The following solutions are required:

Tyrosine reagent, made from phosphotungstic-phosphomolybdic acid, by dissolving 100 grams of sodium tungstate, 20 grams of phosphomolybdic acid, and 50 ml. of 85 per cent phosphoric acid in 750 ml. of water. The liquid is boiled under reflux for 2 hours, cooled, and made up to 1 liter.

Saturated sodium carbonate. Enough sodium carbonate (250 grams) is added to 1 liter of water to supersaturate the solution with respect to sodium carbonate decahydrate and the excess is allowed to crystallize at 20° C. The clear supernatant liquor is the sodium carbonate reagent and is decanted and stored in a rubber-stoppered bottle.

Comparison solution. This solution represents the "standard" for the method and should be made up of exactly the same material that is fed to the boiler. Five grams of a representative

[1] After the type had been set for this publication, the authors became aware of a parallel study of the photocolorimetric determination of tannin in connection with the analysis of whisky, by Rosenblatt and Pelnso (*10*). However, preliminary announcement of the authors' procedure for the determination of lignin and tannin in boiler water was made several years ago (*3*).

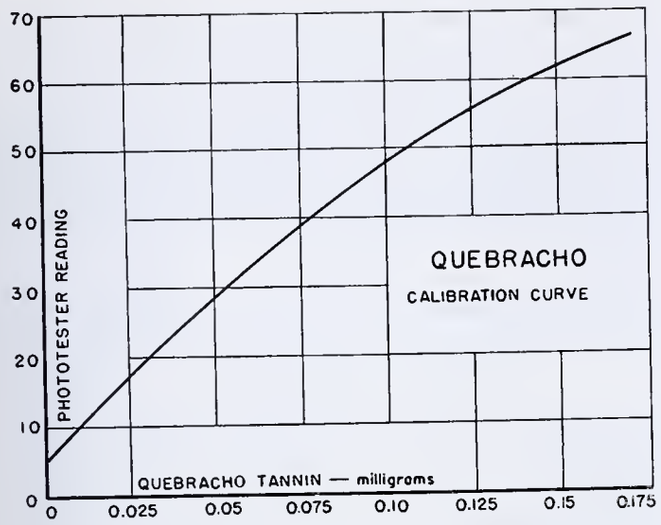

FIGURE 1

sample of the solid or liquid extract used for the boiler feed are weighed out and dissolved in a liter of water, 10 ml. of this solution are pipetted out and diluted to 1 liter. This latter solution is the comparison or standard solution and contains 50 p. p. m. of the original material, but does not represent 50 p. p. m. of pure tannin. Alkaline contamination of the comparison solution results in rapid deterioration due to air oxidation of the tannin and should be avoided. For most tannins, the standard will remain sufficiently accurate for 2 months from the date of preparation.

Master standard solution. To determine the rate of deterioration of the standard solution and to check the uniformity of the extract used, comparison with chemically pure tannic acid is recommended. One gram of tannic acid is weighed out and dissolved in 1 liter of water, and 25 ml. of this solution are pipetted out and diluted to 1 liter for a master standard containing 25 p. p. m. of tannic acid.

PROCEDURE FOR NESSLER TUBES. A 50-ml. filtered, clear, and cooled sample of boiler or feed water containing less than 3 p. p. m. of tannin or 20 p. p. m. of lignin is put into a 100-ml. Nessler tube. (Generally this will require diluting a smaller sample to 50 ml.)

Two or three similar samples are made up from the comparison solution to contain lower and higher concentrations of tannin or lignin than the unknown sample. These reference solutions are also made up to 50 ml. with distilled water, and therefore contain 1 p. p. m. of tannin for every milliliter of comparison solution used.

Each known and unknown is treated with 2 ml. of the tyrosine reagent, stirred, and allowed to stand 5 minutes. Ten milliliters of saturated sodium carbonate are added to each sample, stirred, and allowed to stand 10 minutes.

The blue color of the unknown is matched with the knowns to estimate the amount of tannin or lignin.

PROCEDURE FOR PHOTOELECTRIC CELL COLORIMETRY. This procedure was developed for a phototester with a 15-ml. (0.5-ounce) test bottle (light path 1.83 cm., 0.75 inch). The ratio of the reagents to the volume of solution in the cell should be maintained for other instruments, but the size of the sample may require modification by a factor depending on the dimensions of the test cell. A shorter light path would require a larger sample of a given concentration of reducing agent for a similar effect on the photoelectric cell.

Exactly 1.0 ml. of the sample is pipetted into the test bottle and diluted to half the bottle volume with distilled water.

The tyrosine reagent (0.5 ml.) is added with a calibrated "medicine dropper" pipet. The contents are mixed and allowed to stand 5 minutes. Using a larger calibrated dropper pipet, 2 ml. of the saturated sodium carbonate are added. The bottle is now filled to the base of the neck with distilled water, the screw cap is replaced, and the contents are mixed.

Exactly 10 minutes from the time the carbonate is added, the effect of the blue color developed is measured in the phototester. The reading is converted to milligrams of organic matter, according to a calibration curve for the particular tannin present.

The calibration curve is obtained by subjecting known quantities of the tannin complex to the above procedure and plotting the readings of the microammeter scale against the amount of tannin used. Figure 1 shows a typical calibration curve for quebracho tannin. This curve is steepest and the accuracy highest for the first two thirds of the scale.

TABLE I. TRANSMISSION CHARACTERISTICS OF BLUE SOLUTION[a]

Wave Length, Å.	Transmission, %	Wave Length, Å.	Transmission, %	Wave Length, Å.	Transmission, %
4050	67.5	5300	52.0	6050	46.5
4350	67.0	5450	52.0	6250	45.0
4600	63.0	5600	48.5	6400	45.0
4850	59.5	5760	49.0	6600	45.0
5100	57.0	5900	49.0	6850	45.0

[a] Thickness of solution 915 mm. Color developed (regular phototester procedure) by 0.1 mg. of quebracho tannin.

No light filter is necessary for the phototester. Table I shows that the blue color which is developed will absorb wave lengths over a large part of the spectrum. Partial correction for any color which may be present in the boiler water may be made in the null-point bottle used to set the scale at zero light absorption.

Effect of Variations in Procedure

The effect of variations in procedure and conditions during the development of this color can best be discussed in terms of a typical rate of color-development curve as measured with the photoelectric cell. The curves shown in Figures 3 and 4 are identical and were developed by 0.1 gram of quebracho tannin, using the standard phototester procedure.

Figure 2 shows that change in the tannin content results in a series of similar curves. The color develops very rapidly during the first minute after the carbonate is added, and while it is not a maximum at 10 minutes, the curves have flattened. The 10-minute interval is therefore empirical and may be modified to the requirements of the individual laboratory. Once the interval is selected, however, it must be rigidly adhered to, both for the development of the calibration curve and the analysis of unknowns. For Nessler-tube work this is not so important, since the color of the unknown and of the standards is developed simultaneously.

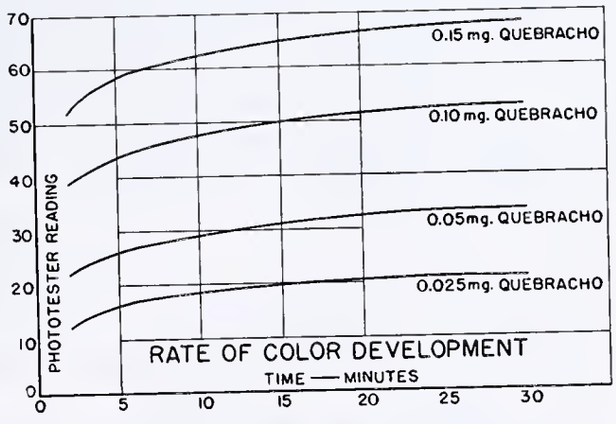

FIGURE 2

Figure 3 (upper) shows that the time that the tyrosine reagent is permitted to react with the tannin before the addition of the sodium carbonate is not critical. The result was the same whether this interval was 2, 5, or 8 minutes. A longer reduction interval of 12 minutes does result in somewhat higher readings.

The quantity of tyrosine reagent used does not materially affect the color development after the first few minutes (Figure 3, center). Excess reagent must be avoided, how-

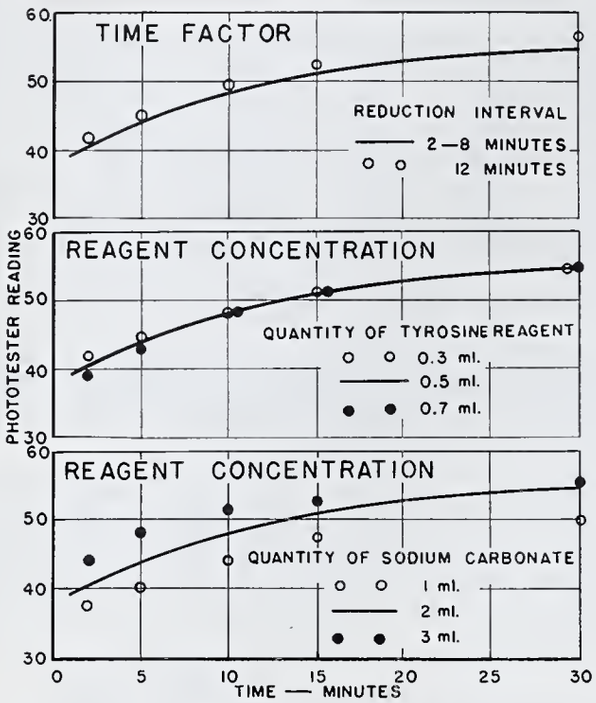

FIGURE 3. EFFECT OF VARIATIONS IN PROCEDURE

ever, since the tungstate and molybdate formed upon addition of the sodium carbonate have a limited solubility.

The quantity of saturated sodium carbonate used is more important, as shown in Figure 3 (lower). Care should be taken to add approximately the same quantity of carbonate for each determination.

EFFECT OF TEMPERATURE. The importance of a controlled room temperature is shown in Figure 4 (upper). Analyses made at 18.5° C. (65° F.) could be referred to a calibration curve obtained at 24° C. (75° F.) without serious error. If the determinations were made at 32° C. (90° F.), however, the results referred to the same curve would be about 10 per cent too high. Where there are pronounced variations in laboratory temperature, it may be necessary to make winter and summer calibration curves. Nessler-tube comparison, however, is not affected by the room temperature.

INTERFERENCE. Figure 4 (center) shows that considerable alkalinity or acidity in the water sample analyzed does not affect the rate of color development for a given amount of tannin. The solution to which sodium hydroxide was added would represent a boiler water containing 4000 p. p. m. of caustic soda for every 100 p. p. m. of quebracho tannin.

Where the alkalinity is very high, however, and the tannin concentration low, poor results may be obtained owing to excessive neutralization of the acid tyrosine reagent. For Nessler-tube comparison under such conditions, 1 drop of dilute sulfuric acid (1 to 5) should be added to every 25 ml. of alkaline boiler water tested, before the tyrosine reagent is added.

Inorganic substances ordinarily present in water do not affect the rate of color development. Figure 4 (lower) shows that a ratio of sodium chloride to tannin of 100 to 1 caused no departure from the normal curve. The effect of sodium sulfite is almost negligible, since 1 mg. of this compound when added to the 0.1 mg. of quebracho caused variations of about 10 per cent.

Ferrous iron will interfere, but in alkaline boiler waters the concentration is usually too low to cause difficulty.

Phenolic compounds which are present in some feed waters will interfere. A blank should be run if their presence is suspected.

SAMPLING. If much time elapses between the sampling of the water and its analysis, appreciable oxidation of the tannin can occur. This action is best prevented by acidifying the boiler water slightly. About 1 ml. of concentrated hydrochloric acid should be added to 1 liter of cooled and filtered water for each 300 p. p. m. of caustic alkalinity. This treatment may cause some of the tannin to be precipitated, so that the sample should be thoroughly shaken before a portion is taken for analysis.

TABLE II. EQUIVALENCE[a] OF 1 P. P. M. OF C. P. TANNIC ACID

	Tyrosine Test *P. p. m.*	Permanganate Number (*1*) *P. p. m.*
Quebracho tannin	1.4	0.9
Cutch tannin	1.9	1.1
Chestnut tannin	1.8	...
Pure lignin	3.3	0.9
Sodium lignin sulfonate	7	1.3
Waste sulfite liquor[b]	16	2.8
Ferrous sulfate	5	18
Sodium sulfite	Over 100	7.3

[a] Quantity of each substance required to produce same result in each standard test procedure as 1 p. p. m. of c. p. tannic acid.
[b] Solution containing 50% solids.

Significance of Results

The tyrosine reagent does not evaluate a particular boiler water in terms of its effect on boiler operation. Neither does it determine the oxidizable matter in solution. Table II lists the equivalence to tannic acid for several substances used for boiler-water conditioning. The tyrosine equivalence is the amount of the substance required to develop the same color in this test procedure as 1 p. p. m. of c. p. tannic acid. The permanganate equivalence is similarly the amount of the substance which will consume as much oxygen from permanganate as 1 p. p. m. of c. p. tannic acid.

The tyrosine test was developed, and is used, to determine concentrations of substances which have aromatic hydroxyl groups. It makes no distinction between such substances, so that the simultaneous use of lignins and tannins is obviously undesirable from the standpoint of analytical control.

It has been found that concentrations as measured by the tyrosine test can be correlated in some degree with quanti-

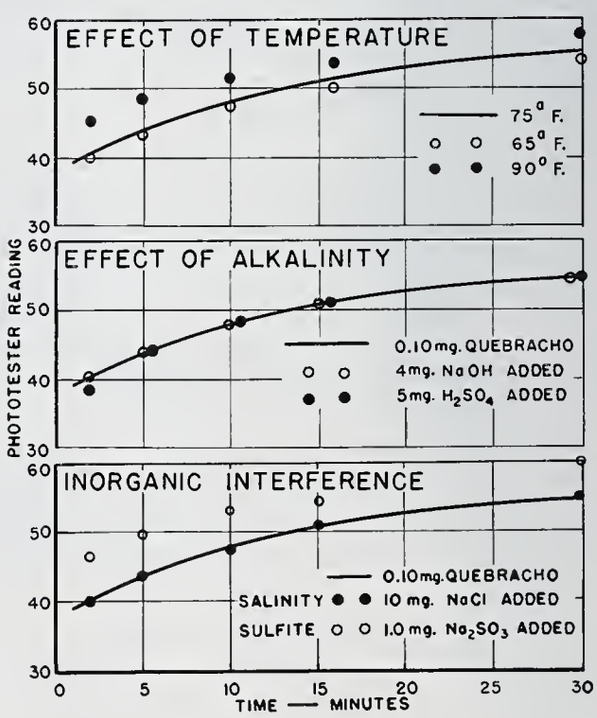

FIGURE 4. EFFECT OF VARIATIONS IN PROCEDURE

ties of tannin fed and with experimental determination of their effect in the boiler water.

Acknowledgment

The transmission characteristics of the blue solution were determined by Morris Slavin, associate spectroscopist of the Bureau of Mines staff. The phototester was supplied by the National Aluminate Corporation and modified to the needs of this laboratory.

The authors are especially indebted to Everett H. Schlie for his assistance in supplying the data for the curves presented in this paper.

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A New Technique for Adsorption Studies

JAMES COULL, University of Pittsburgh, AND H. C. ENGEL AND JOSEPH MILLER, Mellon Institute, Pittsburgh, Penna.

IN THE course of a research program dealing with the production, testing, and evaluation of fundamental properties of activated carbons, an interesting investigational technique for the study of adsorption of gases has been developed. The method comprises batch production of vapor-air mixtures of any desired compositions, and the continuous analysis of the mixture after passage through a bed of activated carbon. The resulting data allow a ready evaluation of the performance of the carbon and yield information as to the efficiency of adsorption (*4*, *5*) after the break point (*4*, *5*) is reached.

Production of Vapor-Gas Mixtures

Numerous investigators have pointed out the importance of evaluating the properties of an activated carbon under the specific conditions for which it is to be used. For testing gas-adsorbent carbons this usually involves the preparation of vapor-gas mixtures of definite compositions; either continuous or batch mixing may be utilized. Considerable work in this laboratory has demonstrated the superiority of the batch method where limited amounts of the mixture are adequate.

The apparatus for making up vapor-gas mixtures is shown in Figure 1. The required amount of liquid, as first approximated from the gas laws, is boiled into the empty tank, *A*. The necessary amount of dry air is pumped in by means of the compressor, *B*.

After analysis by the thermal conductivity method (described below), the composition of the mixture may be adjusted by the addition of either pure air or a rich vapor-gas mixture similarly prepared in the second tank, *C*. Additional mixing is made possible by the valve arrangement which allows circulation of the mixture through the compressor. Where larger amounts of gas are required, both tanks may be operated as a single unit. Mixtures of any composition less than saturation may readily be prepared in this way.

Gas Analysis

Continuous gas analysis is accomplished by means of the thermal conductivity cell (*2*, *3*). The particular instrument used (Figure 2) was designed and furnished by C. C. Minter, 29 Evergreen Place, East Orange, N. J., and is adequately described by Brinker (*1*). Electromotive force measurements were made with a Type 8657-C double-range Leeds & Northrup potentiometer.

Calibration of the cells was accomplished under the conditions of use. Vapor-air mixtures from the gas storage tank, *A*, were passed through a pressure-reducing valve followed by a constant-flow valve in order to maintain a constant rate. The vapor-air

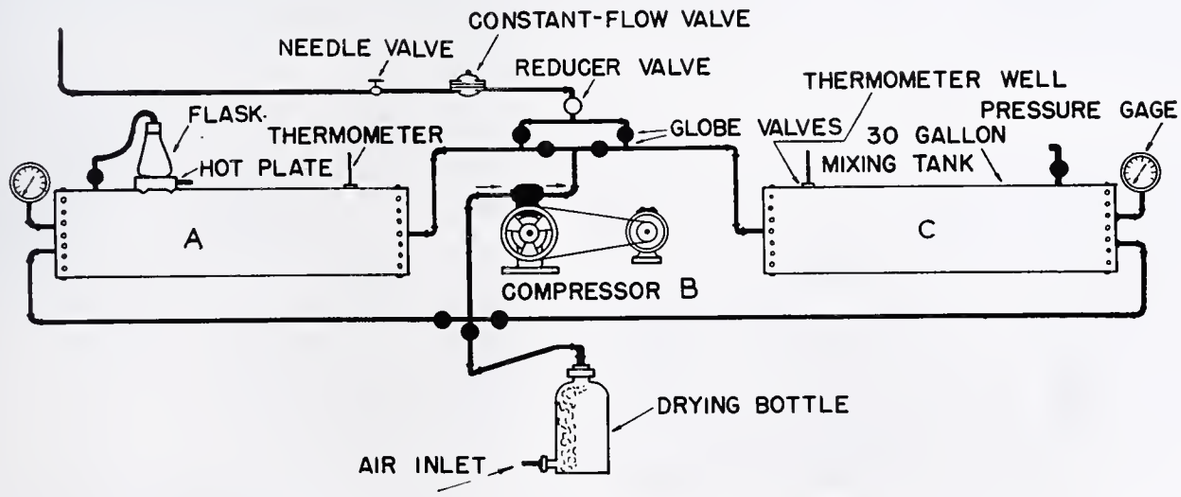


Figure 1. Apparatus for Making Vapor-Gas Mixtures

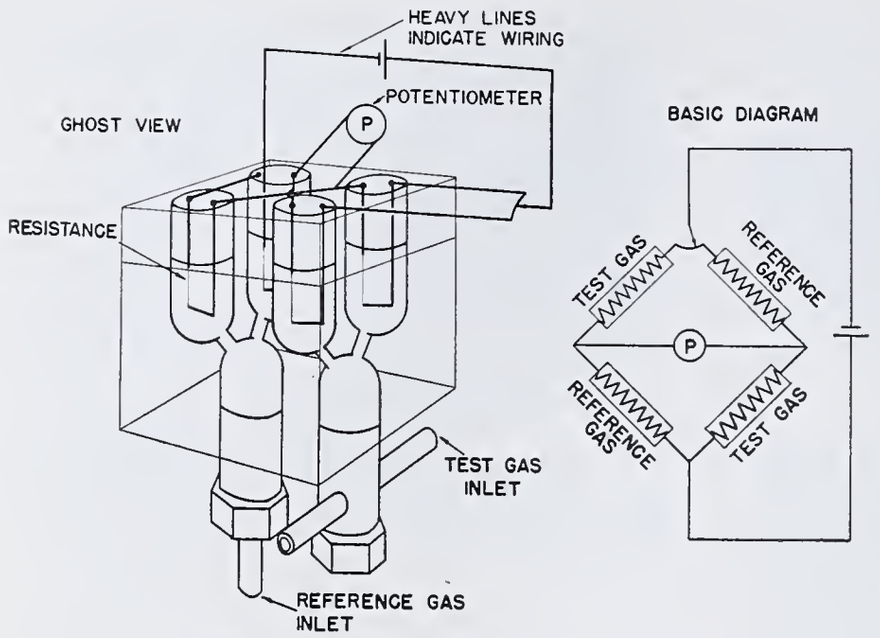


FIGURE 2. THERMAL CONDUCTIVITY CELL

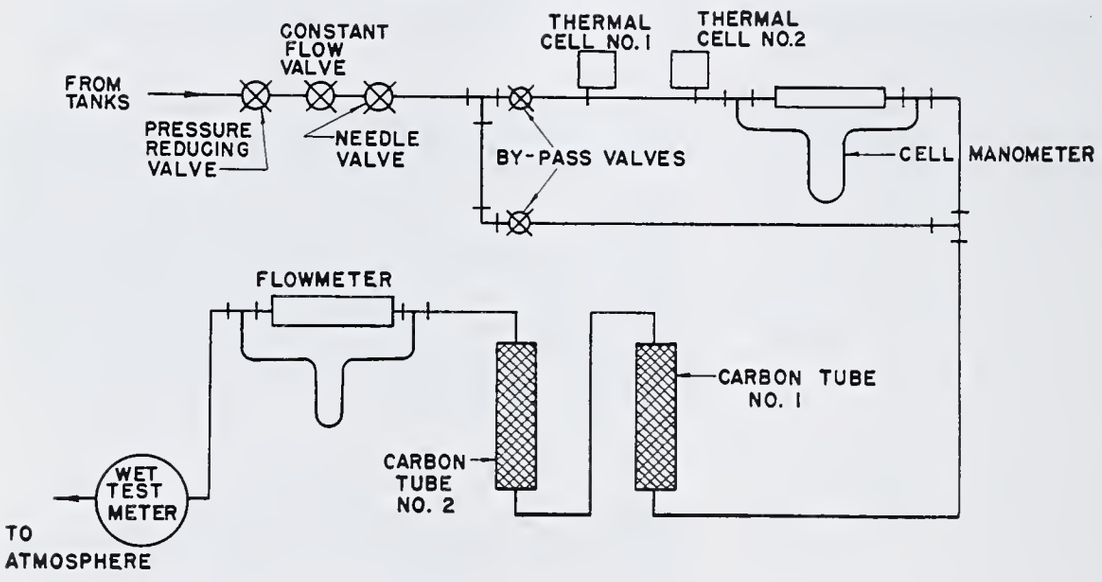


FIGURE 3. APPARATUS FOR CALIBRATION OF THERMAL CONDUCTIVITY CELLS

In actual runs the variable pressure drop through the apparatus produces effects upon the cell readings which require correction. These corrections become increasingly important at the higher rates of flow. However, precise values are possible because increased pressure does not alter the linear nature of the millivolt-composition relation. Therefore, it is only necessary to consider each reading taken during a given run as a fraction of the final, saturation reading. This fraction multiplied by the composition obtained from the calibration curve for an open system millivolt value is the true composition.

Adsorption Studies

The method of conducting adsorption studies is diagrammatically indicated by Figure 5.

A metered stream of the test gas is analyzed by cell 1 in order to check its composition continuously while the remainder is bypassed. The two streams reunite and flow through a carbon-packed tube containing the desired amount of activated carbon. Continuous analysis of the exit gas is made by cell 2. A second carbon tube is inserted to ensure the passage of pure air through the flowmeter and wet-test meter. The indicated valve system (pressure-reducing valves and needle valve) makes possible a constant flow rate. The entire setup is enclosed in an air thermostat.

The agreement between the actual amount of vapor adsorbed as determined by direct weighing of the carbon-packed tube, before and after the run, and the amount of adsorption calculated from the effluent gas analysis has been used as an over-all measure of the success of the experimental method.

The data obtained in a typical run, in which chloroform vapor was adsorbed from a chloroform-air stream, are recorded in Table I. This run was carried out at a rate of flow of 1.98 standard liters of air per minute and a composition of 104 mg. of chloroform per standard liter of dry air.

mixtures then entered a tube packed with bone-dry active carbon (Figure 3). The amount of material adsorbed was determined by direct weighing; a second carbon-packed tube, weighed before and after passage of the gas, was used as a check on completeness of adsorption in tube 1. The volume of the pure air in the total amount of gas was directly measured by a precision wet-test meter.

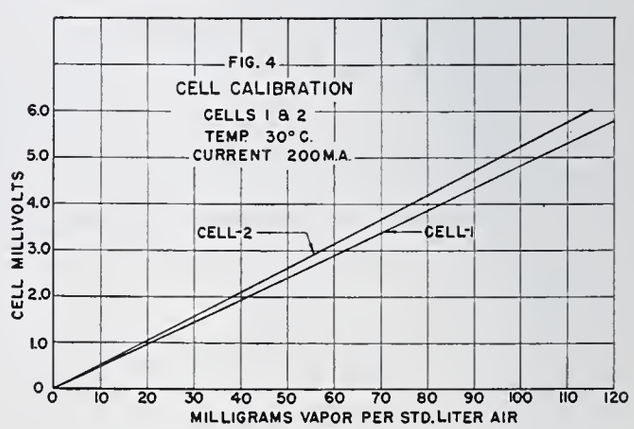
The relation:

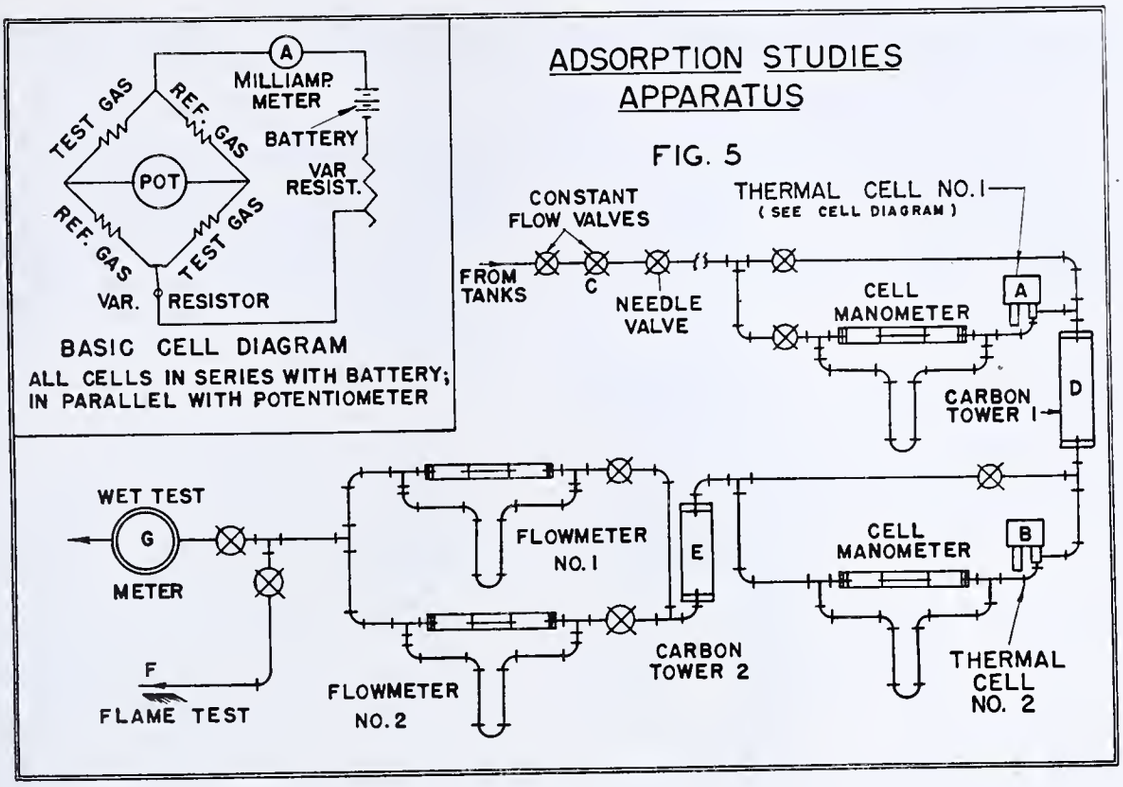
$$C = \frac{W}{L} \qquad (1)$$

where C = composition in milligrams of vapor per liter of air,
L = liters of air passed, and
W = mg. of vapor adsorbed,

allowed ready computation of the desired composition.

Mixtures containing air and benzene, dichloromethane, chloroform, or carbon tetrachloride all showed a simple linear relation between millivolts and composition. The calibration curves for cells 1 and 2 for chloroform-air mixtures are shown in Figure 4. These data were obtained for a cell current of 200 milliamperes and a cell-block temperature of 30° C.





The amount of vapor adsorbed at the break point and at saturation is readily calculated. In any interval of time, dt, the amount of absorbate entering the tower is:

$$C_0 R\, dt \qquad (2)$$

where C_0 = feed composition in weight of vapor per unit volume of dry air and R = volume rate of passage of pure air, and will be constant for any given run

The amount of vapor which passes from the tower without being adsorbed in the same time interval is $CRdt$, where C = the effluent gas composition. Therefore, the amount of vapor adsorbed is

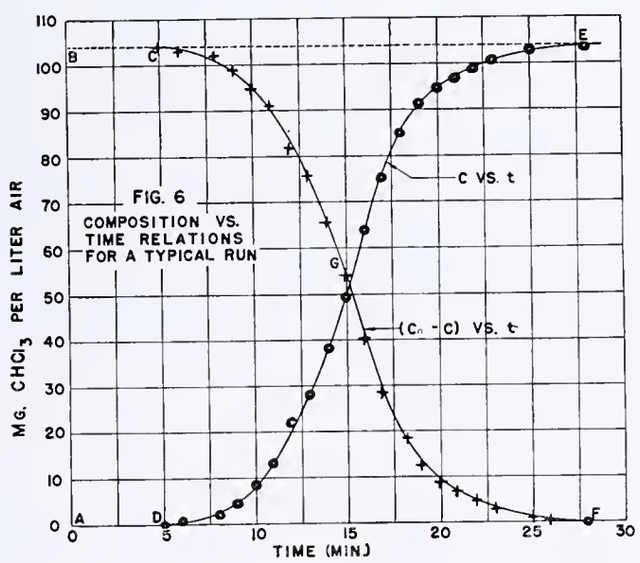
$$(C_0 - C)\, Rdt \qquad (3)$$

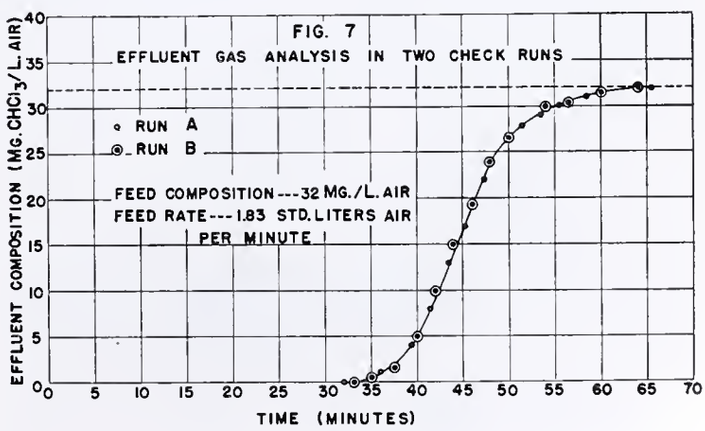
Integrating this expression from $t = 0$ to any time, t, gives the total amount adsorbed—i. e., $\int_0^t (C_0 - C)\, Rdt$. Graphically, this integral is evaluated by determining the area under the curve $(C_0 - C)$ *vs.* t, and multiplying by R.

A good approximation of this area can often be made by treating the total area as a sum of the areas of a rectangle and a right triangle, as indicated by the dotted line in Figure 6. The calculation is indicated herewith for the run repre-

Table I. Adsorption Data for a Typical Run

Time Min.	Mv.	Removal %	C Mg./l.	$C_0 - C$ Mg./l.
0	0	100	0	104
5	Break point	...	..	104
6	0.05	99	1.04	103
8	0.10	98	2.08	101.9
9	0.22	95.5	4.68	99.3
10	0.41	91.5	8.84	95.2
11	0.61	87.5	13.0	91.0
12	1.02	78.8	21.1	81.9
13	1.30	73.0	28.0	76
14	1.78	63.0	38.4	65.6
15	2.29	52.5	49.5	54.5
16	2.98	38.1	64.3	39.7
17	3.50	27.4	75.6	28.4
18	3.95	18.0	85.2	18.8
19	4.25	11.8	91.6	12.4
20	4.40	8.7	95.0	9.0
21	4.50	6.6	97.0	7.0
22	4.59	4.8	99.0	5.0
23	4.68	2.9	101	3.0
24	...	...	..	...
25	4.79	0.6	103	1.0
26	4.81	0.2	103.5	0.5
27	4.82	0.0	104	0
28	4.82	0.0	104	0





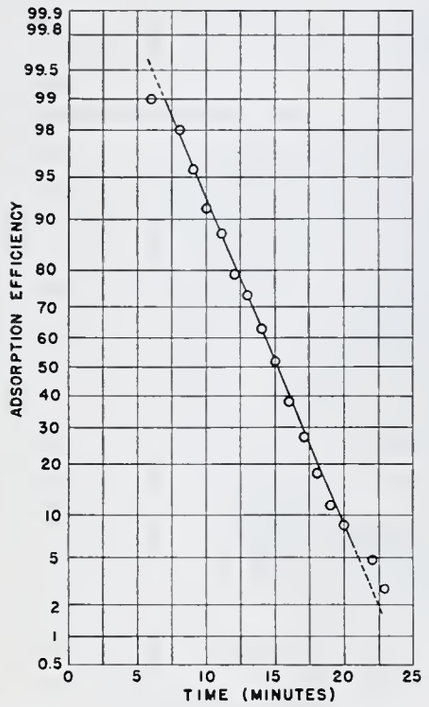


FIGURE 8. ADSORPTION EFFICIENCY *vs.* TIME

Feed composition, 104 mg. of chloroform per liter of air
Feed rate, 1.98 standard liters of air per minute

sented by Table I and Figure 6.

C_0 = 104
R = 1.98
Area $ABCD$ = 520 units
Area $ABCGF$ = 1580 units
Mg. of $CHCl_3$ adsorbed at break point = 1030 mg. per 5.0 grams of carbon
Mg. of $CHCl_3$ adsorbed at saturation = 3120 mg. per 5.0 grams of carbon

A satisfactory agreement has been found between the calculated amount of vapor adsorbed and the amount found by direct weighings; a large number of runs have demonstrated that these two values agree within 5 per cent.

A number of duplicate runs have shown that this method makes possible an accurate reproduction of results. In Figure 7 the effluent analysis *vs.* time curves for two check runs are shown. These data were obtained for 5-gram samples of a coconut-shell carbon, a feed-gas composition of 32 mg. of chloroform per standard liter of dry air, and a gas rate of 1.83 standard liters of air per minute. The carbon bed was 1.8 cm. in diameter and 5.0 cm. in height. Very fair agreement is apparent.

If the efficiency of removal of chloroform from an air-chloroform mixture is plotted *vs.* time on cumulative probability paper, as shown in Figure 8, a very fair straight line results. Although deviations at the two ends of the line are apparent, this plot may in many cases be useful in making comparisons between different carbons. The efficiency-time relation after the break point can be approximated by the relation:

$$y = 50\left[1 - \frac{2}{\sqrt{\pi}}\int_0^{\frac{t-a}{b}} e^{-x^2}dx\right] \qquad (4)$$

At the point of inflection $y = 50$. Therefore, $\frac{t-a}{b} = 0$ and $t = a$. b is readily evaluated because $b = -\frac{t_2 - t_1}{2}$ where t_2 corresponds to $y_2 = 92.3$ and t_1 corresponds to $y_1 = 7.70$.

For the data of Figure 6, $a = 15.0$ and $b = 5.2$; the data reduce to:

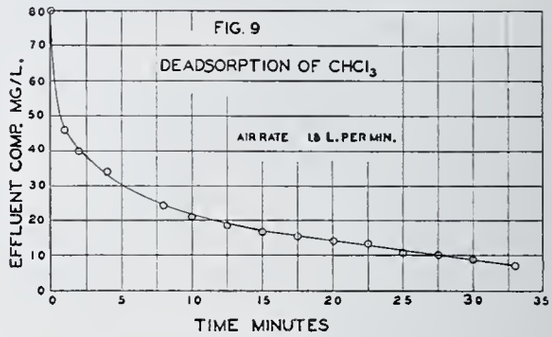
$$y = 50\left[1 - \frac{2}{\sqrt{\pi}}\int_0^{\frac{t-15.0}{5.2}} e^{-x^2}dx\right] \qquad (5)$$

This equation is extremely convenient; the constants can be evaluated almost by inspection. For any value of t, the corresponding value of $\frac{2}{\sqrt{\pi}}\int_0^{\frac{t-a}{b}} e^{-x^2}dx$ is readily found in tables of the probability integral.

A more complete and extensive treatment of adsorption data will be presented in a later paper.

Further Applications

The method described is readily adapted to the investigation of the unsteady state conditions which exist in an adsorption tower. The variables of interest, composition of feed gas, rate of gas flow, mesh size of carbon, height of carbon bed, etc., can all be conveniently studied. Work along these lines will be reported from this laboratory at a later date.



Adsorption phenomena involving a multicomponent gas mixture can be investigated by this technique, provided that the components can be quantitatively and individually removed by specific absorbents or adsorbents.

Deadsorption studies, where an inert gas is used to remove an adsorbed substance from an adsorbent, can be readily carried out. Figure 9 indicates the progress of deadsorption of a carbon which was first saturated with chloroform vapor from a chloroform-air mixture containing 80.0 mg. of chloroform per liter of air at 30° C., 760-mm. pressure. In this case, $R\int_0^t Cdt$ is the amount of vapor removed from the carbon up to time t.

Summary

Batch-mixing of vapor and gas facilitates reproduction of use conditions in adsorbent testing.

Use of the thermal-conductivity cell for analysis of the effluent gases from a carbon-packed tower makes possible accurate determination of the stoichiometry of the adsorption process. This method is especially useful in large-scale equipment for indicating the extent of adsorption.

An empirical equation which can be employed to represent efficiency *vs.* time relations in adsorption studies has been presented.

Some further applications of the method are in the study of desorption of adsorbed vapors and in the testing or investigation of adsorption of vapors from multicomponent systems.

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PART of this work submitted in partial fulfillment of the requirements for the degree of M.S. in chemical engineering.

Assay of Thyroid and Its Preparations

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Analytical Division, John Wyeth & Brother, Inc., Philadelphia, Penna.

Several analytical features of the U. S. P. XI method of assay for thyroid lacked a sufficient degree of refinement to yield reproducible results. The proposed method satisfies this need and eliminates the analytical faults which attended the official method.

IN VIEW of the fact that the U. S. Pharmacopoeia XI method of assay for thyroid was widely considered to be inadequate, this laboratory instituted experimental work in 1937 to standardize the assay procedure. The method which was developed is applicable not only to powdered thyroid but also to tablets.

Several laboratories have made contributions from time to time regarding one or more phases of the analytical procedure. Thus, Hojer (*4*) postulated the existence of chlorates and perchlorates arising from the chlorine-oxidation procedure of the U. S. P. and attributed the gradual and constant shift of the end point observed in the titration to their presence. Burnett and Warkow (*2*) took advantage of the comparative rates of liberation of iodine from potassium iodide by chlorate and iodate, performing the titration at a pH at which the desired reaction by the iodate far exceeded that of the chlorate and thus removed chlorate interference. However, the present authors found that the accurate control of pH was somewhat burdensome. Although the end point was improved, neverthless a gradual shift of the end point was still apparent due to the delayed influence of the chlorate. The coloration produced by the thymol blue indicator was also objectionable.

Hilty and Wilson (*3*) have proposed a modified procedure based on cerate oxidimetry, which necessitates fusion without an oxidizing agent and thus avoids the difficulties attending complete removal of chlorine and nitrite. The authors' experience with this method failed to yield complete recovery of iodine.

While the authors' method was the result of an attempt to improve the entire U. S. P. XI assay method, they cannot overemphasize the improvement contributed by the elimination of nitrite from the solution. Although the A. O. A. C. (*1*) uses sodium bisulfite for removal of nitrites, this reagent is troublesome in that it bleaches the indicator (methyl orange). The authors avoid nitrite interference by the simple expedient of adding urea:

$$CO(NH_2)_2 + 2HNO_2 \rightarrow CO_2 + 2N_2 + 3H_2O$$

Nitrite is produced from nitrate during the initial fusion and, although unstable in acid medium, its complete removal by boiling is difficult and uncertain. The excess of urea is dissipated during the subsequent boiling:

$$CO(NH_2)_2 + H_2O + 2HCl \rightarrow CO_2 + 2NH_4Cl$$

A study of the kinetics showed that the liberation of iodine from a potassium iodide solution by nitrite was at a rate proportional to the square of the concentration of hydrogen ion.

Other notable improvements developed for the assay include standardization of the fusion, partial neutralization of alkaline fusion with hydrochloric acid to yield more rapid elimination of chlorine by the salt-effect (sodium chloride), and standardization of sodium hypochlorite reagent. Blanks are performed for analytical completeness but use of analytical grade reagents requires no correction to be made of the actual determination. Additional evidence of the effectiveness of the assay method is found in the stability of the end point.

In the assay of tablets of thyroid, provision is made for use of additional fusion mixture to ensure complete combustion during fusion.

While the method proposed by Johnson and Nelson (*5*), consisting of alkaline fusion without potassium nitrate and oxidation with bromine instead of hypochlorite, has been found to yield results concordant with those obtained by the authors' method, yet the time consumed is a distinct disadvantage.

TABLE I. EXPERIMENTAL RESULTS ON POWDERED THYROID

Method	Iodine Found %	Blank Cc.
U. S. P. XI	0.212 0.206	End point indefinite
Proposed	0.198 0.198 0.199 0.198 0.199	0.00
Johnson and Nelson (*5*)	0.194 0.194	0.4 0.4
Burnett and Warkow (*2*)	0.202 0.200	0.00
Fusion without KNO_3	0.122 0.143	——

Modified Method

REAGENTS. Sodium Hypochlorite Solution. Dissolve 30 grams of chlorine in a cooled solution consisting of 44 grams sodium hydroxide in 750 cc. of distilled water. Dilute to 1 liter.

Reagent grade hydrochloric acid, urea, sodium carbonate, potassium nitrate, and potassium carbonate.

U. S. P. grade phosphoric acid and potassium iodide.

PROCEDURE FOR THYROID. Weigh accurately a sample of about 1 gram of thyroid and transfer to a nickel crucible (125 cc.). Add 15 grams of U. S. P. XI fusion mixture, and mix thoroughly with the sample. Superimpose a layer of about 5 grams of fusion mixture. Heat the crucible and contents to a dull red heat in a muffle furnace and then maintain this temperature until the carbonaceous matter is completely oxidized (about 20 minutes). Remove crucible and allow to cool. Transfer to a beaker (1 liter) containing about 300 cc. of hot distilled water. After the flux is completely disintegrated and dissolved, remove crucible, rinsing with distilled water. Cool the solution to 15° C. and add, slowly with constant stirring, hydrochloric acid (1 + 4) in amount sufficient to neutralize about 95 per cent of the alkaline constituents of the fusion mixture (*ca.* 20 cc. of hydrochloric acid and 80 cc. of water). Immediately add 50 cc. of the hypochlorite reagent and, while maintaining the solution at 15° C., add 60 cc. of phosphoric acid (1 + 2). Heat to boiling and boil to remove excess chlorine, using moistened starch-iodide paper as indicator. During the boiling, maintain the volume at not less than 300 cc. by addition of distilled water, if necessary. Cool to room temperature and transfer the solution with the aid of distilled water to an Erlenmeyer flask. Add 10 grams urea, heat to boiling, and boil for 30 minutes, maintaining the volume at not less than 300 cc. Cool to 20° C., add 10 cc. of 1 per cent potassium iodide solution, stopper, and allow to stand for 5 minutes. Titrate the liberated iodine with 0.005 *N* thiosulfate, using starch as indicator. Each cubic centimeter of 0.005 *N* thiosulfate is equivalent to 0.0001058 gram of iodine.

PROCEDURE FOR TABLETS. Weigh a counted number of not less than 20 tablets and reduce the weighed tablets to a fine powder. Weigh accurately a sample equivalent to 1 gram of thyroid and transfer to a nickel crucible. For each gram of

sample add 15 grams of U. S. P. XI fusion mixture and mix thoroughly. Superimpose a layer of fusion mixture, using 5 grams for each gram of sample. Continue with method as given above. When neutralizing the alkaline solution of the flux with hydrochloric acid (1 + 4), use about 20 cc. of hydrochloric acid and 80 cc. of water for each 20 grams of fusion mixture employed in the assay.

From the amount of iodine found, calculate to per cent of labeled amount on basis of U. S. P. mean (0.200 per cent) iodine content of thyroid.

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Determination of Salts in Crude Oil

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THE presence of salts in crude oil is one of the most serious problems confronting the petroleum refiner. These salts may contribute to (1) the mechanical clogging of furnace tubes, condensers, and lines by deposition; (2) the corrosion of equipment by the hydrolysis of salts producing hydrogen chloride; and (3) high ash content of still residues.

In predetermining the amount of salt in crude oil for the purpose of plant control, a quick and easy analytical method is of prime importance. A brief review of some of the many analytical methods now in use has been given by Blair (*1*). The more popular methods are essentially alike, in that they involve an intimate mixing of the oil with water, followed by the addition of an emulsion breaker. Frequently an organic solvent for hydrocarbons is added to reduce the viscosity of the oil. Essential differences lie in the manner of mixing the oil and water and in the demulsifying agents employed (*3*, *4*).

The aim of all extraction procedures is the quantitative removal of all the inorganic salts contained in the crude. This extraction is often difficult because the salts may be contained in brine-in-oil emulsions, rendered very stable by naturally occurring emulsifiers in the crude, or they may be present in the form of wax- or asphalt-protected crystals. Analysis of the extract may involve the quantitative determination of sodium, calcium, magnesium, sulfate, and chloride; but for most plant control work, only the chloride is determined. The chlorides are of first importance because of the hydrochloric acid released by the hydrolysis of the calcium and magnesium chlorides by crude oil distillation.

The following procedure for extracting salt from crudes and for determining chloride in the extract has been in use for some time in Continental Oil Company laboratories and has proved to be reasonably accurate. It has the advantage of being rapid, since only one extraction is required.

Procedure

Thoroughly mix the oil sample under test; then transfer exactly 125 ml. of the homogeneous sample to a 1-liter separatory funnel. If the oil is viscous, add 125 ml. of hot xylene, benzene, or toluene and shake until thoroughly mixed. Gasoline has always proved to be a very unsatisfactory diluent. The solvent is unnecessary with most crude oils lighter than 34 A. P. I. gravity, though it may always be used, particularly if an especially clear water extract is desired. For very light crudes, where the addition of a hot diluent may be hazardous, it is preferable to mix the oil and solvent at room temperature, then heat carefully to 60° C. (140° F.). Add 200 ml. of boiling distilled water and shake gently for 3 minutes, frequently relieving the pressure. (Invert the separatory funnel and release pressure through stopcock.) Add 20 ml. of phenol in 30 ml. of boiling distilled water and shake gently for 5 minutes. Better extraction and a cleaner break of the emulsion have been found to result from this stepwise addition of the water and phenol.

Allow the mixture to stand until more than 100 ml. of clear water have separated out. Filter off exactly 100 ml. of the water into a graduated cylinder through two sheets of heavy, qualitative filter paper. If negligible quantities of hydrogen sulfide and mercaptans are present, transfer the 100 ml. of filtered solution to an Erlenmeyer flask, adjust to a pH of 6.5 to 7.0, using 1 ml. of Continental indicator, add 0.5 ml. of a saturated solution of potassium chromate, and readjust the pH to 6.5 to 7.0. The advantage at this point of the use of a universal indicator of the nature of Continental indicator is its clear yellow color at this particular pH range. Experience has shown that a careful pH control at this stage is very important for a satisfactory chromate end point. Exact pH control for this titration has been found necessary in the presence of ammonium salts (*2*), and seems desirable in the presence of phenol. No interference by the pH indicator with the chromate titration has been observed.

Cool the solution to 29.4° C. (85° F.) and titrate with 0.0538 *N* silver nitrate to a light orange end point. Vigorous agitation of the solution during titration is absolutely necessary to ensure complete precipitation of the silver halide before the appearance of the silver chromate end point (*2*). A blank titration may be carried out on 92 ml. of water plus 8 ml. of phenol, the whole having been neutralized to a pH of 6.5 to 7.0 before addition of the chromate indicator. If either hydrogen sulfide or mercaptans or both are present, they must be removed before the halides can be determined. The method of acidifying and boiling is not applicable, since loss of hydrogen chloride may result.

Transfer the 100 ml. of filtered extract to a 250-ml. beaker, add 1 ml. of Continental indicator, and adjust the pH to 6.5 to 7.0 (light yellow). Precipitate the sulfides and mercaptans with an excess of cadmium nitrate. Allow to stand 1 hour, then transfer to a centrifuge tube. Centrifuge until the solution is clear and the precipitate is a dense firm mass. Decant the clear solution into an Erlenmeyer flask, filtering if necessary. Rinse the beaker with 10 ml. of distilled water into the centrifuge tube. Thoroughly wash the precipitate by shaking, and recentrifuge. Decant the clear wash water into the solution in the Erlenmeyer flask and adjust to 6.5 pH (yellow). Titrate for halides. If a centrifuge is not available, filtration through Whatman No. 44 filter paper may be substituted. An alternate method for chlorides in the presence of sulfides and mercaptans is the Volhard procedure.

Calculation.

$$(\text{Ml. of } AgNO_3 - \text{ml. of blank}) \times 10 = \text{grams of NaCl per barrel}$$

This relationship is true only if the exact volumes and concentrations specified are used. If the determination is desired in other units, the silver nitrate solution may be adjusted accordingly. If an appreciable amount of water is present initially in the crude, results will be too low unless corrected as follows:

$$\text{NaCl} \times \frac{250 + 1.25 \times \%\ \text{water}}{250}$$

where the per cent of water is determined by A. S. T. M. method D-95-40.

Discussion of Procedure

The use of phenol promotes the extraction of asphalt- or wax-protected crystals from the crude; hence phenol is essen-

Table I. Effect of Quantity of Phenol on Salt Extraction of Oklahoma City–Edmond, Okla., Crude Samples

Phenol Used	Water Used	NaCl
Ml.	*Ml.*	*Grams/bbl.*
0	250	28
5	245	33
10	240	39
20	230	43
25	225	43

Table II. Comparison of Conoco and Blair Salt-Extraction Methods

Method	Salt Extracted	
	Edmond crude	Oklahoma City–Lucien-Crescent crude
	Grams/bbl.	*Grams/bbl.*
Conoco, no diluent	33	75
Conoco, no diluent	34	74
Conoco + 200 ml. of hot xylene	34	75
Conoco + 125 ml. of hot benzene	35	75
Conoco, no diluent	(H_2S added to crude)	76
Conoco, no diluent	(H_2S and mercaptan added to crude)	74
Blair destabilizer A	34	75
Blair destabilizer A	36	73
Universal Oil Products[a]	34	74

[a] Triple extraction with gravimetric chlorine analysis for control.

tial for complete salt extraction, even when the oil and water do not form an emulsion. Twenty milliliters of phenol have been found to be an optimum quantity, as shown by Table I. Actual separation of the oil and water layers was equally good in all the extractions shown in Table I, but the salt extraction was not complete when less than 20 ml. of phenol was used.

The volume change in mixing phenol and water may be disregarded in these tests.

To check the phenol distribution between the water and crude, 125 ml. of a dry sample of Oklahoma City–Lucien-Crescent fields crude were extracted with 230 ml. of water and 20 ml. of phenol; 209 ml. of water-phenol mixture immediately separated and were drawn off (no filter paper used) and measured. On centrifuging, 38 ml. more of the water-phenol mixture were recovered, thus giving a total recovery of 247 ml., or a volume loss of 1 per cent. This loss is largely due to cooling and in a lesser degree to evaporation. Evidently very little phenol remains dissolved in the crude. Similar results were obtained with a variety of Kansas and Oklahoma crudes.

It will usually be observed that a blank made up of the same proportions of phenol and water as used in the extraction procedure will show a higher acidity than an actual extract water. This effect is attributed to the buffer action of the salts extracted from the crude, rather than to solution of the phenol in the crude. The use of a centrifuge to hasten or secure a more complete separation of the oil and water phases has been found to have no effect on the efficiency of salt extraction.

The data in Table II indicate good agreement between the Conoco and Blair methods, and a similar agreement was found between the Conoco method and triple extraction by the Universal Oil Products method (*4*). In the Conoco method, the use of an oil diluent is frequently unnecessary, as shown in Table II; but with viscous oils, the diluent simplifies the extraction. The hydrogen sulfide and mercaptans were added in the instances noted to check the accuracy of the halide analysis in their presence. In the case of most "sweet crudes" the cadmium separation is unnecessary. If, however, a dark precipitate appears at the start of the silver nitrate titration, the presence of hydrogen sulfide is indicated. In the case of unknown crudes, a second small aliquot portion of the extract water may be checked with cadmium nitrate solution before proceeding with the determination. The success of the cadmium removal of the hydrogen sulfide and mercaptans depends upon the exact adjustment of the pH values. In solutions with a pH but slightly lower than 6.5, the precipitation of the mercaptans is not complete; and some chloride may be carried down with the sulfide. The general effectiveness of phenol as a demulsifier has not been checked on all varieties of crude oils, but it has been used with success on many crudes from Oklahoma, Kansas, Texas, and Louisiana. If slow breaking emulsions are encountered, the difficulty may be due to too vigorous shaking, or it may be that phenol is not the proper demulsifier for the crude. Frequently the addition of a larger proportion of diluent is helpful.

The following Continental indicator mixture has been found very useful in this procedure, particularly for pH adjustment to 6.5 to 7.0. At this value, the indicator gives a clear yellow color which does not interfere with the chromate indicator. If, after the addition of the chromate, the solution is not a bright yellow, readjustment to pH 6.5 to 7.0 can be made by comparing the color with a blank of water and potassium chromate. Exact pH adjustment is very important, as is the quantity of potassium chromate indicator specified. Continental indicator has an advantage over pH paper in that it is more sensitive to slight variations of pH. It is more conveniently prepared, and is generally very useful.

Formula for Continental Indicator

Sodium salt of methyl red (Eastman)	0.20 gram
Bromothymol blue (Eastman) (dibromothymolsulfonphthalein)	0.60 gram
Phenolphthalein (Merck or Mallinckrodt)	0.64 gram

Dissolve the indicators in order in 1 liter of 50 per cent (by volume) ethanol and add sufficient dilute (0.1 *N*) sodium hydroxide to produce a green color (pH = 8). Store in tightly stoppered brown bottles of 100- to 250-ml. capacity. For a pH determination, use 0.10 ml. of indicator per 10 ml. of water sample. The color changes are as follows:

pH up to 3.0	Red	pH 8.0	Green
pH 4.0	Deeper red	pH 8.5	Bluish green
pH 5.0	Orange red	pH 9.0	Greenish blue
pH 5.5	Orange	pH 9.5	Blue
pH 6.0	Orange yellow	pH 10.0	Violet
pH 6.5	Yellow	pH 10.5	Reddish violet
pH 7.0–7.5	Greenish yellow	pH 11.0	Deeper reddish violet

The shaking times specified are minimum. Special care should be exercised in shaking the hot water and oil mixture. If highly volatile fractions are present in considerable quantities in the oil, a cloth should be wrapped around the funnel before shaking and the pressure frequently released.

For calcium and magnesium determination on a crude, the same general procedure may be employed; however, a 200-ml. sample of crude should be used and extracted with 365 ml. of water and 35 ml. of phenol. As large an aliquot as obtainable of the extract water should be drawn off for analysis.

Summary

A simple and rapid method for the quantitative extraction of inorganic salts from crude oils is presented, in which phenol serves as the destabilizing agent.

Provision is made for the separation of hydrogen sulfides and mercaptans, a precaution not taken in some of the common methods for salt extraction and analysis.

Details are given for easy regulation of the pH in the determination of extracted chlorides by the Mohr titration in the presence of phenol.

A formula is given for a simple and generally useful universal indicator to be used in the procedure.

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Determination of Iodate and Other Oxidizing Agents in the Presence of Cupric Salts

Use of the Iodine Monochloride End Point

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IN ORDER to determine iodate in the presence of cupric copper, Kapur and Verma (*2*) have recently suggested that the iodate be allowed to react with an excess of iodide and the iodine thus produced be titrated with a standard thiosulfate solution; by the addition of pyrophosphate the copper is held in the cupric state because of the formation of a stable complex ion. This method has the disadvantage that iodine is liberated very slowly by the iodate-iodide reaction under the conditions necessary for the formation of a stable cupric pyrophosphate complex ion.

If, however, the iodate is titrated with standard iodide solution to an iodine monochloride end point, cupric copper is not appreciably reduced at the equivalence point. The experiments described below show the conditions under which this reaction can be made the basis for accurate volumetric methods for determining iodate and certain other oxidizing agents in the presence of cupric salts.

Substances and Solutions

The chemicals used were of "analytical chemical" grade and were proved to be free from interfering substances.

Potassium iodide and iodate solutions were prepared from the dried salts, and the iodide solution was checked by titration against the iodate solution, using the iodine monochloride end point as recommended by Swift (*5*). A sodium thiosulfate solution was standardized against the iodate solution and a copper sulfate solution was standardized against the thiosulfate under conditions recommended by Foote and Vance (*1*). The carbon dioxide used was from a cylinder, and was proved to be free from significant amounts of reducing agents. Standard potassium bromate and potassium chlorate solutions were prepared directly from the dried salts. A permanganate solution was standardized against the potassium iodide solution, using the iodine monochloride end point.

Determination of Oxidizing Agents in Presence of Cupric Salts

TITRATION OF IODATE-COPPER MIXTURES WITH IODIDE. In the first procedure, 25 ml. of the iodate solution and 10 ml. of the cupric sulfate solution were transferred to a ground-glass stoppered flask. Carbon tetrachloride was added, followed by the volume of concentrated hydrochloric acid required to give the desired acid concentration. Standard iodide was then added from a buret until iodine was present, and the solution was back-titrated with iodate. A series of experiments made under various conditions showed that the iodate thus found was from 0.5 to 5 per cent low, and chlorine or hypochlorous acid was detected above the solutions.

The method was therefore modified by adding an excess of the standard iodide to a neutral solution, then adding the concentrated hydrochloric acid, and back-titrating the iodine present with standard iodate. Table I gives results of these titrations.

The error in titrations 1 to 3 and 6 was subsequently found to have been caused by making the iodate back-titrations too rapidly. In titration 9 the iodate-iodide mixture was acidified with 1 ml. of 6 *N* sulfuric acid before the hydrochloric acid was added.

TABLE I. TITRATION OF IODATE-COPPER MIXTURES WITH IODIDE

Expt.	0.1 *F* $CuSO_4$ Taken	H^+ Final Normality	KIO_3 (0.1000 *N*), Normality Found	Error
	Ml.			%
1	0	3.2	0.0997	−0.3
2	10	3.2	0.0998	−0.2
3	0	2.9	0.0997	−0.3
4	0	3.0	0.1000	0.0
5	0	3.0	0.1000	0.0
6	0	3.0	0.0998	−0.2
7	0	3.3[a]	0.09998	−0.02
8	0	3.4[a]	0.10006	+0.06
9	0	3.4	0.10006	+0.06
10	0	3.4	0.10006	+0.06
11	0	3.4	0.09996	−0.04
12	0	3.4	0.10002	+0.02
13	12.5	3.7	0.10008	+0.08
14	25	3.6	0.10006	+0.06
15	50	3.5	0.10006	+0.06

[a] Solution was first acidified with $HClO_4$, allowed to stand for 3 minutes, then sufficient NaCl added to make the chloride concentration 3.4 formal.

TABLE II. TITRATION OF PERMANGANATE-COPPER MIXTURES WITH IODIDE

Expt.	0.1 *F* $CuSO_4$ Taken	H^+, Final Normality	$KMnO_4$ (0.1068 *N*), Normality Found	Error
	Ml.			%
16	0	4.2	0.1066	−0.2
17	12.5	3.9	0.1066	−0.2
18	50	4.0	0.1067	−0.1
19	0	4.2	0.1066	−0.2
20	0	8.2	0.0990	−7.6
21	0	4.3	0.1067	−0.1
22	12.5	4.0	0.1067	−0.1
23	50	4.3	0.1066	−0.2

TABLE III. TITRATION OF BROMATE-COPPER MIXTURES WITH IODIDE

Expt.	0.1 *F* $CuSO_4$ Added	H^+, Final Normality	$KBrO_3$ Used	$KBrO_3$ Calcd.	Error
	Ml.		*Ml.*	*Ml.*	%
24	0	3.0	25.32	25.37	−0.2
25	12.5	3.0	25.22	25.27	−0.2
26	50	3.0	25.22	25.28	−0.2

TITRATION OF PERMANGANATE-COPPER MIXTURES WITH IODIDE. For experiments 16 to 23 a 25-ml. portion of permanganate was transferred by pipet to an iodine flask, 4 ml. of carbon tetrachloride and the cupric solution added, and then iodide was added from a buret until approximately 0.2 ml. was present in excess. This mixture was acidified with hydrochloric acid and back-titrated with permanganate. Table II lists the results.

The mixture of titration 19 was allowed to stand 15 minutes prior to acidification with hydrochloric acid in order to note if the manganese dioxide formed in the neutral solution caused catalytic decomposition of the permanganate.

In order to prevent the formation of manganese dioxide, titrations 21 to 23 were made by adding the iodide to a permanganate solution which was 0.6 *N* in sulfuric acid, then acidifying with hydrochloric acid and back-titrating.

TITRATION OF BROMATE-COPPER MIXTURES WITH IODIDE. To the bromate-copper solution were added carbon tetrachloride and then iodide until approximately 0.2 ml. of iodide was present in excess. The mixture was acidified with hydrochloric acid and back-titrated with bromate. Table III shows the results.

TITRATION OF CHLORATE-COPPER MIXTURES WITH IODIDE. The procedure used was similar to that used for the iodide titrations of the bromate-copper mixtures. Table IV lists the results. A pronounced tendency toward oxidation of iodide by air is found, since in experiments given treatment *a* no precautions were taken to exclude the air; in treatment *b* the chlorate and hydrochloric acid solutions were saturated with carbon dioxide; in treatment *c* the neutral mixture of chlorate and iodide was saturated with carbon dioxide and the hydrochloric acid saturated with CO_2.

Table IV. Titration of Chlorate-Copper Mixtures with Iodide

Expt.	0.1 *F* $CuSO_4$ Added	H^+, Final Normality	Treatment	$KClO_3$ Taken	$KClO_3$ Calcd.	Error
	Ml.			*Ml.*	*Ml.*	%
27	0	7.0	*a*	25.32	25.21	+0.4
28	0	7.2	*b*	25.11	25.08	+0.1
29	12.5	7.2	*c*	25.18	25.19	0.0
30	25	7.2	*c*	25.18	25.18	0.0

Table V. Titration of Iodide with Iodate-Copper Mixtures

Expt.	H^+, Final Normality	KIO_3 (0.0991 *N*) Normality Found	% Error
31–35	2.9	0.0994[a]	+0.3[a]
36–43	3.4	0.0995[a]	+0.4[a]

[a] Average.

Table VI. Oxidation of Iodide by Oxygen in the Presence of Copper

Expt.	$CuSO_4$ Formality	Treatment	Time	0.01/4 *F* KIO_3 Used
			Min.	*Ml.*
1	0	*a*	70	0.09
2a	0.0024	*a*	17	2.11
2b	0.0024	*b*	17	0.76
3a	0.012	*a*	17	4.19
3b	0.012	*b*	17	1.52

Treatments: *a*, flask open; *b*, solution saturated with CO_2 and flask filled with CO_2.

Table VII. Titration of Iodide with Iodate-Copper Mixtures B

Expt.	H^+, Final Normality	$CuSO_4$:KIO_3 Ratio of Volumes	KIO_3 (0.0991 *N*), Normality Found	% Error	Treatment
44	3.4	2:1	0.1011	+2.0	*a*
31–43	3.2	1:1	0.0995	+0.4[a]	*a*
45	4.8	1:2	0.0995	+0.4	*a*
46	3.4	2:1	0.0993	+0.2	*b*
47	4.8	1:2	0.0992	+0.1	*b*
48	3.4	2:1	0.0992	+0.1	*c*
49	3.4	1:1	0.0991	0.0	*c*
50	3.4	1:1	0.0990	−0.1	*c*
51	4.8	1:2	0.0991	0.0	*c*

[a] Average.

Titration of Iodide with Iodate-Copper Mixtures. In this procedure (which was developed before satisfactory conditions for the direct titration of the iodate-copper mixture had been found) the iodate-copper solution was diluted to an exact volume and a portion of the solution transferred to a buret. An exact volume of the standard iodide solution was then pipetted into a flask. Carbon tetrachloride and concentrated hydrochloric acid were added and this mixture, after being cooled, was titrated with the iodate-copper mixture. In the experiments shown in Table V exactly 10 ml. of the 0.04991 formal potassium iodide solution were taken; and the iodate-copper solution was composed of equal volumes of 0.0991 *N* (0.0991/4 formal) iodate solution and 0.1 formal cupric solution.

It seemed probable that the positive error was due to air oxidation of iodide. To investigate this, similar solutions of iodide and hydrochloric acid at the concentrations prevailing in a pretitration mixture were allowed to react with the same volume of cupric sulfate in the presence of air and under carbon dioxide. The resulting solutions (approximately 40 ml. in volume) were shaken with 4 ml. of carbon tetrachloride, the carbon tetrachloride was separated in a separating funnel and washed with small amounts of water, hydrochloric acid was added, and the iodine was titrated with 0.01 *N* iodate solution to an iodine monochloride end point. The results are listed in Table VI.

The iodine found in experiment 1 was due entirely to uncatalyzed air oxidation of iodide. The iodine of the experiments given treatment *a* was caused by oxidation by cupric ion and air oxidation of iodide, while that of the experiments given treatment *b* was due to oxidation of iodide by cupric ion solely. The oxidation of iodide by oxygen increased with increase of the copper concentration. This oxidation of iodide by oxygen under these conditions is of interest, since it is much larger than that usually experienced in the conventional iodometric determination of copper.

Experiments 44 to 51 (Table VII) were then made. Those experiments indicated as having been given treatment *a* were made as were experiments 31 to 43; in those indicated by treatment *b* the flask was filled with carbon dioxide and the acidified iodide solution saturated with carbon dioxide; in those indicated by treatment *c* both titrating and titrated solutions were saturated with carbon dioxide before the titration and the flask was filled with carbon dioxide. In each experiment 10 ml. of iodide solution were taken.

Thus iodate can be determined accurately in an iodate-copper solution by this method if the solutions are saturated with carbon dioxide previous to titration.

Determination of Total Copper and Oxidizing Agent

Titration of Total Copper and Iodate with Thiosulfate. These titrations were made as though the solutions being titrated contained only a cupric salt.

Iodate and cupric solutions were transferred by pipet to a flask and 1.5 ml. of 6 *N* sulfuric acid were added. Three grams of pulverized potassium iodide were added, the solution was swirled, then titrated immediately with thiosulfate solution. Starch was added as the end point was approached, and finally 2 grams of potassium thiocyanate. Table VIII lists the results.

Attempts were also made to titrate with thiosulfate the copper–iodine monochloride solutions obtained from the iodate determinations. These were not satisfactory because of the large volume resulting from the neutralization of the acid solution, the high salt concentration, and the stable emulsion formed by the cuprous iodide and the carbon tetrachloride.

Titration of Total Copper and Permanganate with Thiosulfate. The procedure used was the same as that for the determination of total copper and iodate except that 1.0 instead of 1.5 ml. of 6 *N* sulfuric acid were used. Table IX shows the results.

Titration of Total Copper and Bromate with Thiosulfate. The procedure was that used for the determina-

Table VIII. Titration of Iodate-Copper Mixtures with Thiosulfate

Expt.	0.0943 *N* $CuSO_4$ Taken	0.1487 *N* KIO_3 Taken	$Na_2S_2O_3$ Used	Na_2SO_3 Calcd.	Error
	Ml.	*Ml.*	*Ml.*	*Ml.*	%
52	20	10	28.95	28.94	0.0
53	10	20	29.40	29.43	−0.1
54	10	20	29.41	29.43	−0.1

Table IX. Titration of Total Copper and Permanganate with Thiosulfate

Expt.	0.0943 *N* $CuSO_4$ Taken	0.1068 *N* $KMnO_4$ Taken	$Na_2S_2O_3$ Used	$Na_2S_2O_3$ Calcd.	Error
	Ml.	*Ml.*	*Ml.*	*Ml.*	%
55	20	10	29.78	29.77	0.0
56	10	20	31.05	31.03	+0.1
57	10	20	31.03	31.03	0.0

Table X. Titration of Total Copper and Bromate with Thiosulfate

Expt.	0.0943 *N* $CuSO_4$ Taken	0.1000 *N* $KBrO_3$ Taken	$Na_2S_2O_3$ Used	$Na_2S_2O_3$ Calcd.	Error
	Ml.	*Ml.*	*Ml.*	*Ml.*	%
58	20	10	29.09	29.09	0.0
59	10	20	29.67	29.66	0.0
60	0	30	30.20	30.24	−0.2
61	10	20	29.64	29.66	−0.1
62	10	20	29.65	29.66	0.0
63	10	20	29.65	29.66	0.0

tion of the total of copper and iodate. Table X gives the results.

The titrations of Table X have a sulfuric acid concentration of 0.15 formal and, where copper is present, no consistent error is noted. Since this acid concentration is somewhat lower than that usually specified for the bromate-iodide reaction (*3*, *4*) especially in view of the results of experiment 60, it is suggested that copper may catalyze this reaction.

Titration of Total Chlorate and Copper with Thiosulfate. The chlorate-iodide reaction requires such a high hydrochloric acid concentration that the iodometric determination of copper under these conditions was not found feasible.

Summary

Iodate, permanganate, bromate, and chlorate in the presence of cupric salts can be determined by adding an excess of a standard iodide solution to neutral solutions of the mixtures, acidifying, and back-titrating with standard oxidizing solutions. An alternative method, investigated in the case of iodate-copper mixtures, consists in titrating a standard iodide solution with the mixture, all solutions having been saturated with carbon dioxide previous to titration.

The total amount of iodate and copper, of copper and permanganate, and of copper and bromate can be determined iodometrically with thiosulfate.

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Determination of Small Amounts of Gold with Stannous Chloride

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Previously known modifications of the widely accepted test for gold, the stannous chloride test, are shown to be qualitatively unreliable because of failure to take into account the factor of acid concentration. So-called colloidal gold may exist in two distinct forms: the yellow form produced in solutions of low acidity, and the purple form produced in solutions of high acidity. The low acid-stannous chloride test developed by the authors is more reproducible, sensitive, and reliable than the high acid test.

THE purpose of this paper is to present a reliable method for the qualitative detection and estimation of gold. Notwithstanding the fact that of all metals gold is second only to iron in economic importance (*11*), experience has indicated that a rapid and reliable chemical method for the qualitative detection and colorimetric determination of traces of gold has not yet been reported in the standard analytical texts. Heretofore, the most widely accepted colorimetric test for gold has been the stannous chloride "purple of Cassius" test (*3*, *6*, *7*, *10*, *12*), which was discovered by Andreas Cassius in 1663 (*8*, *9*). The authors find that previous descriptions of the test are qualitatively erroneous, as the factor of acid concentration has not been taken into account, and report below a reliable method for the detection and estimation of traces of gold.

The following reagents for the detection of gold have been considered, tried, and rejected: hydrogen peroxide, hydrogen peroxide–potassium hydroxide, potassium iodide, potassium iodide–potassium hydroxide, potassium iodide–potassium mercuric iodide–potassium hydroxide, resorcinol, resorcinol–potassium hydroxide, formaldehyde, formaldehyde–potassium hydroxide, benzaldehyde, benzaldehyde–potassium hydroxide, potassium formate–potassium hydroxide, acetylene–potassium hydroxide, sodium thiosulfate, ferrous sulfate, sodium bisulfite, sodium sulfite–potassium hydroxide, hydrogen sulfide, and glucose–potassium hydroxide. When used under the proper conditions, stannous chloride was found to give a more sensitive and more reproducible test than any of the above reagents.

Factors Influencing Stannous Chloride Test

Effect of Concentration of Acid on Tint of Color. The alleged variation in tint of color with variation in the gold concentration is the basis of the colorimetric determination as given in standard analytical texts (*3*, *6*, *7*, *10*, *12*).

Table I. Effect of Acid Concentration on Tint of Color

Series No.	HCl Concn.[a] in Colorimetric Medium *N*	Number of Trials	Maximum Deviation from Mean of Series[b] %	Tint of Color after Standing for 15 Minutes
1	0.002	20	10.6	Yellow
2	0.08	10	8.4	Light brown
3	0.16	20	12.9	Tan with faint purple tinge
4	0.32	10	10.2	Tan with distinct purple tinge
5	0.64	10	13.6	Purple

[a] Includes acid present in stannous chloride reagent.
[b] These values have little bearing on the reproducibility of the test under ordinary analytical conditions, as it is very difficult to prepare standards which have exactly the same ages and compositions as the unknowns. Values are included to show order of magnitude of experimental error.

In order to determine the effect of acid concentration on the tint of color, 40 ml. of a solution containing 0.20 mg. of gold, as bromide, were added to each of ten 50-ml. Nessler tubes, and the solutions were made up to the mark with distilled water and hydrochloric acid. The solutions therefore contained 4 mg. of gold per liter. The Nessler tubes were closed with a rubber stopper (that had been soaked in chlorine water), and inverted twice. To each tube was then added 1 ml. of 0.25 *M* stannous chloride (see Reagents), and the tube was again closed with a rubber stopper and inverted twice. One or two groups of ten trials each were made for each acid concentration. After standing for 15 minutes, one of the tubes of each series was taken as the standard and the rest of the tubes were compared with it by the balancing tube method. The results are given in Table I.

The data of Table I prove that the acid concentration is an important factor influencing the tint of color in the stannous chloride test. No previous investigator has recognized this (*3*, *6*, *7*, *10*, *12*). Gold cannot be determined by the stannous chloride method unless the acid concentration of the colorimetric medium is taken into account.

Effect of Acid Concentration on Velocity of Color Development. As the velocity of color development has an important bearing on the precision and reproducibility of the test, the effect of acid concentration was studied from this

standpoint. Color development takes place more slowly at the lower gold concentrations, and therefore only 0.10 mg. of gold was used in each trial. In other respects, the method of preparing the solutions was similar to the method used in determining the effect of acid concentration on the tint of color. The time of starting each trial was taken as the time when the Nessler tube was first inverted, after addition of the stannous chloride reagent. After standing for 10 minutes, 2.5 ml. of solution were withdrawn from the Nessler tube, a second trial, identical in composition to the previous trial, was started, and the time required for the two trials to match was noted. Three experiments (six trials) were made with each acid concentration. The results are recorded in Table II.

Table II. Effect of Acid Concentration on Velocity of Color Development

Series No.	HCl Concn.[a]	Time, after Start of Second Trial, Required for Colors to Match[b]	Tint of Color after 20 to 30 Minutes' Standing[c]
	N	*Sec.*	
1	0.002	32	Yellow
2	0.08	73	Yellow
3	0.16	400.	Light brown
4	0.64	820	Purple
5	5.0	1420	Dark blue[d]

[a] Includes hydrochloric acid added with stannous chloride reagent.
[b] Average of three experiments (six trials).
[c] With 0.64 *N* and 5.0 *N* hydrochloric acid solutions there was a distinct difference in tint of color between trials 20 minutes and 30 minutes old.
[d] More than 180 seconds required for development of visible coloration.

As shown by Table II, color development takes place more rapidly in the weakly acid solutions. Similar results (not given in Table II) were obtained with gold chloride in place of gold bromide. Moreover, when a limited time is available for the development of the color, the test is more sensitive at the lower acid concentrations.

Effect of Gold Concentration on Color Intensity. Except for the differences in technique indicated below, the procedure used in determining the effect of gold concentration on color intensity was similar to the procedure used in determining the effect of acid concentration on tint of color. The gold was present as bromide and 0.61 ml. of stannous chloride was used for each trial. After standing for 10 minutes, the colorimetric comparisons were begun (see Comparison of Colors below). One of the trials containing 0.50 mg. of gold was used as the standard for the other trials of Table III. Approximately 25 minutes from the time of starting the trials were required to complete the tests of each series.

Table III. Effect of Gold Concentration on Intensity of Color

Series No.	Gold Added per Liter of Colorimetric Medium	Gold Found per Liter of Colorimetric Medium[a]	Average Deviation from Mean of Series
	Mg.	*Mg.*	%
1	0.10	0.09	4.8
2	0.25	0.23	3.7
3	0.50	0.51	3.0
4	1.00	1.07	2.3
5	2.50	2.31	3.8
6	5.00	5.55	3.6

[a] Data are mean of several trials at each gold concentration. One of Nessler tubes of series 3 (0.50 mg. of gold) was used as standard for other trials of table.

Table III indicates the possibility of estimating the gold content of an unknown within about 12 per cent, even though the standard has a gold content very different from that of the unknown.

Other Characteristics of the Low Acid–Stannous Chloride Test. Regarding the stability of the yellow solutions, the authors have never observed any precipitation except in those cases where a flocculent precipitate of stannic oxides or oxychlorides caused coprecipitation of the gold content. The purple form of colloidal gold, in marked contrast to the yellow form, is not stable and invariably precipitates within a few hours from the strongly acid solutions.

In connection with the effect of acid concentration, a very interesting fact was observed which to the authors' knowledge has never been recorded in the literature: On adding hydrochloric acid to the yellow solutions, in amount sufficient to bring the concentration to within the range 2 *N* to 6 *N*, the color changes to blue or purple. This color change has been observed with solutions containing up to 300 mg. of gold per liter. With gold concentrations of 2 to 20 mg. per liter, the addition of hydrochloric acid causes the yellow solutions to become colorless for a period ranging from a fraction of a second to several minutes, after which a blue or purple color develops.

Regarding the stannous chloride concentration, a series of 24 trials with 0.25 mg. of gold as bromide dissolved in 50 ml. of solution, indicated that within the range of 0.0014 *M* to 0.14 *M* stannous chloride in the colorimetric medium, the color intensity is not a function of the stannous chloride concentration when the low acid test is used. Since stannic halides hydrolyze more readily than the stannous compounds, the amounts of excess oxidizing agents in the gold halide solutions should not be much larger than necessary to maintain the gold in solution. Sodium chloride and many other salts accelerate the formation of turbidity in dilute stannous chloride solutions, and for this reason the salt concentrations should be reduced to the lowest practicable values.

Reagents

Stannous Chloride (0.25 *M*). In a 30-ml. test tube were placed 22.6 grams of C. P. stannous chloride crystals ($SnCl_2.2H_2O$) and 2.33 ml. of 11.2 *M* hydrochloric acid. The mixture was heated and maintained at the boiling point for 2 or 3 minutes, and the clear solution was cooled to 40° to 50°, poured into 300 ml. of distilled water, and made up to a final volume of 400 ml. Turbid reagents, or reagents more than about 8 hours old, were not used.

Auric Bromide (500 Mg. of Gold per Liter). In a covered 250-ml. beaker were placed 125 mg. of C. P. precipitated gold powder, which did not have a metallic luster, and 5 ml. of saturated bromine water. Agglomerated particles were crushed with a stirring rod and 10 ml. of bromine water added, 0.5 to 2 ml. at a time; after each addition the beaker was kept covered with a watch glass, to prevent loss of bromine, and swirled. The gold bromide solution was warmed to about 50° in order to remove most of the excess bromine and then poured into a 250-ml. volumetric flask. The undissolved gold remaining in the beaker was treated with 2-ml. portions of bromine water until the gold dissolved, less than 20 ml. of bromine water being required. The gold bromide solution was warmed to 50°, transferred to the 250-ml. volumetric flask, and made up to the mark with distilled water. This reagent, containing 500 mg. of gold per liter, is stable for several months.

Other Reagents. The aqua regia solution of gold chloride was prepared according to Treadwell and Hall (*10*). The aurous cyanide solution was prepared by aspirating air through a suspension of precipitated gold in dilute sodium cyanide solution. The chlorine gas used for the preparation of the chlorine water was generated by the action of hydrochloric acid on calcium hypochlorite. The chlorine concentration in the chlorine water was determined by the potassium iodide method (*5*).

Recommended Procedure

Comparison of Colors. Colors were compared with matched 50-ml. Nessler tubes having a maximum length variation of about 2.5 per cent. The standard and unknown test solutions were diluted until the color intensities of 50-ml. portions were approximately equal. In the cases of the trials of Table IV, the dilutions were made in portions of 20 ml.; in all other experiments the dilutions were made in portions of 50 ml. Final comparison of colors was made by pouring the more highly colored solution from its Nessler tube until the colors matched and measuring the depths of the solutions. Thus a combination of the dilution and balancing tube methods was used.

Procedure for Determination of Gold in Cyanide Solutions. The procedure used was that of Bugbee (*3*), modified as indicated:

One liter of the cyanide solution containing gold was poured into a 4-liter beaker, 0.12 ml. of saturated lead acetate solution added, and the resulting precipitate dissolved by stirring. To the solution was added 0.75 gram of zinc dust, and the solution was swirled. After addition of 20 ml. of saturated sodium cyanide and 20 ml. of concentrated ammonia solution, the solution was vigorously stirred for 5 minutes. The lead sponge precipitate was allowed to settle and all but about 50 ml. of the solution decanted. The precipitate was transferred to a filter containing an 11-cm. Whatman No. 2 paper and washed to the apex of the filter paper. After thoroughly washing with distilled water, dilute (10 to 25 per cent) nitric acid was cautiously added until all the precipitate, with the exception of the black gold particles, had dissolved. The filter paper was washed with distilled water, and the black gold particles were transferred to the apex. A 100-ml. beaker was then placed under the funnel, and 10 ml. of saturated bromine water were passed through the filter paper, 1 ml. at a time, over a period of 15 minutes, the funnel being kept covered to prevent loss of bromine. If black particles remain on the filter paper after this treatment, it is generally an indication of the presence of carbon in the zinc dust rather than of incomplete solution of the gold.

The filter paper was washed with 8 ml. of water, and the filtrate containing the gold heated to about 45° on a hot plate. The beaker was removed from the hot plate and allowed to stand for about 40 minutes, during which time most of the bromine escaped from the solution. The solution was placed in a 50-ml. Nessler tube and made up with distilled water to a volume of 18 ml., and 2 ml. of 0.25 *M* stannous chloride reagent were added. Immediately after the stannous chloride addition, the tube was closed with a rubber stopper that had been washed with chlorine water, and inverted twice. The gold standards, before addition of the stannous chloride, had a gold concentration of 1.73 mg. per liter of gold as bromide, or 0.0311 mg. of gold in 18 ml. of standard solution. After standing for 10 minutes or more, the relative color intensities were compared as indicated above.

Experimental Evidence

Experiments with the low acid–stannous chloride method having demonstrated its superiority to the standard test method in which the acid concentration is both high and uncontrolled, confirmatory trials were made with a synthetic leaching solution. The concentrations of the various constituents were (grams per liter): free sodium cyanide, 0.5; iron, 0.025; copper, 0.025; and calcium oxide, 0.6.

The iron was added in the form of potassium ferrocyanide. Copper was added in the form of sodium cuprocyanide, which was prepared by adding sodium cyanide to copper sulfate solution until the precipitate which first formed had just dissolved. Variable amounts of aurocyanide solution (see Reagents) were also added. The synthetic leaching solution is believed to be similar to those encountered in cyanidation practice. Some results obtained with this procedure as applied to this solution are given in Table IV.

Table IV. Estimation of Gold in Cyanide Solutions by the Low Acid–Stannous Chloride Test

Trial No.	Gold Added[a]	Gold Found[b]	Error
	Mg.	*Mg.*	%
1	0.00	0.004	..
2	0.00	0.000	..
Mean	0.00	0.002	..
3	0.0311	0.026	−16
4	0.0311	0.022	−29
Mean	0.0311	0.024	−22.5
5	0.0467	0.048	+ 3.6
6	0.0467	0.043	− 7.9
Mean	0.0467	0.0455	− 5.8
7	0.0622	0.076	+22
8	0.0622	0.064	+ 3
Mean	0.0622	0.070	+12.5
9	0.0934	0.090	− 3.6
10	0.0934	0.102	+ 9.2
Mean	0.0934	0.096	+ 6.4
11	0.124	0.112	− 9.9
12	0.124	0.112	− 9.9
Mean	0.124	0.112	− 9.9

[a] Dissolved in 1 liter of synthetic leaching solution.
[b] Corrected for blank (trials 1 and 2).

Discussion

The tints of color obtained in the experiments of Table IV (acid concentration below about 0.05 *N*) varied from light yellow to light brown; in no instance was a purple or blue color obtained. Thus, for the first time, the authors have been able to make the stannous chloride test under such conditions that a constant tint of color is obtained, regardless of the gold concentration.

With respect to reproducibility, precision, and accuracy, the method is superior to the stannous chloride test given in the standard texts. Following directions given in standard texts, the authors have found it impossible to obtain the stipulated qualitative and colorimetric results (*4*). The tint of color obtained in the stannous chloride test for gold is primarily a function of the acid concentration rather than of the gold concentration, as shown by Table I.

Table IV indicates that by controlling the acidity, the stannous chloride test can be placed on a semiquantitative basis. The maximum errors were of the order of 0.01 mg. There are no cupellation losses with this method, and special apparatus, such as muffle furnaces and assay balances, is dispensed with. The errors in weighing with an assay balance are generally of the order of 0.01 mg. (*1*), corresponding to 33 per cent error with 0.03 mg., and the partial substitution of filtration for decantation probably reduces the errors due to loss of gold when separating the gold particles from solution (*2*). When less than 0.04 mg. of gold is to be determined, the low acid–stannous chloride test is believed to be superior to the gravimetric assay procedure with respect to accuracy and speed.

When a yellow or brown solution, prepared by the low acid–stannous chloride method and containing gold in the colloid state, is treated with sufficient hydrochloric acid to bring the acid concentration to within the range 2 *N* to 6 *N*, the solution changes from the relatively stable yellow form to the unstable purple form. With gold concentrations of 2 to 20 mg. per liter, there is a distinct time interval during which the solution becomes colorless before development of the purple color.

The time required for development of the color to full intensity is much less at low than at high concentrations of hydrochloric acid, and the test is therefore probably more reproducible at low concentrations of hydrochloric acid.

Within the range of 0.0014 *M* to 0.14 *M* stannous chloride, the tint and intensity of color with the low acid–stannous chloride test are not appreciably affected by change in the stannous chloride concentration.

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Photometric Determination of Potassium

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Potassium is determined photometrically by oxidation of the dipotassium sodium cobaltinitrite precipitate with standard potassium dichromate in the presence of sulfuric acid, and estimation of the resulting colored solution in a photoelectric colorimeter.

THE estimation of potassium in solutions of plant ash and soil extracts by methods based on precipitation as cobaltinitrite has been reported by many workers, using modifications of the original work of Adie and Wood (*1*). Work with some of these proposed modifications (*2*, *4*, *6*) suggested to the writer that further modifications would result in a simpler and more rapid method of determination without sacrifice of accuracy and reproducibility.

The method described takes advantage of the fact that when a solution of potassium dichromate is reduced it changes from bright yellow to green, and that the amount of change depends upon the amount of reduction. The dipotassium sodium cobaltinitrite precipitate formed in the potassium determination is used to reduce a definite volume of standard potassium dichromate, forming various shades of yellow to green solutions. The absorption of these solutions is obtained with a photoelectric colorimeter. In this manner, a standard or working curve is obtained, from which the potassium content of unknown solutions can be determined.

Reagents

Precipitating reagent, an aqueous solution of trisodium cobaltinitrite containing 1 gram of reagent quality salt in 5 ml. of distilled water. The solution is filtered before use.

Nitric acid, approximately 1 *N* (64 ml. of concentrated nitric acid per liter) and 0.01 *N*.

Concentrated sulfuric acid.

Potassium dichromate, exactly decinormal solution prepared by dissolving exactly 4.9035 grams of oven-dry potassium dichromate crystals and diluting to exactly 1 liter with distilled water.

Procedure

Place a 10-ml. aliquot of approximately neutral aqueous solution containing from 1 to 7 mg. of potassium in a 25 × 150-mm. Pyrex test tube. Add 1 ml. of *N* nitric acid, mix, add 5 ml. of trisodium cobaltinitrite reagent, and again mix. Allow the mixture to stand 2 hours at a temperature near 20° C. Wash down the walls of the test tubes and balance them with 0.01 *N* nitric acid. Centrifuge at about 2200 r. p. m. for 10 minutes, pour off the supernatant liquid, and allow the tubes to drain several minutes. Wash the precipitate with 15 ml. of 0.01 *N* nitric acid, being sure to mix the precipitate thoroughly with the wash solution, centrifuge, and drain as before. Add exactly 10 ml. of decinormal potassium dichromate solution, then 5 ml. of concentrated sulfuric acid, and mix thoroughly. Heat will be produced, and the precipitate will be oxidized; a clear solution will result. Place tubes in a cool water bath. When cooled to room temperature, pour the solution and rinse the tube into a 100-ml. volumetric flask and make to volume with distilled water. Mix the solution and read absorption in a photoelectric colorimeter using a filter of about 425 millimicrons transmission. Plot the absorption against milligrams of potassium carried through the procedure as outlined. Unknown concentrations of potassium can then be obtained from this curve.

Discussion

The advantages of the trisodium cobaltinitrite precipitating reagent used in this method are discussed by Wilcox (*6*). This reagent also has the advantage of being easily made up and being immediately ready for use. Only enough should be made up to run the determinations that can be handled in one day.

The temperature at which the precipitation proceeds should be kept nearly constant, about 20° C., since it has been noted (*5*, *6*) that the temperature affects the composition of the precipitate. This can be accomplished by using a water bath. By carrying several known concentrations of potassium through the procedure simultaneously with a set of determinations and referring to the curve to which these known concentrations correspond, the temperature factor can be eliminated.

The effect of length of time of precipitation can be compensated for in a similar manner. The curves shown in Figure 1 confirm the generally recognized observation that the amount of precipitate is influenced by the time permitted for precipitation. It is very important to keep the time of precipitation constant once a working curve is made, and to check by running a few standards with each set of determinations.

Neither the accuracy nor the reproducibility of the method was affected appreciably by washing the precipitate only once with 15 ml. of 0.01 *N* nitric acid. This shortened procedure saves considerable time, a conclusion also reached by others (*2*).

Other ions, such as those of sodium, calcium, barium, magnesium, strontium, iron, zinc, chloride, nitrate, sulfate, and phosphate, in the concentrations usually found in plant tissues and soil extracts, do not interfere with the precipitation of potassium by trisodium cobaltinitrite (*3*, *6*). The ammonium ion is the only one which will interfere seriously, and its presence should be guarded against throughout the procedure. Wet digestions in which both nitric and hydrochloric acid have been used with sulfuric acid will not contain ammonia. There will be no silica in solution with these wet

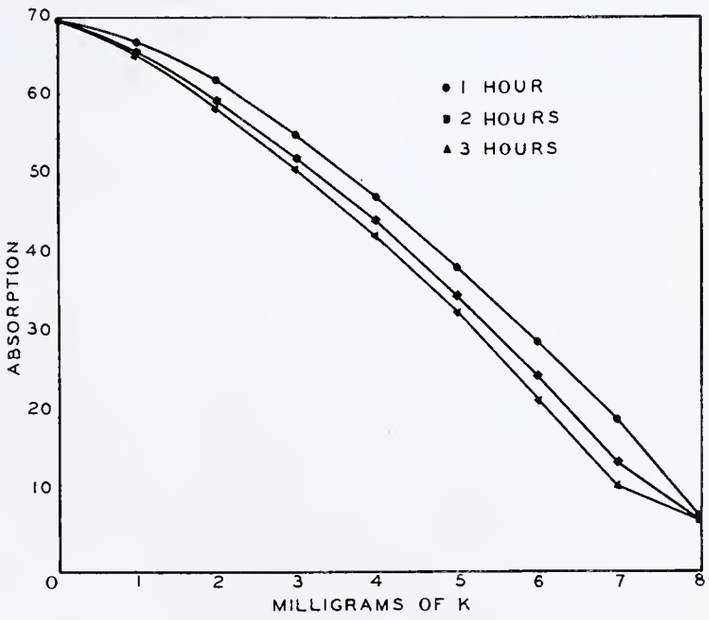

FIGURE 1. EFFECT OF AMOUNT OF POTASSIUM DICHROMATE UPON LIGHT ABSORPTION

Potassium dichromate reduced by dipotassium cobaltinitrite in different lengths of time for precipitation. This working curve was obtained by using for the ordinate the scale A readings (absorption) obtained with a Fischer AC model electrophotometer.

Table I. Recovery of Known Amounts of Potassium as Potassium Chloride Added to Solutions of Plant Ash

Standard Solution, 0.0005 Gram of K per Ml.	Ash Solution	K Found	K Calculated	Error
Ml.	*Ml.*	*Mg.*	*Mg.*	*%*
0	10	3.28	...	...
2	8	3.58	3.62	−1.1
4	6	3.90	3.97	−1.7
6	4	4.25	4.31	−1.4
8	2	4.55	4.65	−2.2
10	0	5.00	5.00	...
0.001 Gram of K per Ml.				
0	5	2.30	...	...
1	5	3.30	3.30	0
2	5	4.23	4.30	−1.6
3	5	5.25	5.30	−0.9
4	5	6.38	6.30	+1.3

[a] Made to 10-ml. volume before precipitation.

digestions either; thus no silica will separate as silica gel when the solutions are acidified.

The light absorption of the various partially reduced dichromate solutions remains the same over the temperature range 15° to 30° C. The color of the solution remains constant for as long as one week after the precipitate is oxidized and the solution is made to volume, if the solution is protected from dust and strong light. It should not, however, be expected that these solutions will remain so indefinitely, because the chromium complex may change from green to violet, especially if considerable reduction has taken place. Absorption readings made on solutions one week old did not vary more than 0.5 absorption unit from those made immediately after the solution was prepared. This variation represents 0.03 to 0.05 mg. of potassium, depending upon the amount of potassium actually present, and this error, which might be attributed to the photoelectric colorimeter, is of no greater magnitude than other errors in the method. Table I presents results obtained on the recovery of known amounts of potassium added in increasing increments to a constant or known volume of unknown solution obtained by wet-digesting plant tissue. These results and the curves in Figure 1 would indicate that 1 to 7 mg. of potassium can be determined within 2 to 3 per cent error.

By using a few simply made solutions and short-cutting the usual methods of color development used in colorimetry, determinations can be made much more rapidly without sacrificing the accuracy of reproducibility expected in this type of determination. Only one easily made and stable standard solution is necessary, whereas two standard solutions are required for the volumetric estimation of the precipitate. The use of a working curve eliminates a factor which presupposes a straight-line function in the development of the precipitate.

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A Fiber Identification Stain

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THE identification of unknown fibers is frequently requested of the laboratory. The rapid procession of new materials in fiber or sheet form makes it impossible to keep up with all of them, but certain dye mixtures give significant colors which aid in the detection of the more common materials.

The National Bureau of Standards (*1*) gives several dye systems for the identification of the rayons. Chief among these are two Hahn stains:

A. 1% picric acid and 0.2% Soluble Blue 2B Extra (presumably Colour Index No. 707)
B. 1% tannic acid and 0.2% Soluble Blue 2B Extra and 0.1% eosin (presumably Colour Index No. 768)

These stains produce the following rayon colors:

	Acetate	Cuprammonium	Nitrocellulose	Viscose
A	Yellow	Deep blue	Colorless	Pale blue
B	Colorless	Deep blue	Lavender	Lavender

A study of these formulas and the color produced suggested that it might be possible to combine them so as to get a more versatile and selective stain. Because of the low solubility of the eosin, it was replaced by acid fuchsin, and the hydrochloric acid was omitted. After trials of other proportions, the following mixture was found to be most useful:

	Grams
Acid fuchsin (Colour Index No. 692)	6
Picric acid	10
Tannic acid	10
National Soluble Blue 2B Extra (Colour Index No. 707)	5

The dyes may be ground together and dissolved together, or dissolved separately in any order and diluted to 1 liter. While the over-all concentration may not be very critical, these ratios of components appear to give the best differentiation. The dye mixture can be dissolved readily only in hot water, but the solution may be used hot or cold. Momentary immersion in the hot solution is sufficient, but commonly something over 2 minutes is allowed for cold dyeing. A thorough rinsing in water completes the test. Some dyed textiles may be identified without previous bleaching. The fibers are treated as usual, and then rinsed. When pressed wet (after rinsing) between white absorbent papers, a dye mixture characteristic of the color which would have been shown by the undyed fibers is transferred to the papers.

The colors shown by common fibers are:

Vegetable fibers	Cotton or linen	Light blue
Synthetic fibers	Acetate or nylon	Pale greenish yellow
	Cuprammonium	Dark blue
	Viscose	Lavender
	Vinyon	Very pale blue
Animal fibers	Wool	Yellow
	Silk (raw)	Black
	Silk (degummed)	Brown

The stain is also useful in the identification of films of cellulose acetate or viscose (cellophane), giving the above colors. The colors realized with any such dye mixture will depend somewhat on the history of the sample tested, and increased confidence follows a check by other dye mixtures.

A solution of this dye mixture is being made available by Eimer and Amend, New York, N. Y.

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Polarographic Determination of Potassium, Sodium, and Lithium

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THE polarographic determination of alkali metals has been investigated by several workers. Heyrovský and Ilkovič (*1*), Majer (*6*), and Peracchio and Meloche (*7*) have made a study of the effects of both the solvent and the supporting electrolyte on the relationship between the diffusion current and the alkali-ion concentration. Other authors have also contributed to the polarographic determination of the alkali metals in various materials (*4*).

Since alkali metals yield diffusion waves at very negative potentials (near −2.3 volts), the tetraalkylammonium halides or hydroxides are the most suitable supporting electrolytes. Tetramethylammonium hydroxide has been recommended as such. The authors found the tetraethylammonium compounds more suitable, since the tetraethylammonium ion is discharged at a potential about 0.2 volt more negative than that of the corresponding methyl ion. Especially with lithium it is very difficult to get a well-defined diffusion current with tetramethylammonium hydroxide as supporting electrolyte, even in relatively concentrated alcoholic medium.

Preliminary investigation confirmed the observations by previous workers that the composition and the degree of purity of the supporting electrolyte are the most important factors in obtaining useful polarographic waves of alkali metals. However, during the course of their work the authors met many difficulties which have not been mentioned in the literature. The main purpose of the present investigation was to find the best general conditions for the determination of the alkali ions. The examination has been extended beyond the exclusive use of the tetraalkylammonium compounds as supporting electrolytes. In fact, lithium hydroxide appears to present some advantages over the tetraalkylammonium compounds in the determination of sodium and potassium.

TABLE I. ANODE POTENTIALS IN DIFFERENT SUPPORTING ELECTROLYTE SOLUTIONS (*vs.* S. C. E.)

Solvent	0.16 *N* Tetraethylammonium Hydroxide	0.1 *N* Tetramethylammonium Hydroxide	0.15 *N* Lithium Hydroxide
	Volt	*Volt*	*Volt*
Water	−0.300	−0.275	...
25% ethanol	−0.305	−0.280	...
50% ethanol	−0.320	−0.290	−0.195
80% ethanol	−0.340	−0.310	−0.220

Experimental

The current-voltage curves were measured with the Heyrovský polarograph, manufactured by V. and I. Nejedly in Prague (Czechoslovakia). The dropping electrode consisted of a drawn-out capillary tube connected to the mercury reservoir by means of rubber and glass tubing. Different capillaries with varying pressures of mercury have been used in order to study the effect of the drop time on the diffusion current. The initial drop times in the authors' experiments were between 1.8 and 15.0 seconds.

An electrolytic cell with a pool of pure mercury as anode was used in all experiments. The anode potential was measured separately against an external saturated calomel electrode (S. C. E.), using an Irving and Smith (*2*) salt bridge filled with the supporting electrolyte solution used in the cell. Since the anode potential remained constant within a few millivolts during the electrolysis, the potential of the dropping electrode was found from the applied e. m. f., taking into account the voltage drop due to the internal resistance of the cell. This resistance never exceeded 1000 ohms. The average values (±10 millivolts) of the mercury anode potentials are given in Table I.

All experiments were carried out in a thermostat at 25° C. Air was always removed from the solution by passing through purified nitrogen.

Materials Used

Four tetraalkylammonium products were used as supporting electrolytes in this work. Kahlbaum's Reagent tetramethylammonium iodide and tetraethylammonium iodide were used as the starting products. Both salts were purified by repeated recrystallizations from ethanol-water mixtures.

The tetraalkylammonium hydroxides were prepared from the iodides according to directions of Peracchio and Meloche (*7*). The authors found that the freshly prepared solutions of the hydroxides yielded relatively large residual currents in the potential region where the alkali metal waves occur. These currents decreased with increasing alcohol concentration but they were still marked even in 80 per cent ethanol.

A precipitate was observed to form in both the aqueous and alcoholic solutions of the hydroxides upon standing. The residual current decreased during the period of separation of this precipitate. This phenomenon is not mentioned in the literature, but it is of great consequence in exact polarographic work. When a solution of tetraethylammonium hydroxide in water was allowed to stand in the dark for 5 to 10 days, the residual current of the filtrate did not decrease upon further standing. The solution remained perfectly clear and could be kept for several weeks without any change in the residual current. The alcohol-water solutions seemed to be somewhat less stable. The authors recommend the preparation of a 0.5 to 1.0 *N* solution of tetraethylammonium hydroxide in water, followed by filtration after about 8 days of standing. The filtrate is used for the subsequent preparation of mixtures with alcohol.

The change of the residual current at various cathode potentials ($\pi_{d.e.}$) of a 0.16 *N* tetraethylammonium hydroxide solution in 50 per cent ethanol with the time of aging of the aqueous solution is given in Table II.

For the lithium hydroxide solutions Merck's Reagent was used. Solutions prepared in 50 and 80 per cent ethanol were turbid, owing to the precipitation of lithium carbonate. The precipitate settled very slowly. After several days of standing the supernatant liquid became clear and was filtered through hardened filter paper. When protected from the air, the filtrate remained clear indefinitely. The residual currents of lithium hydroxide solutions were markedly smaller than those of tetraalkylammonium hydroxides at the same cathode potentials up to −2.2 volts.

Experimental Results

From the analytical viewpoint it is of importance that the diffusion current be proportional to the concentration of the alkali ions. Solutions of potassium, sodium, and lithium chlorides of varying concentrations were electrolyzed in water, and in 25, 50, and 80 per cent ethanol as a solvent, using tetramethylammonium hydroxide, tetraethylammonium hydroxide, and lithium hydroxide as the supporting electrolytes. The diffusion current of sodium and potassium ions was measured in all the work at cathode potentials between −2.20 and −2.40 volts, that of lithium between −2.45 and −2.60 volts (*vs.* S. C. E.).

TABLE II. VALUES OF RESIDUAL CURRENT OF 0.16 *N* TETRAETHYLAMMONIUM HYDROXIDE SOLUTION IN 50 PER CENT ETHANOL

Age of Aqueous Solution	$\pi_{d.e.}$ (*vs.* S. C. E.), volts: −0.450	−0.900	−1.350	−1.800	−2.25	−2.40	−2.55	−2.70
	Microamperes							
3 hours	0.33	0.66	1.12	1.35	1.91	2.25	3.03	4.72
4 days	0.11	0.38	0.60	0.78	1.23	1.46	2.14	3.94
9 days	0.06	0.33	0.55	0.60	0.67	0.90	1.46	3.26

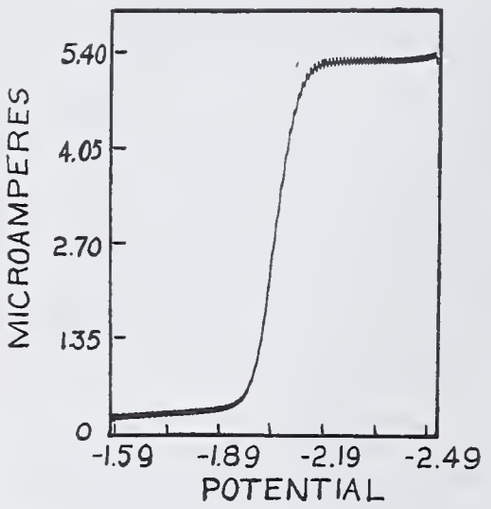

FIGURE 1. POLAROGRAM OF 0.0017 *N* POTASSIUM CHLORIDE IN 80 PER CENT ETHANOL WITH 0.18 *N* TETRAETHYLAMMONIUM HYDROXIDE AS SUPPORTING ELECTROLYTE

Initial drop time, 3.1 seconds. Anode potential, −0.350 volt (*vs.* S. C. E.)

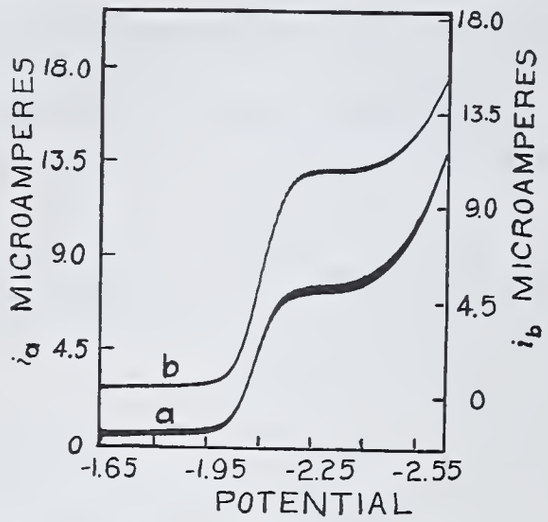

FIGURE 2. PROPORTIONALITY BETWEEN DIFFUSION CURRENT AND CONCENTRATION OF ALKALI IONS

0.0019 *N* (*a*) and 0.0029 *N* (*b*) potassium chloride in 50 per cent ethanol with 0.16 *N* tetraethylammonium hydroxide as supporting electrolyte. Initial drop time, 3.1 seconds. Anode potential, −0.305 volt (*vs.* S. C. E.)

Since the residual currents in water and in 25 per cent ethanol were considerable, solutions of sodium and potassium salts in the concentration range between 0.0005 *N* and 0.008 *N* could not be determined in those media with an accuracy greater than 5 per cent. With alcohol concentrations over 40 per cent the proportionality between the diffusion current and the concentration was found to hold within 2 per cent. Figures 1 and 2 show that the diffusion current of potassium can be measured accurately in both 50 and 80 per cent ethanol. The same is true for sodium.

From the experience gained in a great number of experiments the authors recommend the use of 50 per cent ethanol as the most suitable medium for the polarographic determination of potassium and sodium. Some results obtained with potassium chloride in 50 and 80 per cent ethanol, reported in Table III, show that in the investigated concentration range from 0.0008 *N* to 0.004 *N* the deviation from the proportionality between the diffusion current and the concentration did not exceed 2 per cent. The same accuracy has been found in the case of sodium. All reported values of the diffusion current were corrected for the residual current. If this correction is omitted, no straight-line relation is found between the diffusion current and concentration.

In agreement with Peracchio and Meloche (*7*) the authors found that lithium gives a badly defined diffusion current in aqueous solutions. In 80 per cent ethanol the diffusion current of lithium can be measured accurately (Figure 3). In this medium the diffusion current was found to be proportional to the concentration. The data obtained over a concentration range between 0.0009 *N* and 0.007 *N* lithium chloride are presented in Table IV.

Extensive investigations were made on the effect of the drop time upon the relation between the diffusion current and the concentration of potassium and sodium in 50 and 80 per cent ethanol and of lithium in the latter medium. It was found that the linear relation holds when the initial drop time is equal to or greater than 3 seconds. Moreover, in order to find this linear relation, the concentration of the supporting electrolyte should be at least 50 times as large as the concentration of the alkali ions. Thus, in measuring the diffusion current of a 0.001 *N* solution of an alkali metal the concentration of the supporting electrolyte should be at least 0.05 *N*, and for 0.005 *N* solution, at least 0.25 *N*. If the ratio of the concentration of the supporting electrolyte to that of the alkali ion is less than 50, the limiting current is larger than the diffusion current because the migration current of the alkali ions is not eliminated under these conditions. The diffusion current of the alkali metals is not affected by a large excess of the supporting electrolyte.

Summarizing all the data, potassium and sodium are best determined in a medium of 50 per cent ethanol, the concentration of tetraethylammonium hydroxide should be at least 50 times greater than that of the alkali ions, and the initial

TABLE III. PROPORTIONALITY BETWEEN DIFFUSION CURRENT AND POTASSIUM CHLORIDE CONCENTRATION

[c_1, concentration of KCl in 50 and 80 per cent ethanol. c_2, concentration of tetraethylammonium hydroxide. Initial drop time of capillary, 4.75 seconds. i and i_r, microamperes, measured at $\pi d.e.$ = −2.25 volts (*vs.* S. C. E.)]

Concentration of Alcohol, Volume %	c_1, $N \times 10^3$	$\frac{c_2}{N}$	$\frac{c_2}{c_1}$	Apparent Diffusion Current, i	Residual Current, i_r	$i_d = i - i_r$	Concentration Ratio, R_c	Current Ratio, R_i	$\Delta\% = \frac{R_i - R_c}{R_c} \times 100$
50	0.77	0.09	117	3.04	0.88	2.16	1	1	..
50	1.41	0.12	86	4.94	1.04	3.90	1.83	1.81	−1.1
50	1.96	0.19	97	6.70	1.11	5.59	2.54	2.59	+1.9
50	2.43	0.18	74	8.02	1.07	6.95	3.15	3.22	+2.2
50	3.19	0.28	88	10.25	1.16	9.09	4.14	4.21	+1.7
50	4.25	0.26	61	13.50	1.30	12.20	5.52	5.64	+2.1
80	0.77	0.09	117	2.60	0.68	1.92	1	1	..
80	1.41	0.11	78	4.42	0.96	3.46	1.83	1.80	−1.6
80	1.96	0.19	97	5.83	1.05	4.78	2.54	2.49	−1.9
80	2.43	0.23	95	7.01	1.08	5.93	3.15	3.09	−1.9
80	3.19	0.28	88	9.04	1.17	7.87	4.14	4.10	−0.9

TABLE IV. PROPORTIONALITY BETWEEN DIFFUSION CURRENT AND CONCENTRATION OF LITHIUM CHLORIDE IN 80 PER CENT ETHANOL

[Initial drop time of capillary, 4.5 seconds. i and i_r, microamperes, measured at: (*a*) − 2.44 and (*b*) −2.59 volts (*vs.* S. C. E.). c_1, lithium chloride concentration. c_2, concentration of tetraethylammonium hydroxide]

c_1, $N \times 10^3$	c_2, N	$\frac{c_2}{c_1}$	i		i_r		$i_d = i - i_r$		Concentration Ratio, R_c	Diffusion Current Ratio, R_i		$\Delta\% = \frac{R_i - R_c}{R_c} \times 100$	
			a	*b*	*a*	*b*	*a*	*b*		*a*	*b*	*a*	*b*
0.90	0.09	100	2.35	2.31	0.73	0.80	1.62	1.51	1	1	1	..	..
1.66	0.12	72	3.85	3.84	0.87	1.00	2.98	2.84	1.84	1.84	1.88	±0.0	+2.1
2.34	0.22	94	5.29	5.32	1.03	1.27	4.26	4.05	2.60	2.63	2.68	+1.2	+3.1
4.16	0.28	67	8.72	8.53	1.14	1.36	7.58	7.17	4.62	4.68	4.75	+1.3	+2.9
6.84	0.41	60	14.09	13.77	1.62	1.98	12.47	11.79	7.60	7.70	7.81	+1.5	+2.7

Table V. Half-Wave Potentials (*vs.* S. C. E.) of Alkali Ions in Ethanol-Water Mixtures

Ethanol Concentration, *Vol.%*	$\pi_{1/2}$ of K^+ *Volts*	$\pi_{1/2}$ of Na^+ *Volts*	$\pi_{1/2}$ of Li^+ *Volts*
0	−2.14	−2.12	..
25	−2.12	−2.10	..
50	−2.095	−2.07	−2.31
80	−2.05	−2.035	−2.28

Table VI. Diffusion Currents in Mixtures of Sodium and Potassium Chlorides in 50 Per Cent Ethanol

Concentration of KCl, c_1, $N \times 10^3$	Concentration of NaCl, c_2, $N \times 10^3$	$\frac{c_1}{c_2}$	$\pi_{d.e.}$ (*vs.* S. C. E.)	Diffusion Current Experimentally determined, i'_d	Diffusion Current Calculated, i''_d	$\Delta\% = \frac{i'_d - i''_d}{i''_d} \times 100$
			Volts	*Microamperes per millimole*		
1.06	..	..	−2.29	2.78	..	..
..	2.07	..	−2.29	2.51	..	..
0.97	0.97	1	−2.29	2.65	2.64	+0.4
0.73	2.92	0.25	−2.29	2.48	2.56	−2.7
4.40	1.10	4	−2.29	2.79	2.73	+2.2
1.75	..	..	−2.25	2.80	..	..
..	3.14	..	−2.25	2.50	..	..
1.45	2.90	0.5	−2.25	2.56	2.60	−1.6
4.64	2.32	2	−2.25	2.73	2.70	+1.1
2.80	2.80	1	−2.25	2.60	2.65	−1.9
3.14	..	..	−2.19	2.87	..	..
..	2.20	..	−2.19	2.50	..	..
2.50	1.25	2	−2.19	2.80	2.77	+1.1
2.20	2.20	1	−2.19	2.63	2.70	−2.6

drop time should be greater than 3 seconds. The residual current of the supporting electrolyte must be determined and subtracted from the apparent diffusion current. Lithium is determined in a medium of 80 per cent ethanol, the other conditions being the same as mentioned above. Potassium and sodium in 80 per cent ethanol also yield well-defined diffusion waves.

The experimental error of all determinations is of the order of 2 to 3 per cent. Several "unknowns" have been determined with this accuracy.

Half-Wave Potentials of Alkali Metals in Various Media

The half-wave potentials were measured in various media at different concentrations of the alkali metals. It was found these potentials were independent of the ion concentration Details of these measurements will be given in a subsequent paper. The obtained average values, corrected for the *iR* drop in the cell, are summarized in Table V.

The differences between the half-wave potentials of the alkali ions in the various media were found to remain practically constant. It was also established that the half-wave potentials of the alkali ions are independent of the drop time of the mercury cathode.

Diffusion Currents in Mixtures of Sodium and Potassium

It may be readily seen that the half-wave potentials of sodium and potassium are practically identical. Therefore, sodium and potassium, when present together, yield only one wave. Since the diffusion coefficient of sodium is considerably less than that of potassium, the diffusion current of sodium is notably less than the corresponding value for potassium at the same concentration. At a given total alkali metal concentration the diffusion current of sodium and potassium should be equal to the sum of the diffusion currents of the individual constituents. Although it is claimed (*3*, *7*) that this rule does not hold in aqueous solutions, the authors found in 50 per cent ethanol a good agreement between calculated and experimentally determined values. The results tabulated in Table VI show that the diffusion currents found in mixtures of potassium and sodium chlorides are, within the experimental error, equal to the calculated sums of the diffusion currents of each of the constituents. The capillary used had an initial drop time of 3.75 seconds. The apparent diffusion current was measured at a cathode potential between −2.19 and −2.29 volts (*vs.* S. C. E.). The concentration of the supporting electrolyte was 50 to 100 times larger than the sum of the concentrations of both potassium and sodium ions.

Although the agreement between the calculated and experimentally determined values is within the experimental error, it is evident that the polarographic method cannot yield accurate results in the indirect determination of potassium and sodium in a mixture of both.

Polarographic Determination of Potassium and Sodium with Lithium Hydroxide in Ethanol

From Table V it can be postulated that lithium hydroxide should be a useful supporting electrolyte for the determination of sodium and potassium, provided that the ratio of the concentration of lithium hydroxide to that of sodium and potassium is not too great. In fact, the authors found that when the lithium hydroxide concentration is between 40 and 100 times greater than that of potassium or of sodium or of the sum of both, the diffusion current of the latter can be determined without difficulty in a medium of 50 per cent ethanol. When the concentration of lithium hydroxide becomes less than 40 times the concentration of potassium or sodium ions, the migration current is not eliminated. On the other hand, if the concentration of lithium hydroxide is much greater than 100 times that of potassium and sodium, the potassium and sodium waves go over into the lithium wave without showing a well-defined diffusion current region. Figure 4 demonstrates that a well-defined diffusion current was obtained in a solution of 0.003 *N* potassium chloride with 0.2 *N* lithium hydroxide in 50 per cent ethanol as supporting electrolyte.

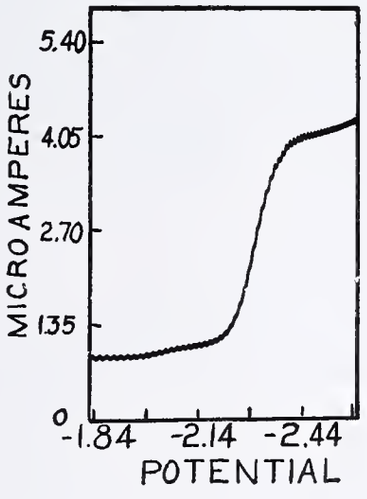

Figure 3. Polarogram of 0.0017 *N* Lithium Chloride in 80 Per Cent Ethanol with 0.25 *N* Tetraethylammonium Hydroxide as Supporting Electrolyte

Initial drop time, 4.6 seconds. Anode potential, −0.325 volt (*vs.* S. C. E.)

The results presented in Table VII show that the diffusion current of sodium in a solution of lithium hydroxide was proportional to the concentration, provided that the concentration of the supporting electrolyte was within the limits given above.

Simultaneous Determination of Potassium or Sodium and Lithium

From Table V it is seen that the half-wave potentials of potassium and sodium are about 0.24 volt more positive than

TABLE VII. PROPORTIONALITY BETWEEN DIFFUSION CURRENT AND CONCENTRATION OF SODIUM CHLORIDE IN 50 PER CENT ETHANOL WITH LITHIUM HYDROXIDE AS SUPPORTING ELECTROLYTE

[Initial drop time, 3.0 seconds. Apparent diffusion current i, microamperes, measured at $\pi_{d.\ e.} = -2.22$ volts (*vs.* S. C. E.)]

Concentration of NaCl, c_1, $N \times 10^3$	Concentration of LiOH, c_2, N	$\frac{c_1}{c_2}$	i	i_r	$i_d = i - i_r$	Concentration Ratio, R_c	Current Ratio, R_i	$\Delta\% = \frac{R_i - R_c}{R_c} \times 100$
0.83	0.06	72	2.91	0.74	2.17	1	1	..
1.43	0.12	82	5.09	1.47	3.62	1.72	1.67	−2.9
2.22	0.14	63	6.99	1.31	5.68	2.67	2.62	−1.9
4.15	0.21	51	11.63	1.87	9.76	5.00	4.96	−0.8

TABLE VIII. CHANGE OF RELATIVE VALUE OF $m^{2/3}t^{1/6}$ WITH CATHODE POTENTIAL

$\pi_{d.\ e.}$ (*vs.* S. C. E.)	Initial Drop Time, t_0, 15 sec. $m^{2/3}t^{1/6}$	Relative values of $m^{2/3}t^{1/6}$	Initial Drop Time, t_0, 3 sec. $m^{2/3}t^{1/6}$	Relative values of $m^{2/3}t^{1/6}$	Average Relative Values Given by Kolthoff and Orlemann (*5*) in Water
Volts	$Mg.^{2/3}\ sec.^{-1/2}$		$Mg.^{2/3}\ sec.^{-1/2}$		
−0.49	7.65	1.000	2.39	1.000	1.000
−0.94	7.52	0.983	2.34	0.980	0.985
−1.54	7.32	0.956	2.27	0.950	0.95
−1.84	7.13	0.932	..	..	0.92
−1.99	7.00	0.915	2.18	0.913	0.89
−2.14	6.84	0.894	2.13	0.891	..
−2.44	6.30	0.823	1.96	0.820	..
−2.74	5.21	0.681	..	..	..

that of lithium. Therefore it should be possible to determine sodium and potassium or the sum of both as well as lithium in a mixture of the alkali ions. As a matter of fact, when dealing with a mixture of potassium and sodium, or each of them separately, with lithium, two distinct diffusion waves are obtained. The first diffusion current corresponds to the sum of the concentrations of potassium and sodium and the second to that of lithium. In order to obtain a well-defined lithium wave it is desirable to work in a medium of 80 per cent alcohol. As a demonstration Figures 5 and 6 present some polarograms.

In Figure 5 the ratio of the concentration of lithium to that of potassium was equal to 1.85, while in Figure 6 the ratio of lithium to the sum of potassium and sodium was only 0.15. The small lithium wave is still measurable in the latter solution.

In the calculation of the concentration of lithium from the second diffusion wave use is made of the relation: $i_d = k_{Li^+} \times c_{Li^+}$, in which k_{Li^+} is the diffusion current corresponding to a concentration of one millimole per liter. In order to find the true diffusion current of lithium it is necessary to subtract from the total current measured at a cathode potential between −2.40 and −2.60 volts, the residual current due to the supporting electrolyte and the diffusion current of sodium and potassium at the same potential. Actually, the diffusion current of both sodium and potassium is measured at a potential between −2.20 and −2.40 volts. The corresponding value is less at the potential where the lithium current is determined, because of the decrease of the product $m^{2/3}\ t^{1/6}$, m being the mass of mercury in milligrams flowing out of the capillary per second and t the drop time in seconds (*5*). This correction is appreciable and cannot be neglected. The authors have determined the $m^{2/3}\ t^{1/6}$ values of mercury dropping into a solution of tetraethylammonium hydroxide in 80 per cent ethanol, using capillaries the initial drop times of which varied between 3 and 15 seconds. From the figures shown in Table VIII it is evident that the relative change of $m^{2/3}\ t^{1/6}$ was practically independent of the drop time.

The following example shows how the concentrations of potassium and lithium were calculated from the measured diffusion currents in a mixture which contained 0.00077 N potassium chloride, 0.0015 N lithium chloride, and 0.18 N tetraethylammonium hydroxide in 80 per cent ethanol.

First the residual current of the supporting electrolyte was determined as well as the constants k_{K^+} and k_{Li^+} with potassium and lithium solutions of known concentrations. The apparent diffusion current of the first (potassium) wave was measured at $\pi_{d.\ e.} = -2.25$ volts and found to be 2.59 microamperes. After subtraction of the residual current $i_r = 0.64$ microampere, the concentration of potassium was calculated by means of the relation

$$c_{K^+} = i_d(K^+)/k_{K^+}$$

$i_d(K^+)$ being equal to 2.59 − 0.64 = 1.95 microamperes.

The sum of the apparent diffusion currents of potassium and lithium was measured at a cathode potential of −2.49 volts and found to be equal to 5.39 microamperes. The residual current (i_r) at this potential was 0.96 microampere. The diffusion current of potassium at $\pi_{d.\ e.} = -2.49$ volts was calculated using the relation

$$i_d(-2.49\ \text{volts}) = i_d(-2.25\ \text{volts}) \times \frac{(m^{2/3}t^{1/6})_{-2.49\ \text{volts}}}{(m^{2/3}t^{1/6})_{-2.25\ \text{volts}}} = 1.95 \times 0.95 =$$

1.85 microamperes

Hence, the true diffusion current of lithium at $\pi_{d.\ e.} = -2.49$ volts was

$$i_d(Li^+) = i_{app.} - i_r - i_{K^+} =$$

5.39 − 0.96 − 1.85 = 2.58 microamperes

From the determined value of k_{Li^+} (= 1.75 microamperes per millimole per liter) the concentration of lithium was calculated. The authors found $c_{Li^+} = 2.58/1.75 = 1.47$ millimoles per liter. The accuracy is quite satisfactory, the true value of c_{Li^+} being 1.50 millimoles per liter.

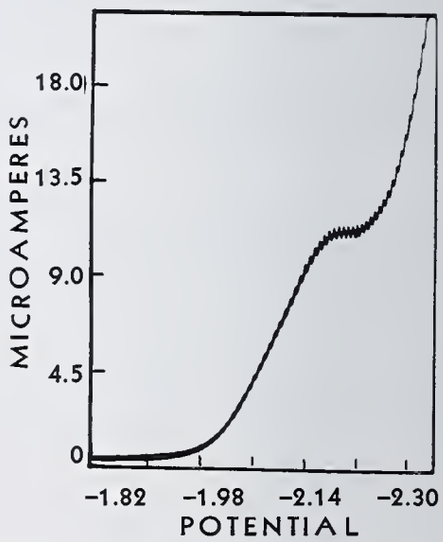

FIGURE 4. LITHIUM HYDROXIDE AS SUPPORTING ELECTROLYTE FOR POTASSIUM DETERMINATION

0.0036 N potassium chloride in 50 per cent ethanol with 0.2 N lithium hydroxide. Initial drop time, 4.3 seconds. Anode potential, 0.190 volt (*vs.* S. C. E.)

A few other results, summarized in Table IX, show that the polarographic analysis of mixtures of potassium or sodium or both with lithium yields satisfactory results.

The concentration of potassium or sodium or of the sum of both can be determined with the ordinary accuracy of about 2 to 3 per cent, even if the concentration of lithium is about 50 times greater than the sum of the other two alkali ions. On the other hand, in order to obtain a satisfactory result in the determination of lithium its concentration should not be more

TABLE IX. POLAROGRAPHIC ANALYSIS OF MIXTURES OF POTASSIUM, SODIUM, AND LITHIUM

Composition of Solution	$\frac{C_{Li^+}}{C_{K^+} + C_{Na^+}}$	$k_{K^+ + Na^+}$ Experimentally Determined at $\pi_{d.e.} = -2.17$ Volts		$\Delta\% = \frac{a-b}{b} 100$	k_{Li^+} Experimentally Determined at $\pi_{d.e.} = -2.49$ Volts		$\Delta\% = \frac{c-d}{d} 100$
		In solution with lithium, a	In solution without lithium, b		In solution with sodium and potassium, c	In solution without sodium and potassium, d	
0.001 N NaCl + 0.004 N LiCl	4.0	2.46	2.41	+2.0	1.76	1.76	±0.0
0.00077 N KCl + 0.0015 N LiCl	1.95	2.70	2.61	+3.5	1.79	1.76	+1.7
0.00167 N NaCl + 0.00334 N KCl + 0.00083 N LiCl	0.16	2.67	2.59	+3.1	1.74	1.76	−1.2

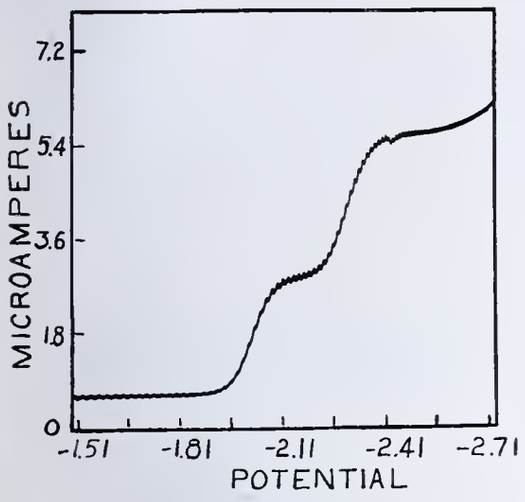

FIGURE 5. SIMULTANEOUS DETERMINATION OF POTASSIUM AND LITHIUM

0.00082 N potassium chloride and 0.0015 N lithium chloride in 80 per cent ethanol with 0.28 N tetraethylammonium hydroxide as supporting electrolyte. Initial drop time, 4.5 seconds. Anode potential, −0.330 volt (*vs.* S. C. E.)

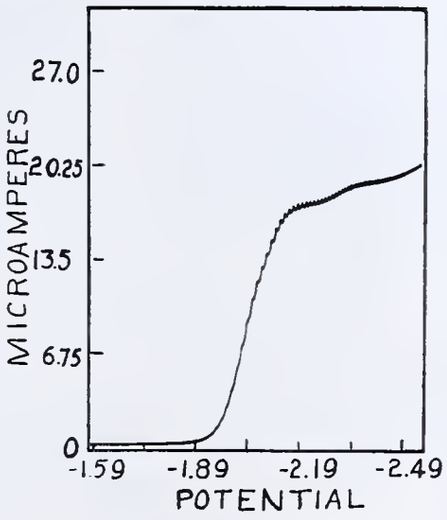

FIGURE 6. SIMULTANEOUS DETERMINATION OF SUM OF POTASSIUM AND SODIUM AND OF LITHIUM

0.0024 N sodium chloride, 0.0041 N potassium chloride, and 0.0009 N lithium chloride in 80 per cent ethanol with 0.3 N tetraethyl ammonium hydroxide as supporting electrolyte. Initial drop time, 4.5 seconds. Anode potential, −0.340 volt (*vs.* S. C. E.)

than 5 to 10 times smaller than the total concentration of sodium and potassium in the mixture.

A large number of "unknowns", containing sodium, potassium, or lithium as well as mixtures of two or all three cations, have been analyzed using the polarograph or the manual apparatus. In general the results obtained agreed within 4 per cent or better with the actual composition of the solution when the initial drop time of the capillary was greater than 3 seconds.

Some capillaries showed an abnormal behavior at potentials at which the diffusion current of lithium was measured. In such cases the drop time became erratic and the value of the current fluctuated in an irregular way with the applied e.m.f. The authors have not been able to establish the cause of this phenomenon. However, no difficulty has been experienced in preparing capillaries which did not exhibit the abnormal behaviour.

Summary

Tetraethylammonium hydroxide is more suitable as a supporting electrolyte in the determination of alkali metals than the tetramethyl compound. A solution of tetraethylammonium hydroxide must be allowed to stand for 5 to 10 days and then be filtered before use as a supporting electrolyte. The filtrate is stable for a relatively long period of time.

Potassium and sodium can be determined polarographically with an accuracy within 3 per cent in a medium of 50 per cent ethanol. The concentration of tetraethylammonium hydroxide must be at least 50 times greater than that of the alkali ions. Lithium is best determined in a medium of 80 per cent ethanol, in which both sodium and potassium yield well-defined diffusion waves.

In 50 per cent ethanol the diffusion current measured in mixtures of potassium and sodium within the experimental error is equal to the sum of the diffusion currents of the individual components.

Lithium hydroxide in a medium of 50 per cent ethanol can be used as a supporting electrolyte for the determination of potassium and sodium or of the sum of both if the concentration of lithium hydroxide is between 40 and 100 times greater than that of the other alkali ions.

In a mixture of potassium, sodium, and lithium with tetraethyl-ammonium hydroxide as supporting electrolyte in a medium of 80 per cent ethanol two diffusion waves are obtained. The first corresponds to the sum of sodium and potassium, and the second to the concentration of lithium. Therefore the sum of the concentrations of potassium and sodium and the concentration of lithium in a mixture can be found from one single current-voltage curve.

Acknowledgment

The authors acknowledge a grant from the Carnegie Corporation of New York City which enabled them to start the present investigation.

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Determination of Oil in Metal Turnings

Improved Method and Apparatus

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BY THE old method for determining oil in metal turnings, a known weight of sample is subjected to repeated washing by decantation at room temperature in a beaker of appropriate size with a chlorinated solvent such as carbon tetrachloride or a low-boiling nonchlorinated solvent such as petroleum naphtha or petroleum ether. This method has certain disadvantages.

1. The operator's complete time is required and he may be subjected to poisonous fumes from the solvent used. Solvents such as petroleum naphtha with a low boiling range constitute a fire hazard.
2. There is considerable loss of solvent by evaporation.
3. Efficiency of extraction is so poor that even after three to four washings with carbon tetrachloride the author has found evidence of residual oil upon drying the turnings after the last washing. Since the higher boiling nonchlorinated solvents have a much lower extraction efficiency than carbon tetrachloride the number of extractions and time required are correspondingly increased.
4. Carbon tetrachloride and other suitable chlorinated solvents are very difficult to obtain because of war conditions, and are relatively expensive.

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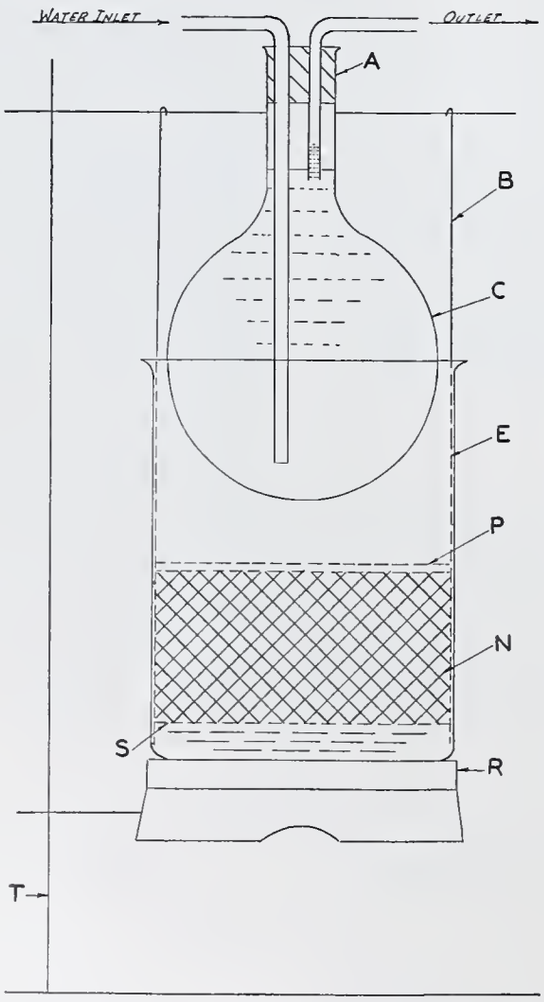

FIGURE 1. APPARATUS DURING FIRST OPERATION

A. Rubber stopper, fastened to flask with copper wire to hold water pressure
B. Copper wire, to fix relative positions of basket and flask
C. Round-bottomed flask as condenser
E. Screen basket
N. Turnings
P. Solvent level
R. Electric hot plate
S. Screen
T. Ring stand

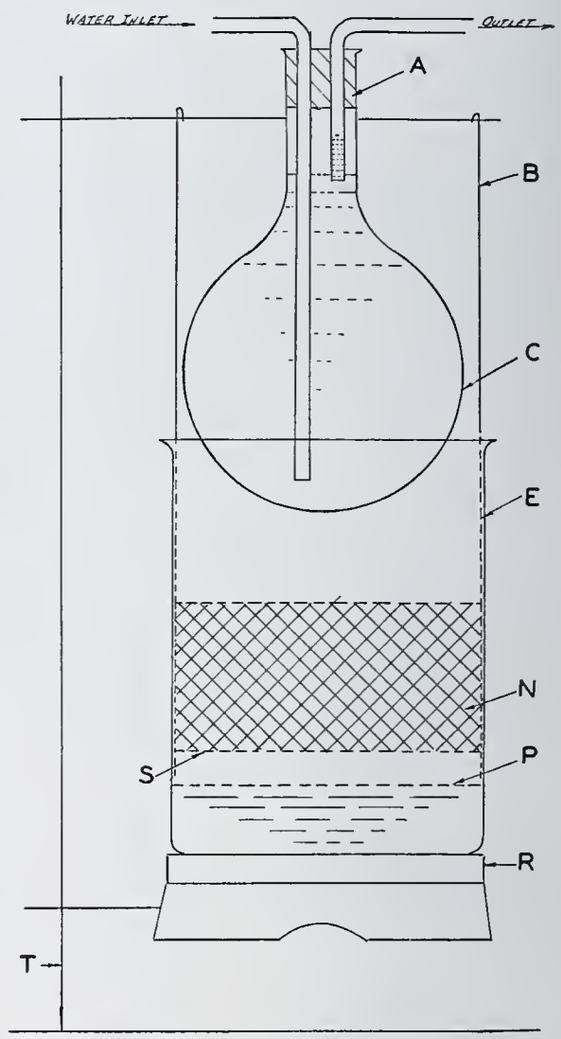

FIGURE 2. APPARATUS AFTER LOWERING OF HOT PLATE AND BEAKER

The following method was developed to overcome these difficulties. There is nothing particularly new about it, but it is simple and requires no expensive apparatus. In principle it is a combination of immersion and vapor degreasing.

APPARATUS. A 2000-ml. Pyrex beaker, a wire basket made from ordinary copper house screening, an electric hot plate, a filter or round-bottomed flask for use as condenser, diameter 1.25 to 2.5 cm. (0.5 to 1 inch) less than the beaker used, an iron ring stand with clamps and fasteners, and some copper wire.

The size of the apparatus can be increased to take care of larger samples, which are often necessary when a lot of material is particularly nonuniform or heterogeneous.

Figure 1 shows the first operation.

PROCEDURE 1. A sample which will occupy not more than about 5 to 7.5 cm. (2 to 2.5 inches) in height in the wire basket is covered with the chosen solvent. The turnings are dried pre-

viously at 110° C. to determine moisture and volatiles at that temperature. The weight need only be sufficient to give adequate accuracy with the balance used for the amount of oil present. The solvent used is petroleum naphtha (boiling point range approximately 110 to 167° C.), which costs about 25 cents per gallon and may be secured without difficulty. The wire basket is cylindrical with a flat bottom and is made to fit snugly to the sides of the beaker, so that the vapors in the second operation must pass through the turnings. If the holes in ordinary screening are too large for the material being extracted, a finer screen or perhaps a specially designed cloth bag is recommended.

2. With the cooling water flowing, the temperature is raised to the boiling point of the solvent and held there for about 15 minutes. This causes intimate contact of each piece of turnings with the solvent, which results in rapid heating of the turnings and maximum solution of the oil in the solvent. The bulk of oil in very oily turnings is thus removed.

3. As shown in Figure 2, the hot plate and beaker are then lowered, so that the level of the boiling solvent is 1.25 to 2.5 cm. (0.5 to 1 inch) below the bottom of the basket. The vapors from the solvent proceed to condense on the turnings and all the oil-bearing solvent finally finds its way into the beaker. The rate of boiling and distance from the bottom of the screen to the top of the solvent should be adjusted with the following points in mind:

The boiling rate should be such that the vapors condense at a slow but noticeable rate on the bottom of the condenser and drip back through the turnings, so that all the turnings are brought in contact with solvent vapors.

The solvent level should be far enough from the turnings so that violently boiling liquid does not come in contact with them. This minimizes the possibility of a residue of oil-bearing solvent on the turnings. A further precaution concerns the possibility of bumping if some fine turnings pass through the screen in step 2. It may be necessary to remove the beaker from direct contact with the hot plate and insert an intervening layer of asbestos if bumping occurs.

In the case of the petroleum naphtha used, the lower boiling fractions do the vapor-degreasing and they are subsequently easily removed from the clean turnings by heating on a hot plate under a hood. For example, from a batch of turnings containing 10 per cent of oil a sample of about 150 grams was taken. The extraction was performed as described above and the clean and dry turnings were weighed. The extraction was repeated in the same way using carbon tetrachloride, and the turnings were again dried and weighed. A difference of only 0.2 gram was observed between the weights obtained in the two extractions. This error is probably due to the crude balance used. For a determination of this sort this can be considered excellent. In other words, the petroleum naphtha was satisfactory.

The errors in sampling far overshadow any errors in this determination. Modifications in the details of the procedure can easily be made according to the product being extracted and the solvent being used.

Many mechanical improvements in the setup could be made, but it is questionable whether the expenditure of time and money is warranted.

Obviously this method can be used to clean oil-covered solids on a laboratory scale.

Determination of Traces of Copper in Wort, Beer, and Yeast

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INCREASED interest has been shown recently in the determination of copper in beer (*2*) and collaborative analytical work has been undertaken by the Association of Official Agricultural Chemists (*1*). The methods studied in this collaborative work, which were considered the most practical and best of those available, were a direct xanthate method involving dry-ashing of the sample and a diethyldithiocarbamate method, including a wet-digestion as well as a hydrogen sulfide and dithizone separation that could be omitted at the discretion of the collaborative analyst.

This increased activity led the author to prepare for publication the copper methods which this laboratory has been using in studies of wort, beer, and yeast. The results of these studies will be published elsewhere.

The details of the methods reported herein were worked out after considerable preliminary experimentation to obtain optimum conditions for the various operations. Originally, a method was developed for determining the copper directly on the sample without preliminary ashing or wet-digestion. While good recovery of added copper was obtained, the results were not completely reliable for the copper originally present in many of the samples. The presence of stable copper complexes and the difficulty of counteracting interferences in the direct method indicated the need for destruction of organic matter present in the samples.

In the present method a dry-ashing is employed rather than a wet-digestion because of its simplicity and the fact that high blanks are obtained due to traces of copper in the large volumes of concentrated acids usually employed in the wet-digestion methods. The copper is determined photometrically as the diethyldithio complex after extraction with amyl acetate (*3*). Iron is the only common metal which is normally present in the wort, beer, or yeast in amounts sufficient to interfere by forming a colored complex with the reagent. This interference is eliminated by the use of 2,2′-bipyridine (α, α-dipyridyl) as suggested by Parker and Griffin (*4*). The 2,2′-bipyridine combines with the iron and prevents it from reacting with the copper reagent. This expedient greatly simplifies the procedure by avoiding the usual lengthy acid–hydrogen sulfide separation. The possible interfering effect of calcium ions is prevented by avoiding alkaline reactions with consequent precipitation of calcium phosphate and by using a large excess of the copper reagent. A further advantage of this method is that most of the reactions are conducted in a single centrifuge tube and in the case of worts and beers no filtrations are involved.

The spectrophotometric data relating to the copper-diethyldithiocarbamate color have been worked out (*3*) and need not be repeated here. Calibration curves (Figure 1) have been made at two separated points on the spectrum (440 and 540 millimicrons), thus extending the range of copper concentrations covered without loss of accuracy. This permits the ready selection of the most suitable conditions (cell length, wave length of light, and optimum reading of instrument) for the amount of copper present in the particular sample used. The instrument employed throughout this work was the Aminco neutral wedge photometer, but the principles involved are readily adaptable to other visual or photoelectric photometers.

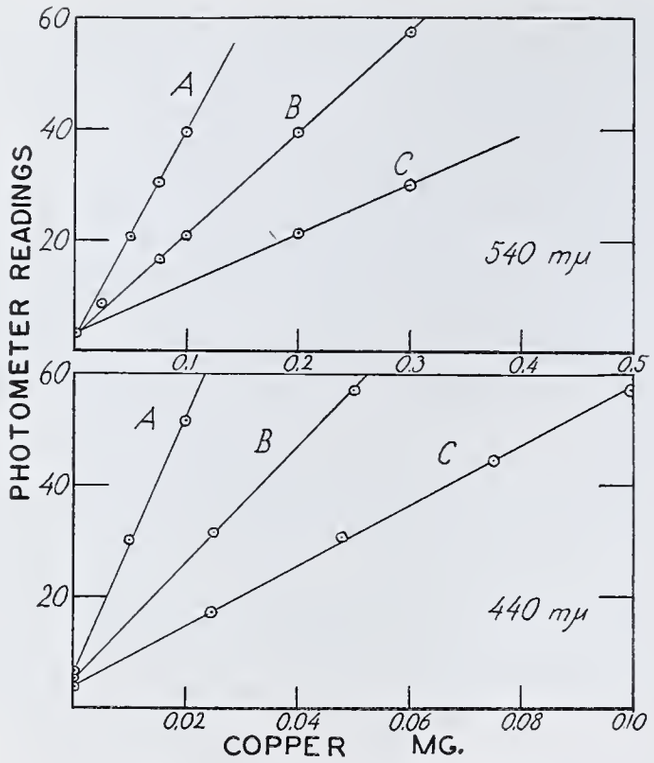

FIGURE 1. CALIBRATION CURVES

A. 2-inch cell
B. 1-inch cell
C. 0.5-inch cell

Determination of Copper in Wort and Beer

REAGENTS. Concentrated C. P. nitric and hydrochloric acids.

Dilute Sulfuric Acid (1 + 6). To 6 parts by volume of water add with stirring 1 part by volume of C. P. concentrated sulfuric acid.

2,2′-Bipyridine Solution. Add 1 ml. of glacial acetic acid to 0.2 gram of 2,2′-bipyridine (α,α-dipyridyl) contained in a beaker, dilute with water, and dissolve. Make up volume to 100 ml.

Saturated *p*-Hydroxyphenylglycine Solution. Prepare fresh before use by stirring thoroughly 0.5 gram of *p*-hydroxyphenylglycine in 100 ml. of 0.1 *N* sulfuric acid. Allow to settle and use the clear supernatant solution.

Sodium Acetate Solution. Dissolve 14 grams of sodium acetate trihydrate in water and dilute to 100 ml.

Isoamyl Acetate. The grade with a boiling range 125° to 140° C. is satisfactory.

Sodium Diethyldithiocarbamate.

The 2,2′-bipyridine, *p*-hydroxyphenylglycine, isoamyl acetate, and sodium diethyldithiocarbamate are obtainable from the Eastman Kodak Co., Rochester, N. Y.

Standard Copper Solution. Weigh out 3.93 grams of clean C. P. copper sulfate crystals ($CuSO_4.5H_2O$), dissolve in water, and dilute to 1 liter (1 ml. = 1 mg. of copper). For the calibration curve prepare a more dilute standard by pipetting 5.0 ml. into a 500-ml. volumetric flask and diluting to the mark with water (1 ml. = 0.01 mg. of copper). The use of copper sulfate crystals as a standard is sufficiently accurate for this purpose and avoids the usual lengthy method involving the dissolution of metallic copper.

METHOD. Measure 100 ml. of beer into a clean silica dish and add 5 ml. of the dilute sulfuric acid. Evaporate, char, and ignite at about 500° to 550° C. in a muffle to obtain a fluffy, white, carbon-free ash, avoiding fusion of the ash. Cool, add 2 ml. of concentrated hydrochloric acid and 1 ml. of concentrated nitric acid, and evaporate to dryness on a steam bath. If any carbon is noted in the ash at this point, put it back in the muffle and reignite to give a completely carbon-free ash. Treat the reignited ash again with the nitric-hydrochloric acid and re-evaporate until the residue is thoroughly dry. Wet the residue with 1 ml. of the dilute sulfuric acid and transfer with hot water to a 50-ml. centrifuge tube (Pyrex No. 2220), keeping the total volume below 20 ml. Add 2 ml. of the bipyridine solution, 1 ml. of the *p*-hydroxyphenylglycine solution, and 6 ml. of the sodium acetate solution. Make the volume up to 30 ml., mix, and warm to about 50° C. for 15 minutes. Cool and pipet into the tube exactly 15 ml. of amyl acetate. Add 0.2 gram of sodium diethyldithiocarbamate, immediately cork the tube, and shake vigorously 60 times. Cool the tube in ice water and reshake twice again, giving 60 shakes each time and allowing the amyl acetate to separate between shakeouts. Centrifuge the cold tube and pour the separated colored amyl acetate into a suitable tube or cell and read in the photometer. If the amyl acetate is not perfectly transparent, the haze, which is due to a dispersion of water in the solvent, can be cleared by warming the cell slightly.

Suitable blank determinations on the reagents should be conducted and correction of the results applied. With good grades of reagents, the blanks will generally run on worts and beers about 0.002 mg. of copper and on yeast about 0.004 mg. of copper.

PREPARATION OF CALIBRATION CURVES. Pipet into the centrifuge tubes quantities of the diluted copper standard sufficient to cover the range desired. This range will depend on the cell depth and wave length of light employed. The calibration plot for the neutral wedge photometer (Figure 1) will indicate the range of concentrations possible on this instrument by this method.

Add 1 ml. of sulfuric acid, dilute to about 20 ml. with water, and then proceed as above, adding the various reagents and shaking out with amyl acetate. Read the clear colored amyl acetate in the photometer, plot the results, and obtain the calibration curves. Beer's law appeared to be followed over the range of concentration employed on the author's photometer.

The quantity of 2,2′-bipyridine used is sufficient to take care of about 0.2 mg. of iron in the sample. If the iron content of the sample is higher, the amount of 2,2′-bipyridine should be increased. The stronger bipyridine reagent listed under the determination of copper in yeast may be substituted.

RECOVERY OF ADDED COPPER. A suitable precise copper method of known accuracy against which this method could be checked is lacking. As a recourse determinations were made to check additions of copper. Table I shows the excellent recovery obtained in the analysis of beers and worts containing added copper. The original iron content of the beers listed was about 0.2 p. p. m, and the wort contained about 0.5 p. p. m. of iron.

The copper in yeast is determined as in wort and beer with a few changes in the ashing technique, necessitated by the presence of quantities of readily fusible salts in the yeast samples.

TABLE I. RECOVERY OF COPPER ADDED TO BEER

Sample	Copper Added	Iron Added	Copper Determined		Recovery
	P. p. m.	*P. p. m.*	*P. p. m.*	*Av. P. p. m.*	%
Beer A	None	None	0.13, 0.15	0.14	...
Beer A	0.25	None	0.38, 0.38	0.38	96
Beer A	0.25	2.0	0.38	0.38	96
Beer B	None	None	0.12, 0.13	0.13	...
Beer B	0.50	None	0.61, 0.61	0.61	96
Beer B	0.50	2.0	0.61, 0.64	0.63	100
Wort C	None	None	0.19, 0.20	0.20	...
Wort C	0.60	None	0.83, 0.77	0.80	100

Determination of Copper in Yeast

REAGENTS. Ammonium Nitrate Solution. Dissolve 50 grams of C. P. ammonium nitrate in water and dilute to 100 ml.

2,2′-Bipyridine Solution. Dissolve 1.0 gram of 2,2′-bipyridine in 2 ml. of glacial acetic acid and dilute to 100 ml. with water.

Other reagents as in the wort and beer method.

PREPARATION OF SAMPLE FOR ANALYSIS. Liquid yeast as obtained from the fermenter of a brewery consists of a viscous suspension of the yeast cells in a more or less fermented beer. If it is desired to determine the copper on the beer-free yeast, the yeast may be washed with ice water on a Büchner suction filter until free of beer and then drained and pressed dry. Since both liquid and pressed yeast contain high proportions of moisture, a determination of yeast solids should be included, so that results may be expressed on a dry basis. Liquid or pressed yeasts are perishable and undergo autolysis and decomposition on storage. Therefore, if some time is to elapse between collection and analysis, the sample should be dried by washing on a Büchner funnel consecutively with cold water, alcohol, and finally with

Table II. Recovery of Copper Added to Yeast

(All results in p. p. m. on dry basis)

Copper Added	Total Copper Found	Copper Recovered	% Recovery
None	31.7[a]	..	..
44.4	73.9	42.2	95
46.8	74.8	43.1	92
89.7	116.8	85.1	95
96.5	127.5	95.8	99

[a] Average of 2 results (30.4 and 33.0 p. p. m.).

ether. After removal of the ether the sample should be a dry powdery solid which may be ground in a mortar to give a uniform stable sample.

Method. Weigh about 0.5 gram of dried yeast or 2 to 3 grams of the pressed yeast into a flat silica dish, add 5 ml. of 50 per cent ammonium nitrate solution, and evaporate to dryness. Char and ignite in muffle at a temperature not exceeding 500° C. Do not permit ash to fuse. When partly ashed, remove from muffle and allow to cool, add 5 ml. of water, and heat on a water bath to dissolve out soluble salts. Filter through a small No. 40 Whatman paper and wash with small portions of hot water. Reserve the filtrate. Ignite the filter containing the water-insoluble material in the original silica dish. The temperature of this ignition may be higher than the first ignition, as all the easily fusible salts have been leached out. Cool the dish after a white ash is obtained and add the above filtrate. Evaporate to dryness and treat with 1 ml. of concentrated nitric acid and 2 ml. of concentrated hydrochloric acid in the usual manner. Evaporate to dryness and proceed from this point as in the previous copper method, using 2 ml. of the stronger bipyridine solution in place of the weaker solution.

Recovery of Added Copper. Table II summarizes and indicates the typical recoveries of copper added to a yeast sample. The iron content of this sample was 165 p. p. m. on the dry basis.

Summary

Methods for the determination of small quantities of copper in wort, beer, and yeast involve dry-ashing the sample, reacting a solution of the ash with 2,2′-bipyridine to prevent interference of iron, and determining the copper photometrically with diethyldithiocarbamate after extraction with amyl acetate.

Acknowledgment

The writer wishes to acknowledge the careful and precise work of Roy W. Seaholm who obtained many of the experimental data.

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Decomposition Temperatures of Some Analytical Precipitates

Barium Carbonate

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THE decomposition temperature of barium carbonate is of interest because it is a weighing form for barium and because of its similarity to calcium carbonate. Moreover, there are very few reliable data of value to analytical chemists, since most of the work has been done to determine when the decomposition of barium carbonate is complete and not when it starts.

The earliest reported work on the decomposition of barium carbonate is by Abich (*1*), who stated in 1831 that it is completely decomposed at a white heat. In 1878, Isambert (*9*) used the gas saturation method to determine the pressure of carbon dioxide in equilibrium with barium carbonate and oxide at 1083° C. By passing nitrogen over the sample at 12 ml. per minute he found the equilibrium pressure was 22 mm. In 1898 Herzfeld and Stiepel (*7*) reported that complete decomposition occurred at about 1450° C., where the compound melted, but Brill (*2*) found that it melted without decomposition which occurred above 1450° C. Pott (*14*), using the static method, determined the dissociation pressures and found the dissociation complete at 1200° C. However, his dissociation pressures for barium carbonate as well as those for calcium and strontium carbonates are higher than the usually accepted values and his work has been criticized by Johnston (*10*).

Finkelstein (*4*), using the gas saturation method, made a very complete determination of the equilibrium pressures of carbon dioxide with barium carbonate and oxide. He used a gas rate of about 33 ml. per minute. In the light of subsequent work, this rate as well as that used by Isambert is much too fast to obtain equilibrium values. Finkelstein also reported that a basic carbonate of the composition $BaO.BaCO_3$ was formed, but Hackspill and Wolf (*5*) have recently proved by x-ray studies that no basic carbonate is formed but that the fusion of a eutectic mixture of barium oxide and barium carbonate takes place between 1070° and 1150° C. Hedvall (*6*) reported the decomposition temperature to be 1361° C., and Hackspill and Wolf (*5*) using a similar method reported that the decomposition begins about 300° C. above the first decomposition of calcium carbonate. They also found that barium carbonate undergoes an allotropic transformation from rhombic to hexagonal at 910° C. Dutoit (*3*), using the gas saturation method with a slow gas rate, determined the dissociation pressures between 1102° and 1236° C. Nakayama (*11*) reported that barium oxalate should be heated

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Table I. Dissociation Pressure of Barium Carbonate

Investigator	Isambert	Pott	Finkelstein	Dutoit
Date	1878	1905	1906	1927
Method	Gas saturation	Static	Gas saturation	Gas saturation
Rate of gas flow, ml. per min.	12	...	33	0.7–0.8
t, ° C.	*p*, Mm. Hg	*p*	*p*	*p*
915	..	...	0.4	...
945	..	...	0.8	...
997	..	0	...	...
1000	..	...	2.7	...
1017	..	5	...	...
1057	..	45	...	...
1083	22	...	...	...
1097	..	120	...	...
1102	..	...	18	26
1114	..	...	21.7	29
1132	..	...	29	27
1137	..	240	...	...
1140	..	...	33.6	31
1157	..	340	...	...
1197	..	675	...	...
1256	..	...	206	199
1300	..	...	381	...

Table II. Dissociation Pressures

No.	Volume *Ml.*	Temp. ° *C.*	Barometric Pressure *Mm. Hg.*	Volume at S. T. P. *Ml.*	Rate *Ml./min.*	Weight of CO_2 *Mg.*	P *Mm.*	log *P*	Temp. ° *C.*
1	1011	22.5	744.8	891	2.2	0.250	0.11	−0.972	846
2	1518	22	743.8	1339	2.0	0.339	0.10	−1.018	846
3	1011	24	741.0	879	2.2	0.247	0.11	−0.972	846
		Av.			2.1		0.11	−0.972	
4	2031	22	742.1	1787	12.5	0.311	0.07	−1.182	846
5	2031	22	743.8	1791	12.3	0.281	0.06	−1.226	846
		Av.			12.4		0.065	−1.187	
		From Figure 4			0.0		0.198	−0.704	
6	1518	25	743.0	1316	2.0	0.776	0.22	−0.651	871
7	1011	25	742.8	871	2.2	0.408	0.18	−0.751	871
8	1518	23.5	741.9	1325	2.0	0.433	0.12	−0.908	871
		Av.			2.1		0.17	−0.762	
9	2031	26	745.1	1757	12.3	0.561	0.12	−0.916	871
10	2031	25.5	740.9	1751	12.3	0.522	0.11	−0.949	871
11	2031	25.5	737.7	1747	12.3	0.498	0.11	−0.970	871
		Av.			12.3		0.11	−0.947	
		From Figure 4			0.0		0.295	−0.530	
12	1518	23.5	733.7	1310	2.0	1.023	0.29	−0.535	893
13	1518	25	731.7	1295	2.0	0.998	0.29	−0.542	893
14	1518	25	734.0	1299	2.0	0.752	0.22	−0.665	893
15	1518	24	732.8	1304	2.1	0.929	0.27	−0.575	893
		Av.			2.0		0.27	−0.579	
16	2031	25	739.2	1751	12.3	0.772	0.17	−0.780	893
17	2031	24	731.7	1743	12.3	0.895	0.19	−0.718	893
18	2031	26	736.0	1745	12.3	1.000	0.21	−0.668	893
		Av.			12.3		0.19	−0.722	
19	2031	24.5	740.3	1759	21.0	0.757	0.16	−0.790	893
20	2031	25	740.5	1755	22.0	0.763	0.16	−0.785	893
		Av.			21.5		0.16	−0.788	
		From Figure 4			0.0		−0.393	−0.406	
21	2031	25.5	731.7	1728	12.4	1.252	0.27	−0.569	911
22	2031	25.5	740.1	1749	13.1	1.225	0.26	−0.578	911
23	2031	25.5	739.6	1749	13.1	1.200	0.26	−0.588	911
		Av.			12.9		0.26	−0.580	
24	2031	25	737.0	1747	1.9	4.1	0.88	−0.055	953
25	1011	25	745.3	880	2.2	2.0	0.86	−0.063	953
26	1011	25	748.6	884	2.9	1.6	0.69	−0.161	953
		Av.			2.3		0.81	−0.093	
27	2031	25.5	739.5	1749	5.9	2.9	0.63	−0.205	953
28	2031	26	740.1	1745	5.7	3.3	0.71	−0.147	953
29	2031	26	742.2	1749	6.4	3.1	0.67	−0.174	953
		Av.			6.0		0.67	−0.175	
30	2031	26.5	747.8	1759	12.5	3.0	0.65	−0.187	953
31	2031	25	751.2	1781	13.4	3.1	0.67	−0.176	953
		Av.			13.0		0.66	−0.181	
32	2031	25	748.2	1773	27	2.8	0.60	−0.220	953
33	2031	25	746.3	1769	27	2.4	0.52	−0.288	953
		Av.			27		0.56	−0.254	
		From Figure 4			0.0		1.07	.030	
34	1518	24	729.9	1299	2.0	5.3	1.52	+0.181	999
35	1518	25	731.7	1302	2.0	6.0	1.72	0.235	999
36	1518	24	747.2	1331	2.0	5.4	1.54	0.189	999
		Av.			2.0		1.59	0.201	
37	2031	26	732.7	1726	6.6	7.0	1.51	+0.180	999
38	2031	25	733.7	1739	6.5	7.7	1.66	0.219	999
39	2031	24.5	744.4	1769	6.5	6.7	1.44	0.157	999
		Av.			6.5		1.53	0.185	
40	2031	25	740.0	1751	13.2	6.7	1.44	+0.159	999
41	2031	25.5	738.3	1735	13.2	6.8	1.47	0.169	999
42	2031	26	736.9	1737	13.2	6.4	1.38	0.140	999
		Av.			13.2		1.43	0.156	
43	2031	25	731.3	1743	28	6.3	1.34	+0.129	999
44	2031	25	731.9	1732	27	6.3	1.36	0.132	999
		Av.			27.5		1.35	0.130	
		From Figure 4			0.0		1.96	0.292	
45	1518	26	738.8	1301	2.1	9.6	2.77	+0.442	1028
46	2031	25.5	730.8	1724	6.8	12.2	2.62	+0.419	1028
47	2031	25.5	739.1	1747	29	9.7	2.09	+0.319	1028
48	2031	26	738.9	1741	29	11.1	2.39	0.378	1028
		Av.			29		2.24	0.350	
		From Figure 4			0.0		3.27	0.514	
49	1518	25	731.6	1295	2.1	10.0	2.87	+0.458	1038
50	1518	26	729.9	1285	2.1	12.4	3.57	0.553	1038
51	1518	27.5	736.8	1287	2.1	11.2	3.25	0.512	1038
52	1518	25.5	736.9	1302	2.1	10.5	3.02	0.480	1038
		Av.			2.1		3.17	0.500	
53	2031	25	734.6	1741	6.8	13.2	2.83	+0.451	1038
54	2031	25	735.5	1743	6.9	13.0	2.78	0.444	1038
55	2031	26	737.6	1733	6.9	13.0	2.81	0.449	1038
56	2031	26	738.2	1741	6.9	13.5	2.90	0.463	1038
		Av.			6.9		2.83	0.451	
57	2031	25	737.5	1747	14.0	13.1	2.81	+0.448	1038
58	2031	25	736.4	1745	14.0	12.6	2.70	0.431	1038
		Av.			14.0		2.76	0.439	
59	2031	24	738.7	1759	26.4	12.3	2.62	+0.418	1038
60	2031	24	738.4	1759	29.0	12.7	2.70	0.432	1038
		Av.			27.7		2.66	0.425	
		From Figure 4			0.0		3.57	0.552	

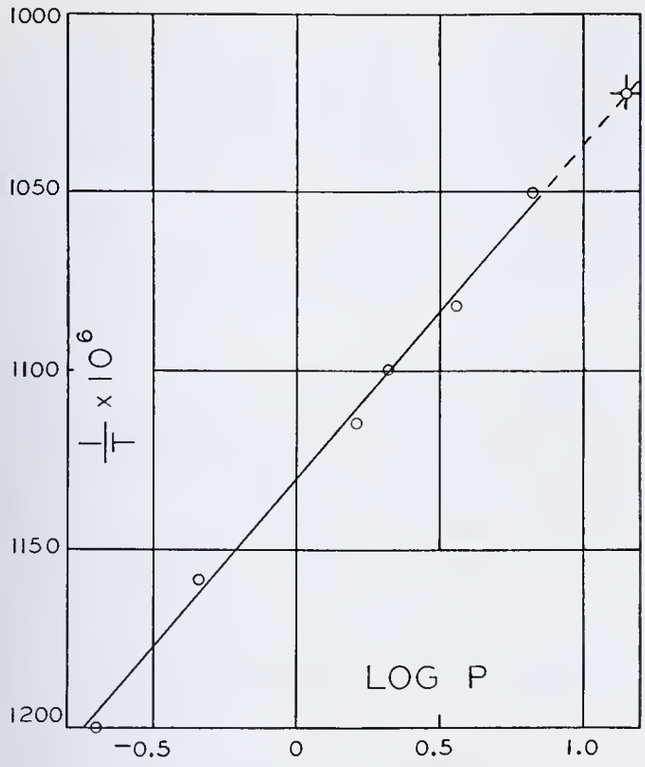

FIGURE 1. EFFECT OF TEMPERATURE ON CALCIUM CARBONATE

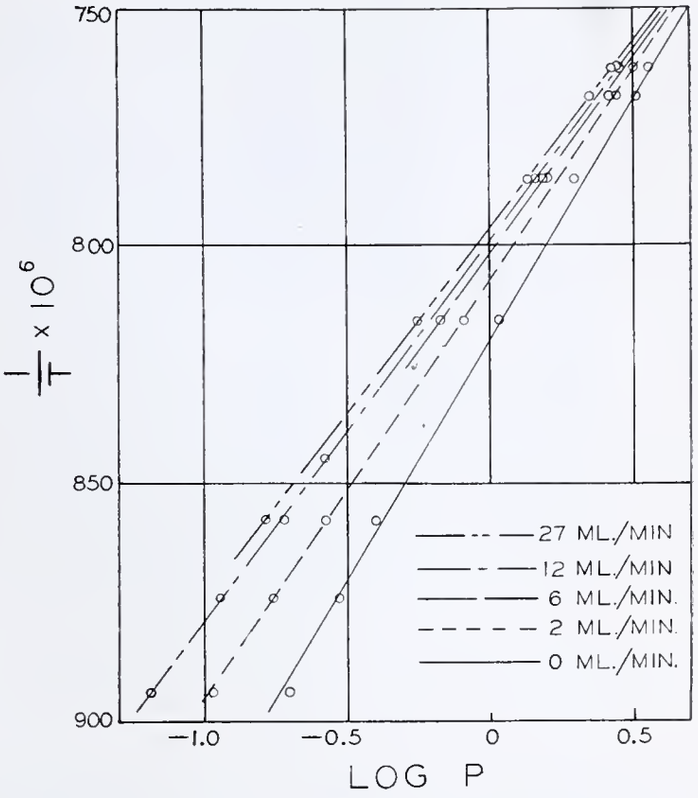

FIGURE 2. EFFECT OF TEMPERATURE

above 520° C. to change it to the carbonate and Ziemens (*15*) found that the dissociation of barium carbonate is complete at 1360° C.

Table I gives the previously determined dissociation pressures of barium carbonate.

The apparatus and method used in this work were those previously described (*13*, Figure 1) except for the following minor modifications: The micro combustion tube, *E*, was Vycor glass; for temperatures below 953° C. a micro absorption tube was used in the absorption train, *G*, and one was also used in the by-pass, *H*, to determine the carbon dioxide liberated during the flushing period; the cold junction of the thermocouple was kept at 0° C.

Before starting the work on barium carbonate, the apparatus was checked by several determinations with calcium carbonate. The purified calcium carbonate was ignited at 500° C. and spectrographic analysis showed the absence of strontium and the presence of only very small traces of other elements. Using 0.5 gram of calcium carbonate and a gas rate of 13 ml. per minute at 705° C., a value was obtained which when plotted with the previously obtained results (*13*) shows fairly good agreement (Figure 1).

Kahlbaum's "purest crystal" barium nitrate was recrystallized three times from distilled water. After the third recrystallization the crystals were filtered on a sintered-glass funnel and dried overnight at 110° C. One hundred and twenty grams of the recrystallized barium nitrate were dissolved in 1.5 liters of distilled water. This and a solution of ammonium carbonate (90 grams in 800 ml. of water) were added, slowly and simultaneously, to 1.5 liters of hot water. The solution in the reaction vessel was stirred constantly during the addition of the reacting solutions and the barium nitrate was always kept in slight excess. After the solutions were mixed, a slight excess of carbonate was added. The barium carbonate was digested overnight on a steam bath, filtered on a sintered-glass funnel, washed free from ammonia, and ignited at 600° C. for 12 hours. Spectroscopic analysis showed that the barium carbonate contained no significant amount of impurity and quantitative analysis by transformation to barium sulfate gave results of 99.94 and 99.95 per cent purity.

The results for the determination of the dissociation pressures at various temperatures and gas rates are tabulated in Table II and are shown in Figure 2 by plotting the logarithm of the pressure against the reciprocal of the absolute temperature. From this it can be easily seen that the rate at which the inert gas is passed over the sample has a profound effect on the pressure of the carbon dioxide.

If the ignition of barium carbonate is carried out in a muffle furnace, the carbon dioxide is in equilibrium with the barium carbonate and oxide; if the ignition is carried out over a blast burner in a covered crucible, the pressure of the carbon dioxide also should be very close to the equilibrium value. Therefore, it is desirable to determine as closely as possible the equilibrium values for the system carbon dioxide–barium carbonate–barium oxide at various temperatures.

In Figure 3 the values for the logarithm of the pressure are plotted against the rate. Because of the sharp rise in these

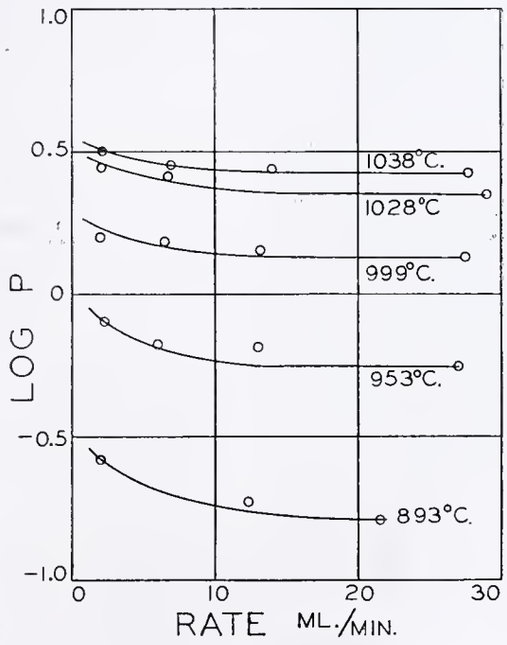

FIGURE 3. EFFECT OF RATE OF GAS FLOW

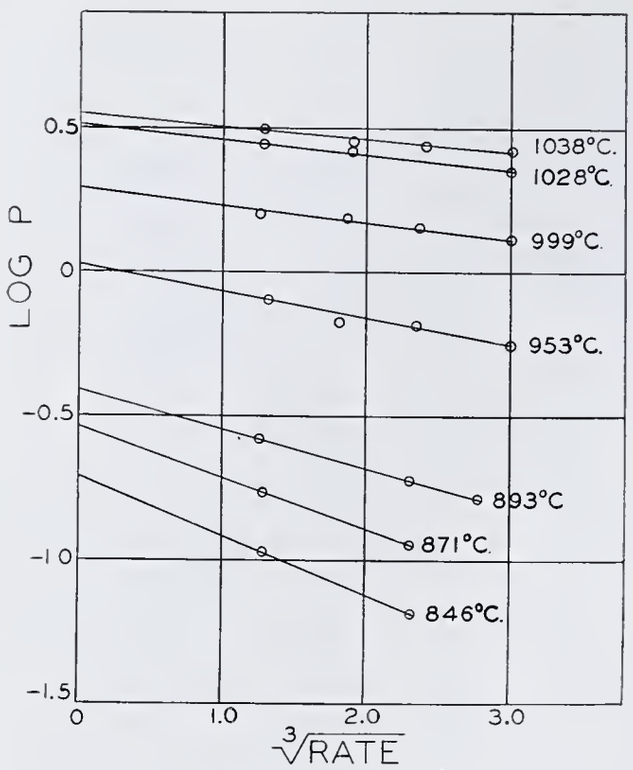

FIGURE 4

curves, the extrapolation to a rate of 0 ml. per minute cannot be made with any degree of accuracy. However, if the cube root of the rate is plotted against the logarithm of the pressure, as shown in Figure 4, a straight line results which can be extrapolated to a rate of 0 ml. per minute. The extrapolated values for the logarithm of the pressure are shown against the reciprocal of the absolute temperature in Figure 2. This line should represent approximately the equilibrium values for the pressure of carbon dioxide in the system carbon dioxide–barium carbonate–barium oxide. If White's data (*13*) from heating calcium carbonate at various rates at 636° C. are plotted by the same method, the extrapolated value for the logarithm of the pressure at zero rate is close to 0.67, which is Johnston's (*10*) equilibrium value for this temperature.

In order to make sure that the decomposition of barium carbonate was proceeding according to the reaction

$$BaCO_3 \rightarrow BaO + CO_2$$

several of the experiments (34, 37, 39, and 42) were checked by weighing the boat and sample before and after and comparing this loss to the total gain in weight of the absorption tube in the train and of the one in the by-pass. The results are given in Table III. The largest difference is 0.5 mg. and in all cases the loss in the sample was less than or equal to the gain in the absorption tubes, which indicates that the above equation correctly represents the reaction occurring at this temperature.

Carbon dioxide is normally present in the atmosphere in a concentration of 0.03 per cent by volume (*8*). Assuming the average barometric pressure in this vicinity is 740 mm., then the partial pressure of carbon dioxide in the atmosphere is 0.222 mm. Decomposition of the barium carbonate on heating should therefore begin when the pressure of the carbon dioxide in equilibrium with barium carbonate and oxide exceeds 0.222. According to Figure 2 the equilibrium pressure of carbon dioxide is equal to 0.222 mm. at about 860° C. It can be predicted therefore that barium carbonate if heated above 860° C. will start to lose weight.

To verify this conclusion, loss-in-weight experiments were conducted with a platinum-wound muffle furnace of about 700-ml. capacity (*12*). The same temperature regulator was used as in the determination of the dissociation pressures and the temperature was measured with a Bureau of Standards calibrated platinum–platinum rhodium thermocouple. The temperatures are believed to be accurate to within ±5° C.

Two samples of barium carbonate were weighed into 10-ml. platinum crucibles and placed in the muffle. One crucible was covered and the other uncovered. After heating the desired length of time at a given temperature, they were removed, allowed to cool for 20 minutes in a desiccator, and weighed. Using the same samples this procedure was repeated, varying the time of heating and the temperature. Although the barium carbonate had previously been ignited at 600° C. and was dried overnight at 110° C., a loss in weight of about 0.2 per cent occurred during the first 2 hours' heating at 845° C. This loss was thought to be due to loss of water and was confirmed in another experiment by first heating the barium carbonate at 300° C. for 20 hours. In this case approximately the same loss occurred at 300° C., at which temperature the barium carbonate would certainly not dissociate. The results, omitting the first 2-hour loss at 845° C., are given in Table IV.

At 865° C. a small but constant loss in weight occurred with both the covered and uncovered crucibles. This loss would not be appreciable in an ordinary analytical determination but it confirms the prediction of the dissociation curve that decomposition should start at this temperature. At 885° C. the loss, although noticeable, is not large enough to cause an appreciable error in an analytical determination if the ignition is made under

TABLE III. COMPARISON OF LOSS BY SAMPLE TO GAIN IN ABSORPTION TUBES AT 999° C.

Run No.	34	37	39	42
Weight lost by sample, mg.	7.5	9.1	9.0	8.9
CO_2 gain in microtube in by-pass, mg.	2.670	2.533	2.480	2.520
CO_2 gain in macrotube, mg.	5.3	7.0	6.7	6.4
Total CO_2 gain, mg.	7.97	9.533	9.18	8.92
Total loss, mg.	7.5	9.1	9.0	8.9
Difference, mg.	+0.47	+0.433	+0.18	+0.02

TABLE IV. LOSS IN WEIGHT OF BARIUM CARBONATE

			Sample 1 (Uncovered), Original Weight 1.4125 Grams				Sample 2 (Covered), Original Weight 1.2979 Grams			
				Loss in Weight				Loss in Weight		
Temp.	Time Each ignition	Time Total at each temperature	Weight after each ignition	Loss in each ignition	Total at each temperature	Loss per hour (av.)	Weight after each ignition	Loss in each ignition	Total at each temperature	Loss per hour (av.)
° C.	Hours	Hours	Grams	Mg.	Mg.	%	Grams	Mg.	Mg.	%
845	2	2	1.4122	0.3	0.3		1.2978	0.1	0.1	
	14	16	1.4122	0.0	0.3		1.2976	0.2	0.3	
	10	26	1.4122	0.0	0.3	0.0006	1.2976	0.0	0.3	0.0006
	12	38	1.4122	0.0	0.3		1.2976	0.0	0.3	
865	2.5	2.5	1.4121	0.1	0.1		1.2976	0.0	0.0	
	7	9.5	1.4118	0.3	0.4		1.2974	0.2	0.2	
	12	21.5	1.4115	0.3	0.7	0.0014	1.2972	0.2	0.4	0.0010
	10.5	32	1.4115	0.0	0.7		1.2971	0.1	0.5	
	12	44	1.4113	0.2	0.9		1.2970	0.1	0.6	
885	2	2	1.4113	0.0	0.0		1.2970	0.0	0.0	
	2	4	1.4112	0.1	0.1		1.2970	0.0	0.0	
	3	7	1.4111	0.1	0.2		1.2969	0.1	0.1	
	12	19	1.4109	0.2	0.4	0.0023	1.2968	0.1	0.2	0.0018
	5	24	1.4107	0.2	0.6		1.2967	0.1	0.3	
	10	34	1.4102	0.5	1.1		1.2964	0.3	0.6	
	12	46	1.4098	0.4	1.5		1.2959	0.5	1.1	
905	2	2	1.4090	0.8	0.8		1.2953	0.6	0.6	
	4	6	1.4078	1.2	2.0	0.023	1.2943	1.0	1.6	0.020
	2	8	1.4072	0.6	2.6		1.2938	0.5	2.1	

conditions similar to these. At 905° C. the loss in weight is appreciable in the course of an hour and an analytical determination would not be satisfactory if the heating were continued at this temperature for more than a few minutes. To be certain that the loss in weight was not due to the volatilization of the platinum, the empty crucibles were heated for 113 hours at 905° C. The total loss in weight of the two crucibles was 0.2 and 0.1 mg.

Summary

The dissociation of barium carbonate has been studied by the gas saturation method of determining vapor pressures.

The extrapolation of the curve of the cube root of the rate of gas flow against the logarithm of the pressure gives values for zero rate of flow or equilibrium pressures which can be used to predict the temperature at which barium carbonate will start to decompose.

A prediction of the temperature at which a noticeable loss in weight will occur when barium carbonate is ignited has been made and experiments to determine the loss in weight have borne out this prediction. Under the conditions described barium carbonate may be ignited for a short time as high as 885° C. without affecting the accuracy of the usual analytical determination, but above this temperature the loss in weight becomes appreciable.

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Glass Valve Pressure Regulator

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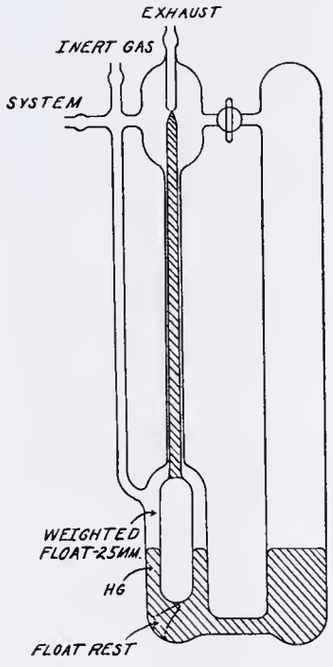
IN THE course of investigations involving the evolution of highly corrosive vapors, it became desirable to conduct experimentation at carefully controlled pressures, both above and below atmospheric pressure. A search of the literature failed to reveal any simple device deemed capable of continued operation under the conditions to be encountered. The glass regulator here described has been found by laboratory trial to meet satisfactorily the conditions imposed.

The apparatus, the construction of which is shown in the diagram, involves the sealed-chamber principle found in the vacuum regulator of McConnell (*1*). It consists of two glass chambers, communicating across the bottom through a mercury pool and across the top through a stopcock. Floating on the mercury in one arm is a lead-weighted float, carrying a glass rod having at its free end a ground-glass tip which serves as a needle to close the ground opening in the exhaust line. The valve parts are held in alignment by a close-fitting sleeve surrounding the float stem. The vertical motion of the valve assembly is limited to about 1 mm. by the valve seat above and the glass stop below.



The dimensions of the regulator may be varied within wide limits; however, it is essential that the weight of the float be sufficient to ensure the opening of the valve against the pressure differential at the valve seat. Rough calculations show that slightly over 10 grams are required to open a 1 sq. mm. opening against a pressure differential of one atmosphere. As this weight should be displaced by the mercury column in as small a pressure range as possible, it is necessary that the cross section of the float be relatively large. A float made from a 25-mm. Pyrex ignition tube has been found to give satisfactory results. In this case, the lead-loaded float weighs approximately 100 grams, giving a sizable safety factor.

In operation, the pressure in the system is allowed to build up to the desired level with the regulator stopcock open. The stopcock is then closed, thereby sealing in the large chamber a body of gas at the selected pressure. As the pressure in the system further increases, the mercury in the float chamber is depressed, allowing the valve to open and exhaust the accumulated gases until the mercury in rising again closes the needle valve. If, in operation, the system does not evolve sufficient gas to maintain the desired pressure, an inert gas may be supplemented, as indicated in the diagram. The pressure fluctuations necessary to actuate the valve mechanism may be largely eliminated at the reaction flask by interposing a bottle of a few liters' capacity to act as a cushion.

This apparatus has been used in the authors' laboratories for the regulation of both positive and reduced pressures. Positive pressures thus far have not exceeded 1500 mm. of mercury, although there appears no reason why higher pressures could not be controlled equally well, provided the weight relationships are kept in balance.

As a reduced pressure regulator, the "exhaust" line is connected to the vacuum system and a controlled air leak is attached to the "inert gas" entrance. It is not expected that the valve should operate at very low pressures, since several millimeters variation in pressure is required to actuate the mechanism. As the control is based upon the equalization of pressure between the system and the sealed chamber, temperature variations within the chamber directly affect the regulator precision. Under proper operating conditions, the apparatus has been found capable of controlling pressure either above or below atmospheric pressure, to about 1 mm. of mercury.

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Contribution No. 272, Department of Chemistry, Kansas State College Manhattan, Kans.

Ozone as an Analytical Reagent

Determination of Vanadium, Cerium, and Manganese

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OZONE is an exceedingly powerful oxidizing agent. The normal oxidation potential as given by Kassel (*3*) for the reaction

$$O_3\ (g) + 2H^+ + 2e \longrightarrow O_2\ (g) + H_2O \qquad (1)$$

is 2.07 volts. Only fluorine and atomic oxygen have higher oxidation potentials. Ozone is much easier to produce and to handle than either fluorine or atomic oxygen, and, therefore, should be useful where a very powerful oxidizing agent is required. Furthermore, ozone is a gas and its decomposition and reduction product, oxygen, is also a gas, and both may easily be removed merely by warming or by passing some inert gas through the solution. Thus oxidations may be produced without adding any foreign matter.

The action of ozone on inorganic compounds has been previously investigated by a great number of workers. In practically all cases prolonged action results in the formation of a compound of the highest stage of oxidation of the element under consideration. Most investigators did not determine whether a given compound could be quantitatively changed to a definite oxidized state.

Jannasch and Gottschalk (*2*) devised a method for the determination of manganese by oxidizing manganous salts to manganese dioxide by means of a current of ozone. Noyes and Garner (*4*) used ozone to prepare thallic nitrate from thallous nitrate and found that the oxidation was quantitative.

Experimental

APPARATUS. The ozonizer employed in this investigation (Figure 1) was essentially that described by Noyes, Hoard, and Pitzer (*5*). It was constructed from two coaxial Pyrex cylinders, *F* and *G*, with an air space of about 3 mm. between them. The diameter of the inner cylinder was about 25 mm. and the length 45 cm. The outer tube was sealed to the inner tube at the top and the inner tube was sealed off at its lower end. The lower end of the outer tube was drawn down and sealed to a smaller tube bent at right angles and carrying the male half of a standard-taper glass joint, *J*. Oxygen entered through a side arm, *B*, at the top of the apparatus and left through the standard-taper glass joint. A coil of aluminum sheet, *H*, placed inside the inner tube served as one electrode and a similar coil, *E*, wound around the outer glass tube served as the second electrode. The outer electrode was surrounded by a glass cylinder, *D*, through which tap water was circulated to cool the whole apparatus. Water entered at *I* and left at *K*. Rubber stoppers, *C*, were used to hold the cooling jacket in place. A 15,000-volt, 850-watt, 60-cycle transformer supplied current to the two electrodes through connections *A* and *L*.

The ozone was led into the solutions by means of a bubbler tube which was constructed by cutting off the top of a Jena fritted-glass funnel approximately 32 mm. in diameter. The edges were ground down until there was no projection of glass above the fritted-glass disk. The stem of the funnel was sealed to a Pyrex tube bent at right angles and terminated by the female half of a standard-taper glass joint. The bubbler tube reached nearly to the bottom of a large 25-cm. (10-inch) test tube. Rapid oxidation depends largely upon getting intimate contact between the gas and solution—that is, the gas bubbles should be as small as possible.

Oxygen was supplied from a tank and was dried by passage over anhydrous magnesium perchlorate. The ozonizer was placed in a hood, since ozone is very irritating to the mucous membranes of the body. Rubber tubes carrying oxygen and water were partially protected from the deteriorating action of ozone by dipping the tubes in molten beeswax.

A T-tube was placed in the oxygen inlet line, so that carbon dioxide or nitrogen could be substituted for oxygen in order to sweep out oxygen and ozone from the solutions.

The gas issuing from the ozonizer at the rate of about 10 liters per hour contained approximately 5 per cent ozone by weight if the outer electrode was not grounded and about 2 per cent by weight when the outer electrode was grounded.

PREPARATION OF REAGENTS. Potassium permanganate solution was prepared in the usual way and standardized against Bureau of Standards sodium oxalate by the procedure of Fowler and Bright (*1*). This was reduced with hydrogen peroxide in dilute perchloric acid solution and diluted to a known volume to give a standard solution of manganous perchlorate.

Standard solutions of ferrous sulfate were kept under carbon dioxide. The ferrous sulfate was standardized regularly against standard potassium permanganate solutions.

A solution of cerous nitrate was prepared from pure cerous nitrate crystals. This solution was standardized by oxidation with persulfate, using silver ion as catalyst, and subsequent titration of the ceric ion with standard ferrous sulfate according to the method of Willard and Young (*7*). The ferrous sulfate was standardized against potassium dichromate.

The silver nitrate solution used as a catalyst contained 35 grams of silver nitrate per liter.

Determination of Manganese

In perchloric acid solutions containing a little silver ion as catalyst, manganous ion is rapidly oxidized to permanganate ion. In nitric acid, phosphoric acid, or sulfuric acid solutions oxidation is incomplete or manganese dioxide is precipitated.

GENERAL PROCEDURE. A measured quantity of the standard manganous perchlorate solution was added to the desired quantity of perchloric acid and diluted to 100 ml. in a 25-cm. (10-inch)

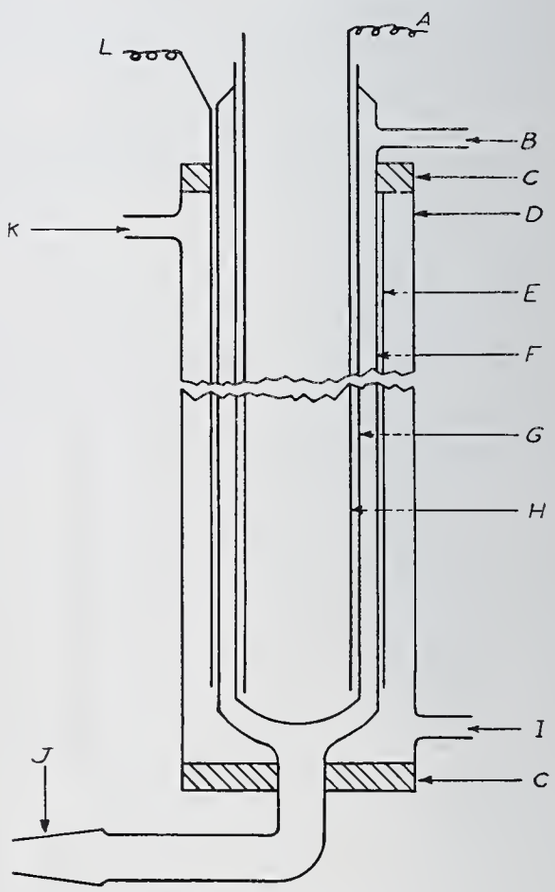

FIGURE 1. OZONIZER

[1] Present address, Wayne University, Detroit, Mich.

Table I. Influence of Perchloric Acid Concentration

Perchloric Acid *Molarity*	Mn Present *Mg.*	Mn Found *Mg.*	Error *Mg.*
1.16	3.22	3.16	−0.06[a]
1.39	3.22	3.24	+0.02
1.39	3.22	3.19	−0.03
1.74	3.22	3.29	+0.07
1.74	3.22	3.26	+0.04
2.32	3.22	3.23	+0.01
3.48	3.22	3.26	+0.04
3.48	3.22	3.01	−0.21
4.64	3.22	3.11	−0.11
5.80	3.22	1.87	−1.35
5.80	3.22	2.55	−0.67

[a] A slight precipitate of MnO_2 was noted on bubbler tube.

test tube. Silver nitrate solution was added and the solution so prepared was subjected to a stream of ozone from the ozonizer. The gas flow was always about 10 liters per hour. After the desired period of oxidation, carbon dioxide was substituted for oxygen and the excess oxygen and ozone were thus swept out of the solution; this required 15 minutes.

The bubbler tube was rinsed into the solution with distilled water and a measured excess of standard ferrous sulfate was added. The excess ferrous sulfate was back-titrated with standard potassium permanganate. The small amount of potassium permanganate necessary to give the pink color at the end point was determined each time by adding standard permanganate solution to an equal volume of acid until the same shade of pink was obtained. This usually amounted to 0.03 to 0.05 ml. of 0.1 *N* permanganate and was subtracted from the amount used in the back-titration.

Influence of Perchloric Acid Concentration. Solutions containing 3.22 mg. of manganese and 35 mg. of silver nitrate were treated as described above (Table I). Ozone was passed through the solutions for one hour. The concentration of perchloric acid present was varied from 1.16 *M* to 5.80 *M*. With a concentration of perchloric acid less than 1.16 *M* a precipitate of manganese dioxide always appeared. The results became erratic at concentrations of acid higher than 2.32 *M*.

Influence of Silver Nitrate Concentration on Time Necessary for Complete Oxidation. With no silver nitrate present in the solutions about 6 hours were required for complete oxidation; with 17.5 mg. of silver nitrate per 100 ml., 2 hours; with 35 mg., 1 hour; and with 70 mg. 0.5 hour. In these trials an ozone concentration of about 2 per cent by weight was used, rather than 5 per cent, since the effect of silver nitrate is more pronounced with the lower ozone concentrations. In subsequent experiments a concentration of 35 mg. of silver nitrate per 100 ml. of solution was employed.

Influence of Ozone Concentration on Speed of Oxidation and on Amount of Manganese Oxidized. If a gas stream having an ozone concentration of 2 per cent by weight was employed, only 5 mg. of manganese could be oxidized without precipitation of manganese dioxide. The time necessary for complete oxidation was from 60 to 75 minutes. With an ozone concentration of 5 per cent, about 9 mg. of manganese could be oxidized in 15 minutes without precipitation of manganese dioxide.

Interferences. All substances which are oxidized by ozone and subsequently reduced by ferrous sulfate but not reoxidized by potassium permanganate will interfere; this includes cerium, cobalt, and chromium. Vanadium interferes unless care is taken that the pink color is permanent in the back-titration with permanganate.

Chlorides and all other ions which precipitate silver must be absent. Large concentrations of nitrates will interfere, since they oxidize ferrous salts.

Recommended Procedure for Determining Manganese in Steel and Iron Ore

Steel. Dissolve 1 gram of the steel, or a sample which will contain not more than 9 mg. of manganese in about 5 ml. of nitric acid diluted with 15 to 25 ml. of water. After all the steel has dissolved, add 25 ml. of 70 to 72 per cent perchloric acid and evaporate until fumes of perchloric acid appear, to remove nitric acid. Allow the beaker and contents to cool and then add 1 ml. of a silver nitrate solution containing 35 grams of silver nitrate per liter. Transfer the solution to a 25-cm. (10-inch) test tube and dilute to approximately 100 ml. with water. Bubble 5 per cent ozone through this solution for 15 minutes.

After 15 minutes of oxidation, pass carbon dioxide through the solution for 15 minutes, remove the bubbling tube from the ozonizer, and rinse it several times with distilled water. Add an accurately measured excess of standard ferrous sulfate (20.00 ml. of 0.05 *N* ferrous sulfate is sufficient) and back-titrate immediately with standard potassium permanganate until a faint pink color persists. Determine the excess permanganate necessary to give this pink color by adding permanganate to the same volume of water or dilute acid until the colors in blank and experiment match. Usually 0.03 to 0.05 ml. of 0.10 *N* potassium permanganate is required. Subtract the blank from the volume of standard permanganate used in the experiment.

Iron Ore. Dissolve 1 gram of the ore, or a weight which contains not more than 9 mg. of manganese, in 40 ml. of hydrochloric acid (3 to 1). After all iron oxide has gone into solution and only flocculent white silica remains, add 25 ml. of concentrated nitric acid and 25 ml. of 70 to 72 per cent perchloric acid, and evaporate the solution to fumes of perchloric acid. After the solution has cooled, transfer it to a 25-cm. (10-inch) test tube, add 2 ml. of silver nitrate solution (35 grams of silver nitrate per liter), and dilute to about 100 ml. Pass ozone through the solution for 15 minutes and proceed exactly as described above for a steel.

Results of Analyses. Results of analyses on National Bureau of Standards samples of iron ores and steels according to the procedures described above are given in Table II.

Table II. Determination of Manganese in Steel and Iron Ore

Sample	Weight of Sample *Gram*	Mn Found %	Certificate Value %
Bur. of Standards iron ore No. 28	0.9380	0.449	0.465
	0.8992	0.439	
Bur. of Standards steel No. 96	1.0040	0.733	0.747
	1.0713	0.739	
Bur. of Standards steel No. 11c	1.0278	0.429	0.435
	1.2600	0.436	
	1.2469	0.426	
	1.7630	0.433	

Determination of Cerium

Preliminary experiments showed that ozone did not oxidize cerous salts in perchloric acid solution and only partially in sulfuric acid solution, but it quantitatively oxidized them to ceric salts in solutions containing both sulfuric and phosphoric acids, although the oxidation with gas containing 2 per cent ozone was very slow. Ceric phosphate, being much more insoluble than cerous phosphate, separates out of the solution in the form of a thick white gel. Unless the cerium is precipitated in this way, oxidation is incomplete.

Procedure. Take for analysis a sample containing not more than 100 mg. of cerium, and to this solution add 2 ml. of concentrated sulfuric acid and 5 ml. of sirupy phosphoric acid (80 per cent). Dilute the solution to 100 ml. and treat with 5 per cent ozone for 60 minutes. Add concentrated sulfuric acid until the white gel of ceric phosphate dissolves completely and a clear yellow-orange solution results. About 15 to 20 ml. of acid is required. Pass carbon dioxide or nitrogen through the solution for 15 minutes to remove oxygen and ozone. Remove the bubbling tube from the solution and carefully rinse with distilled water. Add 2 drops of *o*-phenanthroline ferrous sulfate solution (0.025 *M*) and titrate the ceric sulfate with standard ferrous sulfate until the indicator changes to a bright pink.

In the presence of large amounts of iron, 7 ml. of phosphoric acid, rather than 5 ml. as specified, are added to the solution.

Results of Analyses. Results of several analyses by the method described above are listed in Table III. Amounts of cerium greater than about 100 mg. are not readily handled,

Table III. Determination of Cerium

Cerium Taken	Other Additions	Cerium Found	Error
Mg.		*Mg.*	*Mg.*
100.00	....	99.83	−0.17
77.37	....	77.40	+0.03
72.03	....	71.87	−0.16
57.64	....	57.61	−0.03
57.62	....	57.94	+0.32
43.22	....	43.16	−0.06
43.22	....	43.13	−0.09
28.81	....	28.78	−0.03
14.41	....	14.21	−0.20
93.64	2 mg. Cr^{+++}	93.43	−0.21
86.44	1 gram Fe^{+++}	86.09	−0.35
86.44	1 gram Fe^{+++}	86.21	−0.23
72.03	2 mg. Cr^{+++}	72.00	−0.03
72.03	1 gram Fe^{+++}	71.94	−0.09
28.81	1 gram Fe^{+++} and 2 mg. Cr^{+++}	28.95	+0.14

owing to the large amount of gelatinous ceric phosphate which is formed, and the oxidation takes longer than one hour.

Interferences. It is apparent from Table III that iron and small amounts of chromium do not interfere. All substances which are oxidized by ozone in acid solution and are reduced by ferrous sulfate must be absent. This includes manganese, cobalt, vanadium, and large amounts of chromium salts. Chlorides and large concentrations of nitrates should not be present.

Determination of Vanadium

In preliminary experiments it was found possible to oxidize vanadyl salt solutions completely to vanadates by means of ozone. The oxidation of chromium was very slow in the cold and in the absence of a catalyst. These two observations suggested that ozone could be substituted for potassium permanganate in the Willard and Young (*6*) method for the determination of vanadium in chromium-vanadium and chromium-vanadium-tungsten steels. Such was found to be the case.

The completion of the oxidation of the vanadyl ion may be easily judged by adding a small amount of manganous salt to the solution before oxidation. When all vanadyl ion is oxidized, the ozone will begin to oxidize manganese to permanganate. The appearance of a permanganate color thus serves as an indication of the complete oxidation of all vanadium. Many steels contain sufficient manganese without further addition.

Procedure for Chromium-Vanadium Steels. Weigh out a sample of approximately 5 grams of the chromium-vanadium steel and dissolve in approximately 30 ml. of water and 3 ml. of concentrated sulfuric acid with 1.5 ml. additional acid for each gram of steel. After all action has ceased evaporate the solution until considerable salts begin to separate, in order to decompose carbides. Add approximately 50 ml. of water, and then add to the boiling hot solution concentrated nitric acid drop by drop until the vigorous oxidation of ferrous salts is completed. After boiling for 2 to 3 minutes to remove oxides of nitrogen, cool the solution to room temperature, add 25 ml. of 1 to 1 phosphoric acid, dilute the solution to 300 ml., and connect to the ozonizer. The concentration of ozone in the gas should be about 5 per cent by weight. (With this concentration the oxidation of vanadyl salts is complete in about 5 minutes.) As soon as a definite permanganate color appears remove the solution from the ozonizer and carefully rinse the bubbling tube into the sample. Remove the permanganate by reduction with azide or nitrite and titrate the solution with ferrous sulfate, using oxidized diphenylamine as indicator as described by Willard and Young (*6*), or potentiometrically.

If tungsten is present, it is kept in solution as complex fluoride and the procedure of Willard and Young (*6*) is followed.

Results of Analyses. Results of a series of determinations according to the method described above (Table IV) are slightly higher than those recommended by the Bureau of Standards for the chromium-vanadium-tungsten steels.

Action of Ozone on Compounds of Other Elements

Chromic ion in acid solution was scarcely oxidized unless silver nitrate was present as catalyst and even then only in small amounts.

Iodide in alkaline solution was oxidized to periodate and this reaction will be described in a subsequent paper. Bromide under similar conditions was partially oxidized to bromate. Chloride ion was not affected.

Selenite and tellurite ions were quantitatively oxidized to selenate and tellurate in alkaline solution, but there was only slight oxidation in acid solution.

Trivalent arsenic and antimony were completely oxidized in acid or alkaline solution.

Mercurous perchlorate was readily oxidized to the mercuric form.

Lead in alkaline solution was partially oxidized and bismuth formed a brown precipitate of a higher oxide, probably Bi_2O_4.

Cobaltous and nickelous hydroxides formed the corresponding trivalent hydroxides.

Nitrite was oxidized to nitrate; hypophosphite and phosphite were oxidized to phosphate.

Table IV. Determination of Vanadium in Steels

Sample	Vanadium Present	Vanadium Found	Error
	%	%	%
Bur. of Standards Cr-V steel No. 30c	0.235	0.231	−0.004
		0.237	+0.002
		0.234	−0.001
		0.236	+0.001
Bur. of Standards Cr-V stee No. 30a	0.20	0.199	0.00
		0.204	0.00
		0.199[a]	0.00
Steel sample No. 6	0.218[b]	0.215	−0.003
		0.217	−0.001
Steel sample No. 19	0.230[b]	0.228	−0.002
		0.228	−0.002
Steel sample No. 20	0.139[b]	0.143	+0.004
		0.144	+0.005
Bur. of Standards, Cr-V-W steel No. 50a	0.976	0.998	+0.022
		0.994	+0.018
		0.990	+0.014
		0.984	+0.008
Bur. of Standards Cr-V-W steel No. 50	0.756	0.783	+0.027
		0.765	+0.009
		0.762	+0.006
		0.746	−0.010

[a] End point determined potentiometrically.
[b] Value determined by permanganate method of Willard and Young (*6*).

Summary

Manganese can be quantitatively oxidized to permanganate by ozone if the amount of manganese does not exceed 9 mg. and the oxidation is carried out in a perchloric acid solution between 1.16 and 2.32 *M*, using silver nitrate as catalyst. Oxidation requires about 15 minutes if oxygen containing 5 per cent ozone by weight is used. Procedures are given for the determination of manganese in steel and iron ore.

Up to 100 mg. of cerium can be oxidized in 1 hour to ceric phosphate by ozone in a solution containing sulfuric and phosphoric acids. Ceric phosphate separates in the form of a white gel which is dissolved by sulfuric acid and the ceric ion is titrated with ferrous sulfate.

Vanadium in chromium-vanadium and chromium-vanadium-tungsten steels may be determined after oxidation by ozone to vanadic acid.

Iodides, selenites, tellurites, nitrites, hypophosphites, and phosphites are oxidized by ozone. Mercurous, arsenious, and antimonous ions are also oxidized. Chromic and bromide ions are only partially oxidized.

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Determination of Iodides

OZONE can be used in the determination of manganese, vanadium, and cerium (*3*). It will also quantitatively oxidize iodide ion to periodate ion in solutions alkaline with sodium or potassium hydroxide. In sodium carbonate solutions iodide is oxidized mainly to iodate, but there is some further oxidation to periodate. Some iodine is lost as iodine pentoxide which is carried out of the solution in the form of white fumes by the bubbles of oxygen and ozone. Quantitative oxidation of iodide to iodate could not be secured, even though buffered solutions of varying pH values and sodium and potassium carbonate solutions of varying concentrations were tried.

Periodates may be determined by either of two methods. In a buffered neutral solution periodate reacts with iodide ion to form iodine and iodate according to the equation:

$$H_2O + KIO_4 + 2KI \longrightarrow KIO_3 + I_2 + 2KOH$$

Willard and Boyle (*2*) have found that a buffer consisting of 5 grams of boric acid and 5 grams of borax per 100 ml. of solution is satisfactory for this reaction. The liberated iodine is titrated with standard, neutral, arsenite solution. Periodates may also be determined by allowing them to react with excess iodide in the presence of excess acid:

$$8HCl + KIO_4 + 7KI \longrightarrow 8KCl + 4I_2 + 4H_2O$$

The liberated iodine is titrated with standard sodium thiosulfate.

The first method of determining the periodate formed by the oxidation of iodide by ozone should be especially useful for large amounts of iodine, since only two atoms of iodine are formed for the final titration from each iodide ion present at the start. The second method of titration is especially useful for the determination of small amounts of iodide ion, since eight atoms of iodine are present for the final titration for each iodide ion in the original solution.

Experimental

PREPARATION OF SOLUTIONS. Standard Iodide Solution. An approximately 0.1 *M* solution of potassium iodide (Mallinckrodt's analytical reagent grade) was carefully standardized against a solution of potassium permanganate which, in turn, was standardized against Bureau of Standards arsenious oxide. The procedure was that described by Kolthoff, Laitinen, and Lingane (*1*). Weight burets were used and all end points were determined potentiometrically. The solution was found to contain 0.015688 gram of iodine per gram of solution (weighed in air against brass weights).

Sodium Thiosulfate Solution. An approximately 0.1 *N* sodium thiosulfate solution was carefully standardized every few days against iodine which had been sublimed once from potassium iodide and then twice resublimed and dried over calcium chloride. Portions of this purified iodine were fused in a tared weighing bottle, cooled, and weighed, and the bottle was opened under a 20 per cent solution of potassium iodide. The iodine was titrated with the sodium thiosulfate solution using starch as indicator. This procedure was also checked by standardizing the thiosulfate against the potassium permanganate used to standardize the iodine solution described above. The results agreed within 3 parts in 10,000 with the standardizations against purified iodine. Weight burets were used in all titrations.

Another solution of thiosulfate was prepared in a similar manner, but ordinary volume burets were used for the titrations.

Standard Arsenite Solution. Bureau of Standards arsenious oxide (6.5200 grams) was dissolved in 100 ml. of water containing 15 grams of sodium hydroxide crystals (monohydrate). This solution was diluted to about 1.5 liters, saturated with carbon dioxide, and then diluted to exactly 2 liters in a volumetric flask. One milliliter thus corresponds to 0.004183 gram of iodine originally present, since two atomic weights of iodine are formed by each mole of periodate.

PROCEDURE. A weighed portion of the standard iodide solution was added to the desired amount of sodium or potassium hydroxide containing any other desired additions. This solution was diluted to approximately 100 ml. in a 25-cm. (10-inch) test tube and connected to the ozonizer described in the previous paper (*3*). The gas containing about 5 per cent by weight of ozone was passed into the solution at such a rate that the bubbles from the bubbling tube rose to form a fine layer of foam on top of the solution (about 10 liters of gas per hour). After the desired time of oxidation, the current was turned off and the oxygen was stopped. Nitrogen was passed through the solution for 15 minutes to remove all dissolved ozone and ozygen. The solution was rinsed into a flask and titrated by one of the two following methods.

1. *Reduction of Periodate to Iodate.* Eight grams of boric acid were added to the solution for every gram of sodium hydroxide (or 1.5 grams of sodium hydroxide crystals) present in the original solution. The solution was stirred and warmed slightly, if necessary, to dissolve the boric acid. This amount of acid is enough to convert the sodium hydroxide to borax and to give an equal weight of excess boric acid. Ten to 20 grams of potassium iodide were added and the liberated iodine was titrated with standard arsenite solution using starch as indicator.

2. *Reduction of Periodate to Iodine.* Ten grams of potassium iodide were added to the solution, which was then acidified with 6 *N* sulfuric acid, using 5.5 ml. of acid for every gram of sodium hydroxide (or 1.5 grams of sodium hydroxide crystals) originally present. The liberated iodine was titrated with standard thiosulfate solution, using starch as indicator.

TABLE I. PERMISSIBLE LIMITS OF ALKALI CONCENTRATION

Alkali Concentration	Time of Oxidation	Iodide Taken	Iodide Found	Error
	Min.	*Gram*	*Gram*	*Gram*
2% Na_2CO_3	90	0.1704	0.1261	−0.0443
4% Na_3PO_4	90	0.1553	0.1298	−0.0255
1% NaOH	30	0.0695	0.0695	−0.0000
2% NaOH	40	0.1498	0.1497	−0.0001
10% NaOH	30	0.0640	0.0639	−0.0001

PERMISSIBLE LIMITS OF ALKALI CONCENTRATION. Several analyses were made by the second procedure, using known quantities of iodide ion and varying the concentration of alkali present (Table I). It is obvious that any concentration of sodium hydroxide from 1 per cent to at least 10 per cent may be present during the oxidation. Sodium carbonate or tribasic sodium phosphate solutions are not alkaline enough for complete oxidation. Oxidation in these solutions has been shown to stop with the iodine largely present as iodate.

TABLE II. AMOUNT OF IODINE OXIDIZED

Time of Oxidation	Iodide Taken	Iodide Found	Error
Min.	*Gram*	*Gram*	*Gram*
15	0.0541	0.0272	−0.0269
15	0.1680	0.0954	−0.0736
20	0.1651	0.1653	+0.0002
30	0.1866	0.1864	−0.0002
30	0.1992	0.1992	±0.0000

AMOUNT OF IODIDE OXIDIZED IN A GIVEN TIME. Analyses were carried out by the procedure described above, using 2 per cent sodium hydroxide solutions for the oxidation (Table II). All titrations of periodate were made according to method 1, using neutral arsenite solution.

It is apparent that 20 minutes is the minimum time for the complete oxidation, using a gas containing about 5 per cent ozone by weight. Thirty minutes is recommended. With 2 per cent ozone the time is greatly extended. In one experiment only 4.4 per cent of the theoretical amount of periodate had been formed after 80-minute oxidation of a solution containing 0.17908 gram of iodine.

About 0.2 gram of iodine as iodide is the upper limit for convenient handling. More than this amount requires more than 50 ml. of 0.1 *N* arsenite solution and some of the iodine liberated is liable to volatilize from the solution and be lost. About 0.05 gram of iodine is the upper limit for convenient handling when the periodate is titrated with thiosulfate by method 2.

INTERFERENCES. Tests were carried out by the procedures described above on solutions containing known amounts of iodide ion with various other additions (Table III). It is apparent that bromide ion interferes with either method of titration of the periodate but that chlorides do not interfere. Potassium hydroxide may be substituted for sodium hydroxide. If other iodine compounds are present they will also be oxidized to periodate.

TABLE III. EFFECT OF CHLORIDES AND BROMIDES

Addition	Method of Titration	Iodide Taken	Iodide Found	Error
		Gram	*Gram*	*Gram*
2 grams NaCl	Arsenite	None	0.00000	0.00000
3 grams NaCl	Arsenite	0.08554	0.08581	0.00027
	Arsenite	0.15533	0.1550	−0.0003
	Arsenite	0.16800	0.16853	0.00053
	Thiosulfate	0.01104	0.01102	−0.00002
	Thiosulfate	0.01157	0.01151	−0.00006
3 grams KCl[a]	Arsenite	None	0.0000	0.0000
3 grams KCl[a]	Arsenite	0.16731	0.1678	0.0005
3 grams KCl	Arsenite	0.04760	0.04745	−0.00015
	Thiosulfate	0.01264	0.01258	−0.00006
0.6 gram KBr, 2 grams NaCl	Arsenite	None	0.00807	0.00807
2 grams KBr, 2 grams NaCl	Arsenite	None	0.00636	0.00636
2 grams KBr, 2 grams NaCl[b]	Arsenite	None	0.00900	0.00900

[a] Potassium hydroxide substituted for sodium hydroxide.
[b] Solution kept near boiling during oxidation.

Recommended Procedure

To a portion of the solution containing not more than 0.2 gram of iodine as iodide or iodate 2 grams of sodium hydroxide (or 3 grams of sodium hydroxide crystals, $NaOH.H_2O$) were added. The clear solution was diluted to 100 ml. in a 25-cm. (10-inch) test tube and ozone was passed through it for 30 minutes. The gas should contain about 5 per cent ozone by weight and a speed of about 10 liters of gas per hour is sufficient. Nitrogen was substituted for oxygen and the solution was swept free of dissolved ozone and oxygen for 15 minutes. The bubbler tube was removed and both bubbler tube and solution were rinsed into a large wide-mouthed flask.

Two procedures may be followed from this point. Procedure A is recommended for small amounts of iodine (up to about 0.05 gram) and B for large amounts (0.05 to 0.2 gram).

PROCEDURE A. TITRATION WITH THIOSULFATE. To the solution in a large flask 10 grams of iodate-free potassium iodide were added and then 11 ml. of 6 *N* sulfuric acid (5.5 ml. for each gram of sodium hydroxide). The liberated iodine was titrated with standard sodium thiosulfate solution. When the solution was still faintly yellow 1 ml. of 2 per cent starch solution was added as indicator, and it was titrated to a clear, colorless solution. In determining small amounts of iodine a blank should be run.

PROCEDURE B. TITRATION WITH ARSENITE. Sixteen grams of boric acid (8 grams for each gram of sodium hydroxide) were added to the solution contained in a wide-mouthed flask, warming slightly if necessary to dissolve the boric acid. The solution was cooled to room temperature and 10 grams of potassium iodide were added. The liberated iodine was titrated with standard arsenite solution, and when the solution became faintly yellow, 1 ml. of 2 per cent starch indicator solution was added and the titration continued until the dark starch-iodine color disappeared.

Results of Analyses

Results of a series of analyses by the procedures described above are listed in Table IV.

TABLE IV. DETERMINATION OF IODIDE BY OZONE

Iodide Present	Other Additions	Titration Method	Iodide Found	Error in Iodide
Gram			*Gram*	*Gram*
0.16356	...	A[a]	0.16352	−0.00004
0.10563	...	A[a]	0.10533	−0.00030
0.06720	..	A	0.06731	+0.00011
0.01408	...	A	0.01408	±0.00000
0.00729	...	A	0.00725	−0.00004
0.00462	...	A	0.00461	−0.00001
0.00051	...	A[b]	0.00051	±0.00000
0.00051	...	A[b,c]	0.00043	−0.00008
0.04970	3 grams NaCl	A	0.04945	−0.00025
0.01157	3 grams NaCl	A	0.01151	−0.00006
0.00863	3 grams NaCl	A	0.00854	−0.00009
0.19917	...	B	0.19924	+0.00007
0.10136	...	B	0.10109	−0.00027
0.06769	...	B	0.06774	+0.00005
0.1870	3 grams NaCl	B	0.1870	±0.0000
0.08554	3 grams NaCl	B	0.08581	+0.00027
0.06378	3 grams NaCl	B	0.06406	+0.00028

[a] Weight burets employed in final titration.
[b] Approximately 0.001 *N* thiosulfate, freshly prepared from 0.1 *N* solution, used in titration.
[c] Dilute iodide solution had remained in glass flask overnight before oxidation.

Summary

Iodide and other iodine compounds can be quantitatively oxidized to periodate by ozone in sodium or potassium hydroxide solutions. The periodate formed may be determined by allowing it to react with iodide in excess in a neutral or acid solution, the iodine liberated being titrated with either arsenite or thiosulfate solution.

Amounts of iodide ion varying from several hundredths of a milligram to 0.2 gram can be determined in this manner with considerable accuracy.

Bromides interfere, at least in concentrations greater than 0.6 gram. Chlorides do not interfere.

Complete oxidation requires from 20 to 30 minutes using a gas containing 5 per cent ozone by weight. The sodium hydroxide concentration may vary from 1 per cent to at least 10 per cent.

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Determination of Palladium with Beta-Furfuraldoxime

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THE Group VIII metals are known to form inner-complex and penetration compounds readily and hence it is not surprising to find a considerable number of organic reagents proposed for the quantitative and qualitative analysis of some of these metals. The familiar reaction of dimethylglyoxime with nickel and palladium salts was apparently the first application of this type of compound to inorganic analysis (*11*). Since that time various dioximes, as well as other types of organic compounds, have been proposed as reagents for the detection and determination of palladium. As representative of these may be mentioned benzoylmethylglyoxime (*3*), α-nitroso-β-naphthol (*8*), salicylaldoxime (*5*), *p*-dimethylaminobenzalrhodanine (*2*), and *p*-nitrosodiphenylamine (*12*).

It has been convincingly demonstrated (*1*) that the formation of the chelate compounds with dioximes is due to the presence of the system —N=C—C=N—. The compound used in this work, beta-furfuraldoxime, does not possess this atomic grouping, but was found to precipitate palladium completely. Therefore it is natural to find a different type of compound formed, one which is evidently an addition rather than an inner-complex compound. Beta-furfuraldoxime was found to be well adapted to the gravimetric determination of palladium, as well as to the separation of this metal from most others.

Materials Used

The palladous chloride solutions were prepared by dissolving a known weight of the pure salt in a definite volume of 0.1 *N* hydrochloric acid, and were then standardized both by precipitation with dimethylglyoxime (*10*) and by reduction to the metal with formic acid (*10*). The palladous nitrate solution was prepared from the pure metal by solution in aqua regia and subsequent repeated evaporation with nitric acid until no test for chloride ion could be obtained. Standardization was by the same procedure as with the palladous chloride solutions. The palladous sulfate solution was also prepared from the metal by dissolving in hot concentrated sulfuric acid, followed by appropriate dilution and standardization by the procedures indicated above.

The beta-furfuraldoxime was obtained from the Eastman Kodak Company and used without further purification. It had a melting point of 88–90° C. A solution of the reagent was prepared shortly before use by dissolving 10 grams of the oxime in 100 ml. of alcohol.

The solutions of inorganic ions were made up from salts of reagent grade. In most cases the concentrations were adjusted so as to give closely 1 mg. per ml. of the ion in question.

All other reagents used were of C. P. grade.

Qualitative Tests

The action of beta-furfuraldoxime was first studied qualitatively with various ions. The procedure followed in most cases was to add 1 ml. of the alcoholic solution of the oxime to 5 ml. of the solution to be tested. As far as solubility relations allowed each ion was tested with the reagent in neutral, slightly acid, and slightly ammoniacal solution. The "neutral" solutions were those obtained by dissolving the pure salt in water, disregarding changes in acidity due to hydrolysis. With some of the less common ions the amounts used were smaller and the reactions were observed on a spot plate. The following ions were tested and gave negative results: Li, Na, K, Rb, Cs, Cu^{++}, Ag, Be, Mg, Ca, Sr, Ba, Zn, Cd, Hg^{+}, Hg^{++}, Al, Tl^{+}, BO_3^{-}, Sc, La, Tb, Er, Sm, Nd, Pr, Yt, Ti^{++++}, Zr, Sn^{++}, Sn^{++++}, Ce^{+++}, Pb, Th, PO_4^{---}, VO_3^{-}, As^{+++}, As^{+++++}, Sb, Bi, CrO_4^{--}, WO_4^{--}, MoO_4^{--}, Cr^{+++}, Mn^{++}, MnO_4^{-}, Fe^{+++}, Co, Ni, Ru^{+++}, Rh^{+++}, Os^{++++}, Ir^{++++}, and Pt^{++++}.

Ceric solutions were found to give a yellow precipitate which, however, was far from quantitative. Auric ion gave, on standing, a brown precipitate which is probably chiefly metallic gold. Ferrous ion gave a red color with the reagent in both neutral and acid solution. Palladous ion yielded a heavy yellow precipitate from acid solution, and this precipitate was found to be quantitative for palladium.

Procedure for Determination of Palladium

The palladous chloride solution, containing about 0.03 gram of palladium, is diluted to about 100 ml. and the acidity is adjusted to approximately 3 per cent hydrochloric acid (specific gravity 1.19, 38 per cent hydrochloric acid by weight) by volume. Close control of the acidity is not essential, as good results were obtained in solutions containing more than 10 per cent hydrochloric acid by volume. The palladium is then precipitated by the addition of 2 ml. of the alcoholic solution of the oxime. The curdy, light yellow precipitate which forms immediately is stirred briefly and allowed to settle for an hour or longer. Precipitation is evidently complete in a very short time, as variations in the time of settling ranging from a few minutes to several days caused no noticeable difference in the results.

The precipitate is next filtered on a weighed Gooch filter and washed with about 50 ml. of cold 1 per cent hydrochloric acid, followed by the same volume of water. Considerably larger volumes of the wash liquids may be used without affecting the results. After washing, the precipitate is dried for 2 hours at 110° C. and weighed. The time of drying may be safely extended without fear of decomposition of the precipitate but the temperature must not be allowed to rise much above 110° C. The theoretical factor for palladium, corresponding to the formula $Pd(C_4H_3OCHNOH)_2Cl_2$, is 0.2669 and this value is used in calculating the results. Further evidence in support of this formula is given below.

Table I gives the results of a few determinations in solutions containing palladium alone.

TABLE I. DETERMINATION OF PALLADIUM

Palladium Present (as $PdCl_2$)	Palladium Found	Difference	Palladium Present (as $PdCl_2$)	Palladium Found	Difference
Gram	*Gram*	*Mg.*	*Gram*	*Gram*	*Mg.*
0.0018	0.0019	0.1	0.0227	0.0229	0.2
0.0018	0.0018	0.0	0.0227	0.0227	0.0
0.0018	0.0018	0.0	0.0227	0.0228	0.1
0.0179	0.0177	−0.2	0.0227	0.0228	0.1
0.0179	0.0178	−0.1	0.0300	0.0300	0.0
0.0179	0.0177	−0.2	0.0300	0.0299	−0.1
0.0186	0.0186	0.0	0.0300	0.0300	0.0
0.0186	0.0186	0.0	0.0300	0.0301	0.1
0.0186	0.0187	0.1	0.0300	0.0299	−0.1
0.0186	0.0186	0.0	0.0300	0.0299	−0.1

Composition of Precipitate

Several grams of the precipitate were prepared, carefully washed, and dried to constant weight. Qualitative analysis indicated the presence of chlorine, nitrogen, and palladium. The quantitative analysis for chlorine was carried out by the Stepanov method (*9*), for nitrogen by the Kjeldahl procedure (*4*), and for carbon and hydrogen by both the semimicrotechnique of ter Meulen and Hesslinga (*6*) and the conventional macrocombustion with the apparatus slightly modified, as

described by Renoll, Midgley, and Henne (*7*). The palladium content was determined by the gravimetric method with dimethylglyoxime (*10*). The following results were obtained:

	Found	Average	Calculated
	%	%	%
Carbon	29.85, 29.97, 30.99	30.27	30.04
Hydrogen	2.51, 2.63, 2.55	2.56	2.52
Nitrogen	6.97, 6.95, 7.01	6.98	7.00
Chlorine	17.89, 17.48, 17.77	17.71	17.73
Palladium	26.68, 26.74, 26.70	26.71	26.69

The average values indicate the formula $Pd(C_4H_3OCHNOH)_2Cl_2$, which is a coordination compound similar to that formed with *p*-nitrosodiphenylamine and palladous chloride (*12*).

Table II. Separation of Palladium from Platinum, Rhodium, Ruthenium, and Iridium

Palladium Present (as $PdCl_2$)	Metals Added	Palladium Found	Difference
Gram	*Gram*	*Gram*	*Mg.*
0.0018	0.02 each of Pt^{++++}, Ru^{+++},	0.0019	0.1
0.0018	Rh^{+++}, and Ir^{++++}	0.0018	0.0
0.0186	0.04 each of Pt^{++++}, Ru^{+++}, and	0.0186	0.0
0.0186	Rh^{+++}	0.0187	0.1
0.0186		0.0186	0.0
0.0227		0.0227	0.0
0.0227		0.0225	−0.2
0.0227		0.0226	−0.1
0.0300	0.06 of Pt^{++++}, 0.04 of Ru^{+++},	0.0299	−0.1
0.0300	0.02 each of Rh^{+++} and Ir^{+++}	0.0301	0.1
0.0300		0.0300	0.0

Determination of Palladium

In the Presence of Other Cations. To test the efficiency of the method for separating and determining palladium in the presence of other metallic ions, synthetic samples were prepared and the palladium was determined by the regular procedure. Table II gives results for the determination of palladium in the presence of other platinum group metals. All were present as the chlorides and of the valences indicated. The theoretical factor 0.2669 was used in calculating the palladium content.

In addition to the other platinum metals, separations from many of the more common metallic ions are readily possible. Various salts were added to the palladous chloride solutions to give the concentrations of metallic ions indicated in Table III. Where antimony was present the acid concentration was about 7 per cent by volume and approximately 1 gram of tartaric acid was added to keep the antimony in solution. With this exception the regular procedure was used without modification and the results were calculated using the theoretical factor 0.2669.

In the Presence of Anions. Determinations of palladium in the presence of various anions are also readily feasible, as Table IV shows. The procedure was the same as for a pure palladium sample and the results were again calculated by means of the factor 0.2669.

Table III. Separation of Palladium from Metals

Palladium Present (as $PdCl_2$)	Metals Added	Palladium Found	Difference
Gram	*Gram*	*Gram*	*Mg.*
0.0227	0.1 each of Fe^{+++}, Co^{++}, Cu^{++},	0.0227	0.0
0.0227	Ni^{++}, Mn^{++}, and Hg^{++}	0.0227	−0.1
0.0227		0.0227	0.0
0.0300	0.1 each of Cu^{++}, Cd^{++}, Bi^{+++},	0.0298	−0.2
0.0300	Hg^{++}, Sn^{++++}, Sb^{+++}, Zn^{++},	0.0300	0.0
0.0300	and Al^{+++}	0.0300	0.0
0.0300		0.0300	0.0
0.0300		0.0299	−0.1
0.0186	0.1 each of Th^{++++}, Ti^{++++},	0.0186	0.0
0.0186	Zr^{++++}, Mn^{++}, and Cr^{+++}	0.0187	0.1
0.0300		0.0300	0.0
0.0300		0.0301	0.1
0.0186	0.1 each of Ca, Sr, Ba, Mg, Na,	0.0187	0.1
0.0186	and K	0.0186	0.0
0.0300		0.0300	0.0
0.0300		0.0299	−0.1

Table IV. Separation of Palladium from Anions

Palladium Present (as $PdCl_2$)	Negative Ions Added	Palladium Found	Difference
Gram	*Gram*	*Gram*	*Mg.*
0.0186	1.0 of PO_4^{---}, 0.2 each of	0.0185	−0.1
0.0186	AsO_4^{---}, and VO_3^{-}. 0.1 each	0.0186	0.0
0.0186	of MoO_4^{--} and WO_4^{--}	0.0186	0.0
0.0186		0.0186	0.0
0.0300	0.2 each of BO_3^{---} and SeO_3^{--}	0.0300	0.0
0.0300		0.0300	0.0
0.0300		0.0300	0.0

Interfering Ions. Gold was found to be partially reduced to the metal by the reagent and the direct separation of gold and palladium by this method is not possible. Ceric ions yield a precipitate with the oxime and hence also interfere. Those ions which form insoluble chlorides are also precipitated and hence must be removed before the determination of palladium is attempted.

Table V. Determination of Palladium in Presence of Nitrate and Sulfate

Palladium Present	$NaNO_3$ Added	Na_2SO_4 Added	Palladium Found	Difference
Gram	*Grams*	*Grams*	*Gram*	*Mg.*
0.0300[a]	2	0	0.0300	0.0
0.0300[a]	2	0	0.0301	0.1
0.0300[a]	2	2	0.0300	0.0
0.0300[a]	2	2	0.0300	0.0
0.0300[a]	10	0	0.0299	−0.1
0.0300[a]	10	0	0.0299	−0.1
0.0300[a]	0	10	0.0301	0.1
0.0300[a]	0	10	0.0300	0.0
0.0300[a]	5	5	0.0301	0.1
0.0300[a]	5	5	0.0300	0.0
0.0122[b]	0	1	0.0122	0.0
0.0122[b]	0	1	0.0122	0.0
0.0122[b]	0	1	0.0121	−0.1
0.0139[c]	1	0	0.0139	0.0
0.0139[c]	1	0	0.0140	0.1
0.0139[c]	1	0	0.0139	0.0

[a] Palladium present as palladous chloride.
[b] Solution of palladous sulfate taken, precipitated and let stand for 2 hours in absence of chloride. Then 1 gram of sodium chloride added and determination completed and calculated according to regular procedure.
[c] Solution of palladous nitrate taken, precipitated and allowed to stand for 2 hours in absence of chloride. Completed as in [b].

Precipitation of a Complex Palladous Sulfate and Nitrate. When the formula of the palladous chloride–beta-furfuraldoxime precipitate had been established, it became of interest to ascertain whether analogous compounds were formed from palladous sulfate and palladous nitrate with the reagent. It was found that a precipitate could be readily obtained in each case, and several grams of each were prepared by direct precipitation of the palladous sulfate and palladous nitrate solutions. After washing and drying to constant weight, these precipitates were subjected to analysis, with the results indicated below. The same procedures of analysis were used as with the chloride precipitate, although more difficulty was encountered in the combustion.

Analysis of Palladous Sulfate Complex. Found: C, 37.34; H, 2.37; N, 8.85; Pd, 16.12; SO_4, 14.80. Calculated for $C_{20}H_{20}N_4O_8PdSO_4$: C, 37.12; H, 3.12; N, 8.65; Pd, 16.50; SO_4, 14.84. This composition corresponds reasonably well to the formula $Pd(C_4H_3OCHNOH)_4SO_4$, which is different from the compound formed with palladous chloride.

Analysis of Palladous Nitrate Complex. Found: C, 41.81; H, 3.30; N, 10.42; Pd, 19.47. Calculated for $C_{20}H_{18}N_4O_8Pd$: C, 43.75; H, 3.30; N, 10.20; Pd, 19.44. This composition agrees fairly well with the formula $Pd(C_4H_3OCHNOH)_2(C_4H_3OCHNO)_2$, which would indicate still a third type of compound.

The evidence in support of the formulas given above is not conclusive. A complete investigation was not made, since it

soon became apparent that neither precipitate was well suited for the quantitative estimation of palladium because of solubility.

Determination of Palladium in Presence of Nitrate and Sulfate. It was essential to determine whether sulfate and nitrate would interfere in the determination of palladium as the chloride complex. Using the procedure already described and adding varying amounts of sulfate and nitrate, the first ten results given in Table V were obtained. The last six were obtained in a somewhat different manner.

Table V shows that even in the presence of large amounts of nitrate and sulfate the procedure gives accurate results, and that even though the nitrate and sulfate complexes are first formed they are rapidly transformed into the chloride compound upon the addition of chloride ion, indicating that the latter is the least soluble.

Summary

A method for the gravimetric determination of palladium involves the precipitation of the compound $Pd(C_4H_3OCH\text{-}NOH)_2Cl_2$ with beta-furfuraldoxime. It is shown to give accurate results for amounts of palladium ranging from 2 to 30 mg.

By this method palladium may be directly determined in the presence of platinum, ruthenium, rhodium, iridium, iron, cobalt, nickel, copper, manganese, mercury, zinc, aluminum, antimony, bismuth, tin, cadmium, calcium, strontium, barium, magnesium, sodium, potassium, chromium, thorium, titanium, zirconium, molybdate, vanadate, tungstate, phosphate, arsenate, borate, selenite, sulfate, and nitrate ions.

The determination of palladium in the presence of gold, silver, mercurous, lead, and ceric ions is impossible.

The method is believed to have three advantages over the familiar dimethylglyoxime procedure: The precipitate has a higher molecular weight, and hence lower palladium content; as the reagent, beta-furfuraldoxime, is water-soluble, there is no danger of the reagent precipitating out; and furfuraldoxime precipitate is much easier to handle in filtration.

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Semiautomatic Fractionation

A Rapid Analytical Method

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A still head with a constant take-off rate has been developed for a laboratory column. Using the packed column described, an analysis by fractionation may be accomplished in approximately one hour, with sufficient accuracy for most plant control purposes. The analytical still is semiautomatic in operation, one man being able to operate as many as five stills simultaneously.

THE requirements of analytical methods used in routine control of a continuous chemical process are distinct from those of methods used in research and development work. It is important that any routine analysis selected give necessary and sufficient data for routine plant control. In addition, the test should consume as little time as possible, so that rapid results may be obtained. The simplest technique is usually best for the control laboratory and least likely to involve serious errors by the operators.

In a petroleum refinery, still performance can usually be determined by simple physical tests on the still products: specific gravity, viscosity, color, and boiling range. These properties sufficiently define the relatively wide-boiling bands of similar hydrocarbons found in a finished petroleum product. In a chemical plant making careful separation of close-boiling and often homologous hydrocarbons, however, these tests are unsatisfactory for still control. Only occasionally can satisfactory still performance be defined in terms of product density, refractive index, or by simple chemical test, and the common "boiling range" gives only a vague idea of the percentage composition of a mixture.

In the absence of simple analytical controls it is most reasonable to rely on analytical fractionation by distillation, thereby using the same method for analysis that is used for separation in the plant process. But an analytical fractionation is customarily time-consuming and requires more or less constant attention while running.

Thus, when routine analysis by distillation was deemed necessary for chemical plant control, it became imperative to develop a method for analytical fractionation sufficiently accurate and rapid for the needs of still control, and requiring a minimum of man power for operation. (By continuous distillation cuts are made which vary in composition from C_3 hydrocarbons, through C_4 and C_5 olefins and diolefins, benzene, toluene, and xylene, to aromatic mixtures boiling as high as 300° C.) For this purpose the analytical still shown in Figure 1 was constructed, incorporating the novel features of a constant product take-off rate and automatic temperature recording.

Apparatus

The refluxing distillate drains from the bulb condenser into cell *K*, first filling *K* (capacity 2 to 3 ml.) and then overflowing

back to the fractionating column. At 30-second intervals the 50-ohm solenoid magnet, *A*, is activated by an electric timer. This attracts the iron core in the glass tube extending down through the bulb condenser and connected with a glass dumbbell in *K*. The dumbbell is thus raised long enough to drain the contents of *K* into the product take-off line, while the upper portion of the dumbbell seals the top of *K* and prevents additional liquid from entering. The dumbbell then drops into its former position, sealing the product take-off line, while the reflux fills *K* and overflows as before.

The period when the magnet is activated may be lengthened to permit more viscous distillate to drain from *K*, without altering the reflux ratio or take-off rate. The product taken off each cycle is constant to within 0.05 ml., and as this error shows no definite trend through the distillation, it is well within the limits of accuracy of the method.

Funnel *E*, supported by glass prongs, acts as a reservoir, keeping the column packing wet during the take-off period.

Resistance thermometer *B* is connected to a recorder which plots vapor temperature. Pressure is read on a closed-end manometer connected at *C*. Tie rod *D* strengthens the still head and reduces breakage. The vacuum-jacketed fractionating column has glass bellows *F* to allow thermal expansion. A screen resting on glass prongs at *H* supports carding teeth column packing. Three-way stopcock *G* permits venting receiver to air and also connects to a 19-liter (5-gallon) surge bottle, vacuum control, and pump.

The bulb condenser design, consisting of bulbs of decreasing size, is particularly efficient. Most of the vapor is condensed in the larger bulbs, where the large openings prevent choking, while the upper and smaller bulbs condense the last traces of vapor and prevent carry-over.

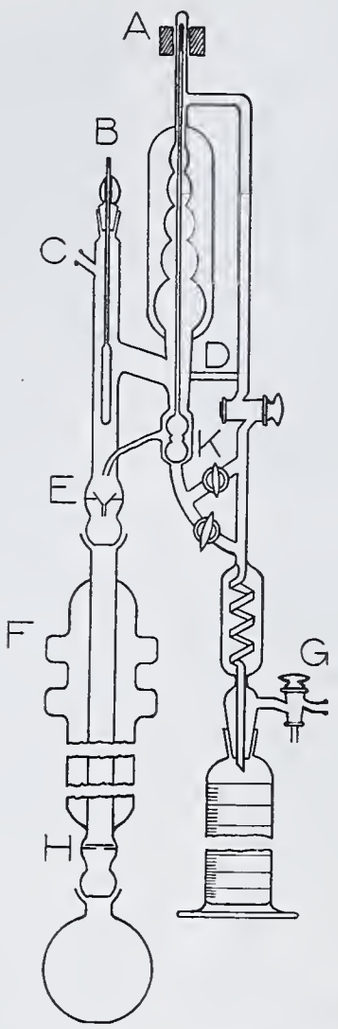

FIGURE 1. ANALYTICAL STILL

The still, using a conventional packed column, is operated at approximately maximum throughput. The take-off device takes a definite amount of product at regular time intervals. The overhead temperature is recorded on a chart moving at a uniform rate, so that a curve of temperature against distillation time is plotted. Since the rate of production is constant, the same curve represents overhead temperature *vs.* per cent distilled. With this method of operation the reflux ratio varies with the throughput, which is kept so high that the ratio is always at least 5 to 1.

With this apparatus, the automatically recorded curve permits consistent and reasonably accurate analysis of the hydrocarbon mixture into its component parts. The still column selected must be efficient enough for the most difficult separation encountered in the given system of analysis, and must be of sufficient capacity and throughput rate to process the required amount of sample within a given time, which for the author's purpose is one hour or less.

A vacuum-jacketed, silvered glass column was chosen having the following dimensions and characteristics:

Inside diameter	3.1 cm. (1.25 inches)
Packing length	62.5 cm. (25 inches)
Packing material	4 × 6 mm. iron carding teeth
Efficiency under total reflux	10 plates
Throughput at 760 mm.	4 liters per hour
Throughput at 40 mm.	1.5 liters per hour
Operating holdup	60 to 75 ml.

(This column, as well as the still head, is supplied to the Ugite Sales Corporation specification by Ace Glass, Inc., Vineland, N. J., which also supplies glass still pots with interchangeable ball and socket joints, glass vacuum control apparatus to Ugite Sales specifications, etc.)

For fractionation of materials boiling between propane (−44.5° C.) and benzene (79–81° C.), the still is placed in an insulated box which is cooled either by packing the top with solid carbon dioxide or by mechanical refrigeration to prevent superheating of vapors, and toluene is circulated through the condenser, after being cooled by passing through a coil immersed in toluene and solid carbon dioxide. A box temperature of −25° C. and condenser inlet temperature of −65° C. may be obtained, and are found satisfactory for the propane-butane separation. An electric heater is used to supply heat to the still pot. For samples boiling above room temperature, water is used in the condenser and no insulating box is used to enclose the still. A Bunsen burner is normally used for heating.

Since the required accuracy of temperature reading could not be approached by thermocouples unless individually calibrated, a six-point TAG recording potentiometer together with resistance thermometers was obtained from the C. J. Tagliabue Co., Brooklyn, N. Y., to serve a battery of six analytical stills. A special scale of −20° to +150° C. supplied the range required, and a chart speed of 45 cm. (18 inches) per hour gives an optimum curve dimension.

With this column 200 ml. of sample may be distilled at 40-mm. pressure, at 5 to 1 reflux ratio, in 40 minutes after reflux has been established. A 200-ml. sample is used as standard, although a larger charge is employed in certain analyses to permit increased accuracy.

Procedure

Using a sample which is a mixture of benzene, toluene, and xylene, 200 ml. are measured into the still pot at a given temperature, normally room temperature. Sufficient heat is applied to the pot to start the sample refluxing in the column, and then to attain maximum throughput in the column, which occurs just below the flood point. Incipient flooding in the column may be taken as an indication that maximum efficiency is being obtained.

After reflux has been established (this requires 10 to 15 minutes) the vapor temperature-recording device is allowed to come to a constant (minimum) temperature (requiring an additional 5 to 10 minutes) and the switch is then thrown, allowing the electric timer to activate the product take-off device at regular intervals. The distillation is continued until an overhead temperature of 135° C. is reached, when the distillation is stopped. The volume of the distillate is observed and written directly on the distillation curve, together with the distillate temperature if it varies from the temperature of the charge. All volumes may then be corrected to a common temperature if necessary, before calculation of results.

It has been found advantageous to record also the volume distilled at the several significant temperatures or "cut points" selected for calculation purposes, in this case 81°, 109°, 111.5°, and 135° C.

Figure 2 shows the distillation curve plotted by the vapor temperature recorder. This curve shows directly per cent distilled *vs.* vapor temperature. The calculation is made as follows:

1. All the material between 79° and 81° C. is considered benzene.
2. All the material between 109° and 111.5° C. is considered toluene.
3. All the material above 135° C. is considered xylene.
4. The area under the intermediate portions of the curves (81° to 109° and 111.5° to 135° C.) is integrated graphically and the average boiling point of the intermediate fraction is thus determined. Reference to a prepared table will give the percentage composition corresponding to any average boiling point. This table (or curve) may be taken from the literature or calculated from the McCabe-Thiele diagram for the alpha of the two components under consideration, and should be available to the analyst making routine calculations.

TABLE I. ANALYSIS OF SYNTHETIC MIXTURES

Test Mixture No.	Actual Composition						Test Analysis					
	C_4	C_5	C_6	C_7	C_8	$>C_8$	C_4	C_5	C_6	C_7	C_8	$>C_8$
	%	%	%	%	%	%	%	%	%	%	%	%
1	..	3.8	91.3	4.9	..	..	..	3.1	91.5	5.4	..	..
2	..	..	0.7	99.3	..	..	..	..	0.7	99.3	..	..
3	..	..	..	..	3.3	96.7	..	..	..	..	4.5	95.5
4	..	..	1.8	97.0	1.2	..	..	..	1.6	96.6	1.8	..
5	..	..	..	0.8	99.2	..	..	..	..	0.8	99.2	..
6	..	20.6	79.4	..	..	..	..	22.0	78.0	..	..	..
7	..	99.0	1.0	..	..	..	..	98.5	1.5	..	..	..
8	..	0.5	99.5	..	..	..	..	0.6	99.4	..	..	..
9	98.0	2.0	..	..	..	..	97.5	2.5	..	..	..	..
10	0.5	99.5	..	..	..	..	0.4	99.6	..	..	..	..

C_4. Mixture of butylenes and butadiene (1,3)
C_5. Mixtures of isoprene, piperylene, and pentanes
C_6. 2° benzene C_7. 2.5° toluene C_8. 5° xylene
$>C_8$. Mixture of hydrocarbons boiling above 147° C.

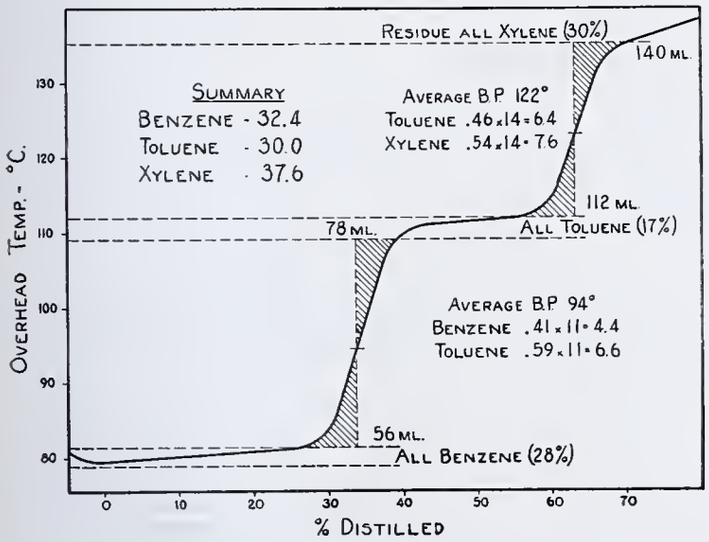

FIGURE 2. DISTILLATION CURVE

5. The several portions of each component are added, deductions are made for any flux oils added, and the finished result is obtained.

The two or more components may be present in nearly equal or in very different proportions. The operating hold-up of the still selected is 60 to 75 ml. This means that if the highest boiling component comprises less than 30 per cent of the total sample, it will be insufficient in quantity to force all the lower boiling material overhead. Therefore, the use of a variety of flux oils according to the needs of the individual analysis is incorporated as an integral part of this method. For example, a benzene stream containing 0 to 10 per cent of toluene has 125 ml. of C. P. toluene flux added to the standard 200-ml. sample, and the distillation is carried to the initial boiling point of C. P. toluene, 109° C. An overall material balance is unnecessary if normal precautions are maintained to avoid distillate losses; the total material boiling below toluene subtracted from the 200-ml. total sample gives the toluene content of the sample.

A flux oil is also required in the case of a small quantity of component of intermediate boiling range. Analysis of mixtures of pure compounds depends on the presence of any intermediate component in sufficient quantity to establish a well-defined distillation "flat" in the boiling range of the pure component.

For example, consider again the benzene-toluene-xylene mixture.

In the exceptional case when toluene is present in this system in insufficient quantity to establish a toluene flat, a correct analysis by fractionation cannot be obtained, for, in the extreme case where no toluene is present, the distillation curve will still pass through the toluene boiling range and the method outlined above will show some toluene to be present. Therefore, sufficient C. P. toluene is added to provide a toluene flat. The analysis may then be performed and the calculation made in the usual way, subtracting the added toluene from the final result.

If, instead of single compounds, it is required to distinguish between groups of compounds in succeeding boiling ranges, a somewhat more empirical method must be employed. A cut point is selected such that the amount of distillate coming over below the cut point will be equivalent to the total amount of the lower boiling group present. The cut points and method of calculation for any type of sample can best be determined by experiment, either with synthetic mixtures of pure compounds, or with typical samples, accurately analyzed by independent means. This is a method for the oft-repeated routine analysis, where the general nature of the sample to be analyzed is known, and where rapid results are important.

There are three cases where a larger amount of sample than the standard 200-ml.-charge may be used to advantage:

1. Where the sample containing a small quantity of low-boiling material is being analyzed for the sole purpose of determining the quantity of the low boiler, a large charge may be distilled up to the point where all the low boiler has come off in less time than it takes to distill a charge of standard size to completion. Hence, a 400-ml. charge is customarily used and greater accuracy is thereby obtained.
2. Where a special test requiring more than routine accuracy is to be run or a large number of fractions is to be determined

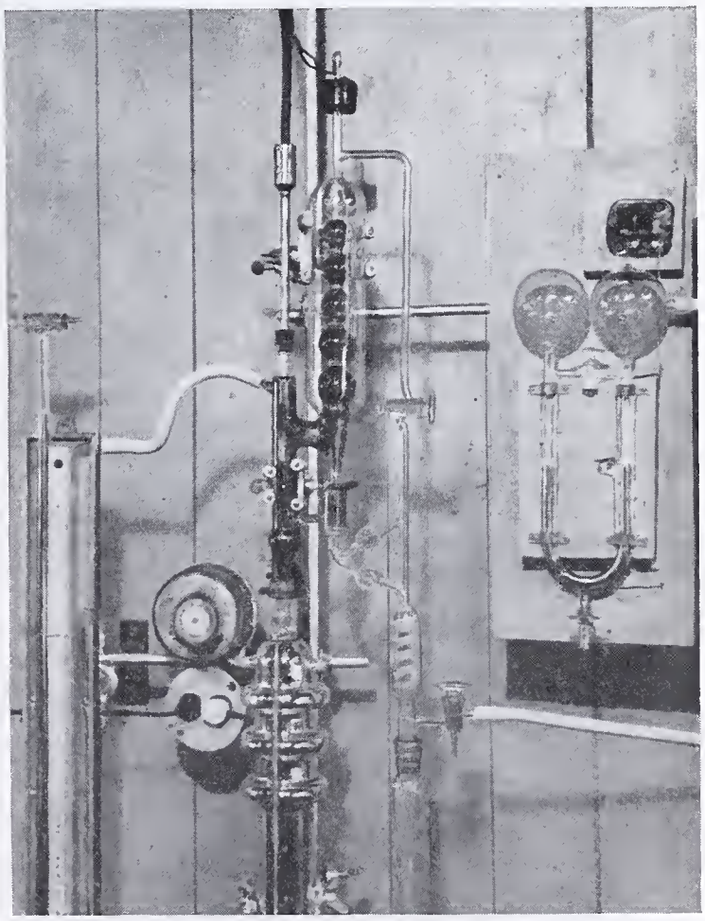

FIGURE 3. FRACTIONATION APPARATUS

in a cut of wide boiling range, an increase in the charge size is desirable to sharpen the analytical tool. Samples of 300 to 2000 ml. have been used for this type of work, depending on the time interval available and the complexity of the separation.

3. Where it is desired to separate two close-boiling groups and consistent results cannot be obtained using the standard charge, a larger charge may be required to decrease the effect of column hold-up, even though speed must be sacrificed.

Test Results

Table I gives the results of analysis of several synthetic mixtures, using the still and analytical methods described herein. The degree of accuracy shown in this table has proved to be dependable, in the main, under routine control laboratory conditions during a period of more than a year. The accuracy of this method cannot be compared to that of inorganic quantitative analysis, but where approximations of the order shown are sufficient, it has proved of value in getting results in a quick and relatively simple way.

Acknowledgment

The author wishes to acknowledge the helpful information and suggestions furnished by Edward H. Smoker, the work of Mark D. Snyder, Louis T. Lazzarini, and others of the Ugite Sales Corporation laboratory, and the cooperation of Floyd Hughes and others of Ace Glass, Inc., in fabrication of the apparatus. The photograph of the apparatus (Figure 3) by Theodore Shmanda, and the drawing of Figure 2, by Robert Kauffman, both of the Ugite Sales Corporation, are acknowledged with thanks.

The Film Balance

As an Analytical Tool for Biological and Food Research

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Surface films may be used to detect minute amounts of certain insoluble organic substances, and to identify unknown compounds when some knowledge of the homogeneity of the material is available. Data obtained from film pressure-area measurements are often of decisive value in the proof of the structure of complex organic molecules. Recent refinements in the technique appear to afford a method for determining the molecular weight of large molecules such as proteins, polymers, etc.

The most widely used tool in the study of insoluble films is the combination of the surface trough and balance. The vertical and horizontal balance types of film balances are in use in this laboratory and a detailed description and theoretical discussion of the former are given, with a discussion of the technique of their use and the limitations and precautions involved.

Accessory tools, such as the dark-field ultramicroscope with a cardiod condenser and apparatus for the determination of surface potential and surface viscosity, are described. Measurements of the permeability of films may be made. Temperature control is of great importance.

To illustrate the nature of the data obtained, application of the method of surface films to a number of recent diverse scientific problems is described.

AN OIL may be spread on water or mercury as a monomolecular or polymolecular film, but all films of the latter type, commonly designated as duplex, are unstable and change spontaneously into a monolayer and a lens (*6*).

Since films and membranes play a predominant role in biology, and are of fundamental importance in many lines of industry, it is essential to know not only what types of apparatus may be used to reveal their characteristics, but also what techniques are best suited. One of the most remarkable features of the film balance is that it is possible by its use to determine the length, breadth, and approximate molecular weight of certain types of large molecules by only an hour's work.

The volume of any pure oil which covers 1 sq. cm. of the surface of water is given by the thickness of the film. If the thickest monolayer of a pure organic substance which can be obtained by compression is about 4.5 Å., the molecules lie flat on the surface. With thicker films the interpretation should be based upon various types of data. Often the position of a polar group in molecules of compounds such as vitamin D (calciferol), or in other sterols, can be shown by the film balance either to disagree or to agree with that given by organic chemists who have studied their structure. In cases of disagreement it has been necessary to change the earlier structural formulas.

The Film Balance

The most valuable of all the types of apparatus for the study of oil films is known as the film balance, or filmometer, and this is used to determine the film pressure, π, at any given molecular area, σ. The pressure of any film is, by definition, the difference between the surface tension of the clean surface of the subphase, γ_0, and that of the subphase when covered by the film, γ_f, or

$$\pi = \gamma_0 - \gamma_f \qquad (1)$$

The justification for the designation of the difference between two tensions as a pressure lies in the fact that two-dimensional $\pi\sigma$ and $\pi\sigma T$ diagrams (Figure 1) resemble very closely three-dimensional P-V and P-V-T diagrams, respectively. Thus, in the two-dimensional gas law, $\pi\sigma = nkT$, the value of k is that of the Boltzman constant of a three-dimensional gas ($k = 1.371 \times 10^{-16}$ erg deg.$^{-1}$) which plays an important part in the theory of two-dimensional systems.

Thus, from Equation 1, any apparatus, by means of which both of these surface tensions may be determined, could be considered as a film balance. The first of these was used in 1891 by Pockels (*12*). The apparatus consisted of a long rectangular trough, filled with water to the brim. The surface of the water was made clean by "sweeping" or "scraping" the surface by the use of a barrier which consisted of a

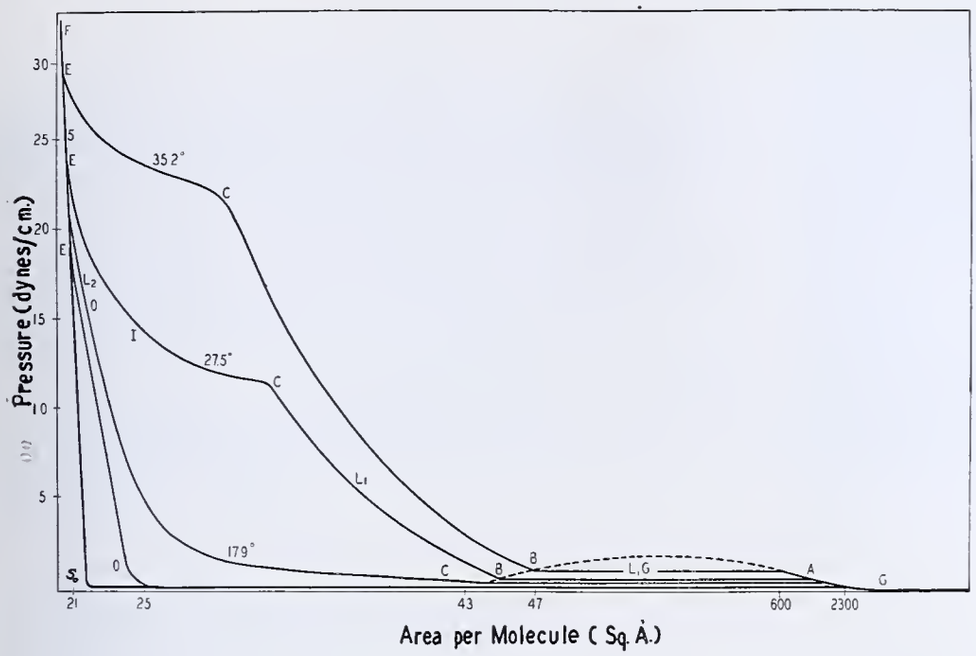


FIGURE 1. GENERAL PHASE DIAGRAM FOR MONOLAYERS ON A LIQUID SUBPHASE

G. Gas
A. Transformation of liquid expanded (L_1) phase begins
L_1G. Two-phase region
B. Transformation to L_1 completed
BC or L_1. Liquid expanded
CD or *I*. Intermediate phase
DE or L_2. Liquid condensed phase
EF or *S*. Solid phase

The presence of calcium ions, when the film consists of a long-chain paraffin acid, such as stearic acid, extends *FE* to very low pressure where the transition, S_0, is between low-pressure vapor and solid film.

transverse sheet of metal 1.5 cm. wide. In the earliest work the surface tension of both the clean and the film-covered water was obtained by the determination, by means of an ordinary balance, of the force necessary to pull from the surface either a small disk (6 mm. in diameter) or a large ring (114 mm. in circumference).

The measurements of film pressure may be made more exact if the difference between the two surface tensions is determined directly, as in the film balance of Lord Rayleigh (*13*), which makes use of the Wilhelmy slide.

This apparatus gave excellent results, since with a monolayer of stearic acid the area at which the film pressure begins to rise corresponded to 22.1 Å.^{2} at 15° C., while the most recent value, as determined in this laboratory, is about 21 Å.^{2}, when a minute amount of calcium ion is present in neutral water. This is presumably the kind of water used by Miss Pockels.

Modern Film Balances

A modern film balance is a device which measures directly the difference between two surface tensions, $\gamma_1 - \gamma_2$, commonly to determine $\gamma_0 - \gamma_f$, which (Equation 1) is defined as the film pressure, π. For such a balance the surface must be divided into at least two parts. The vessel which contains the liquid is usually designated as the "trough", though two separate vessels or troughs may be used if the film balance is of a type which allows such a separation.

VERTICAL FILM BALANCE. The simplest, the most generally applicable, and probably the most accurate film balance is that in which the surface tension exerts a downward pull upon the vertical face of a sheet of glass or other suitable material, as in the surface tension method of Wilhelmy. On account of its inherent difficulties this surface tension method is not in use, but it was found by Harkins and Anderson (*7*) that these difficulties disappear if no attempt is made to determine the surface tension (γ) itself, but the principle of the vertical pull is used in the development of a balance which measures π directly. The vertical pull may be applied in two ways, one of which is described below, and is illustrated in Figure 2.

A clean slide of glass, quartz, or platinum, or any other material which gives a zero contact angle, is suspended in the water in the trough from one arm of an ordinary balance as shown. The surface of the water is swept carefully both before and after the slide is dipped into the water. If the film is insoluble it is then spread on the surface, and the film pressure, π, is given directly by the deflection of a beam of light as read on a scale at a distance of several meters.

The vertical film balance exhibits the following advantages over the horizontal type commonly used:

It is much cheaper for any given accuracy.

It is much simpler and may be installed in a few minutes, since an ordinary balance and a microscope slide give results of a high degree of accuracy.

It is specially useful in studies of the effects of metal ions, since the slide, trough, and barriers can all consist of quartz, so that the solution and its surface come in contact with quartz alone.

In a simple form it will give the film pressure of a soluble film, while the use of the horizontal types involves the very elaborate movable partition of the PLAWN trough of McBain.

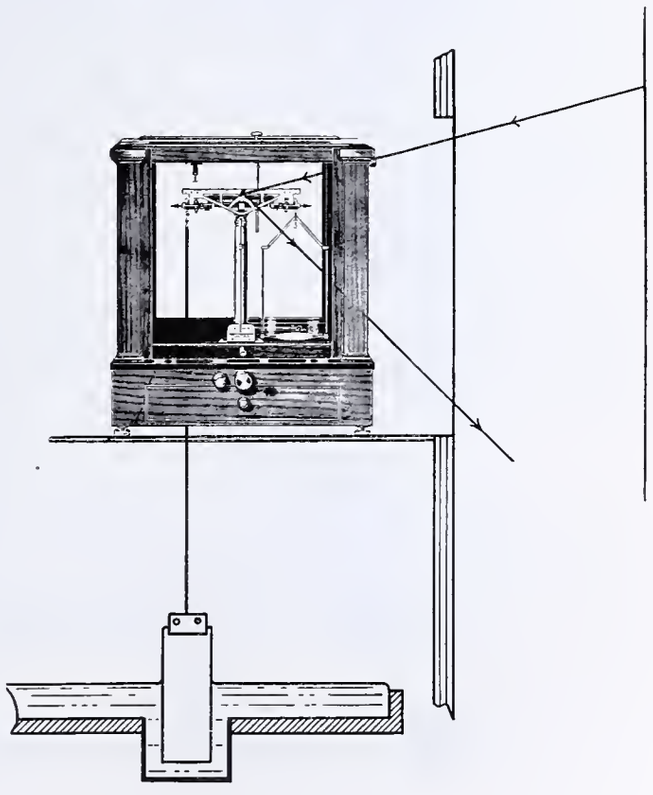


FIGURE 2. ARRANGEMENT OF VERTICAL TYPE OF SURFACE BALANCE FOR RESEARCHES WITH SOLUBLE AND INSOLUBLE FILMS

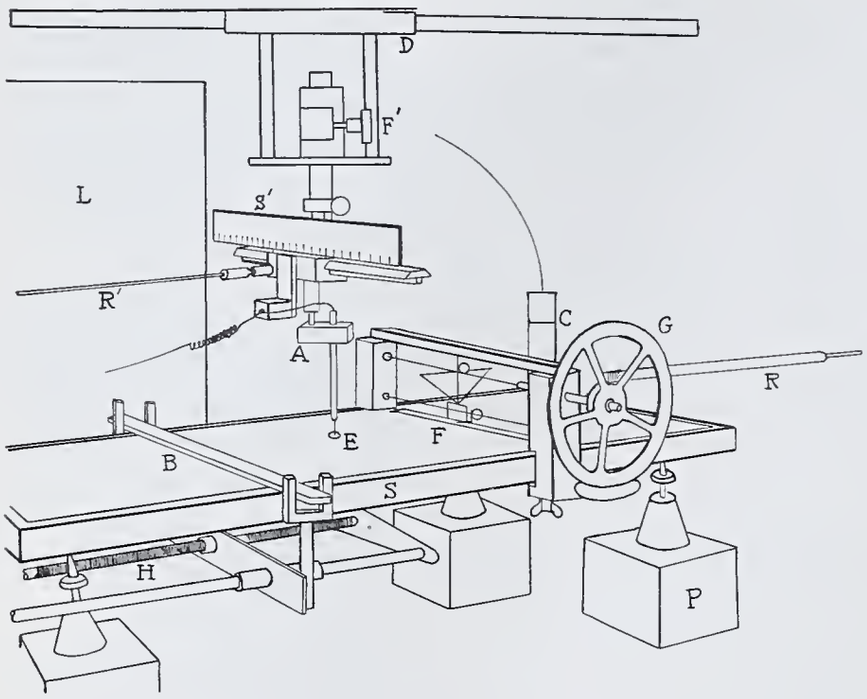


FIGURE 3. SCHEMATIC DIAGRAM OF HORIZONTAL TYPE OF SURFACE BALANCE FOR RESEARCHES WITH INSOLUBLE FILMS

A. Amber block supporting electrode
B. Compressing barrier
C. Calomel half-cell
D. Rack for horizontal movement of contact potential electrode
E. Silver electrode coated with polonium for measurement of surface potential
F. Movable float of torsion balance
G. Divided circle
H. Calibrated lead screw
P. Paraffin blocks
R, R'. Control rods extending to outside of housing
S. Brass, stainless steel, or silica trough
S'. Scale for electrometer

It is very easy to give the method a very high sensitivity, since all that is required is to make the slide or slides very thin. With wide sheets of glass of the thickness of microscope cover glasses, surface pressures as low as 0.003 dyne per cm.$^{-1}$ may be measured. With thinner sheets 0.001 dyne per cm.$^{-1}$ or even less could be detected. This should make it feasible to determine the molecular weights of certain film-forming substances by the use of the two-dimensional gas law.

The vertical film balance seems to require less elaborate supplementary equipment for the continuous recording of the film pressure, as in the apparatus of Dervichian, than is needed in conjunction with the horizontal type.

If the slide or sheet is w cm. wide and t cm. thick, the perimeter, p, is $2\ (w + t)$ and the downward pull of the surface tension is $2(w + t)\ \gamma$ or $2p\gamma$. Let W be the apparent weight in grams of the slide partly immersed in water. If the surface tension is reduced by the spreading of a film the slide rises, but if W is kept constant the decrease in the downward pull, F_d, of the surface tension must be compensated for by a decrease in the buoyancy, F_u, of the submerged portion of the slide,

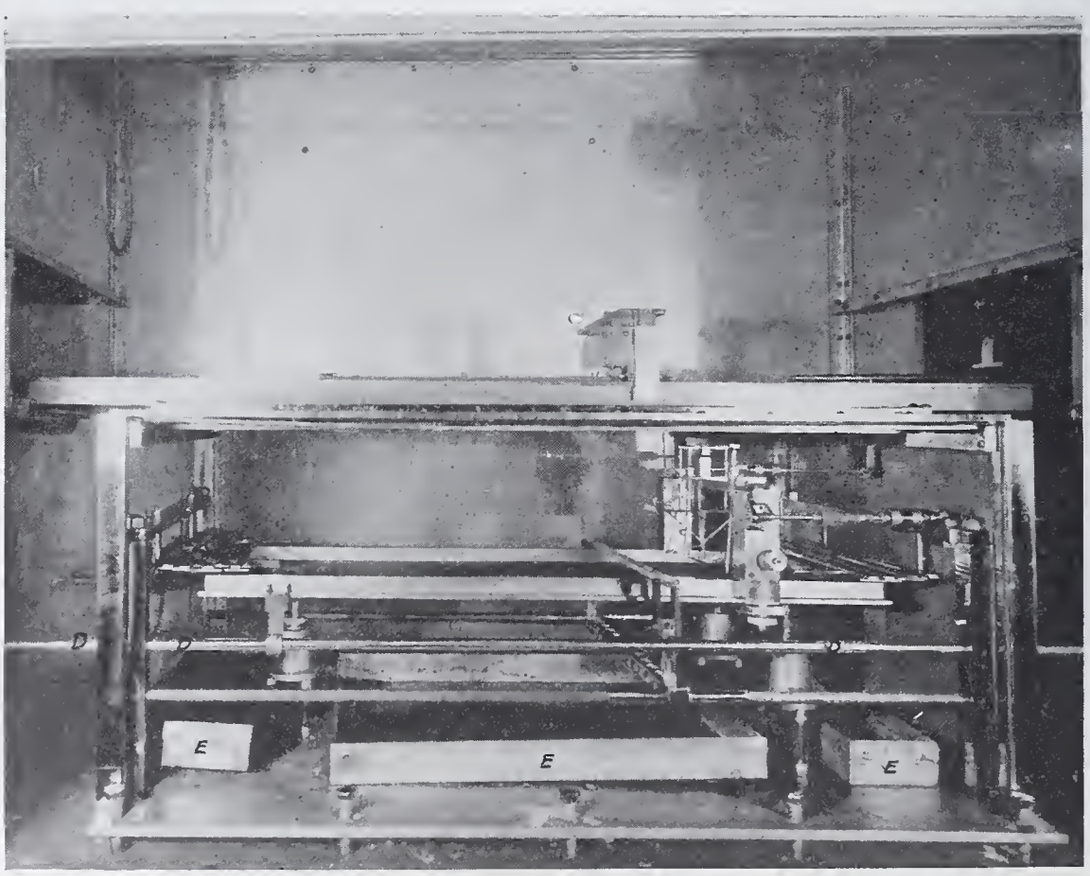


FIGURE 4. TROUGH AND FILM BALANCE INSIDE GLASS BOX WITH FRONT REMOVED

Outer box of metal is air thermostat
D. Sweeping mechanism
E. Pan of water to ensure saturation of air inside glass box

or,

$$\Delta F_d = \Delta F_u$$

$$2p\,\Delta y = g\,ptw\,\Delta h$$

where g is the acceleration of gravity, and h is the change in height of the slide measured by the use of a cathetometer. In practice it is much simpler to mount a galvanometer mirror with its center in the axis of rotation of the beam, and to observe the deflection of the beam by a telescope and illuminated scale, or to use a beam of light and observe its deflection on a scale. For a segment of a circular scale, Δh is proportional to ΔS, the change in the scale reading, or

$$-p\ \Delta\gamma = k\ \Delta S$$

where the value of the constant k is determined by observing the values of ΔS associated with different weights on the balance pan with the slide immersed in water which has a clean surface.

$$k = -g\Delta M/\Delta S$$

Thus the reduction in the surface tension, $-\Delta\gamma$, which is the film pressure π, caused by spreading and compressing the film, is

$$\pi = -\Delta\gamma = k\Delta S/2p$$

In the determination of the film pressure of a soluble substance the trough is filled with pure water, the surface is swept, and the scale reading of the beam of light is set to zero. Then either of two procedures may be followed:

1. The solute is added and the solution stirred until the concentration is uniform throughout. The surface is swept again, and the scale reading, taken when equilibrium is attained, gives the film pressure.
2. The pure water is removed from the trough, and replaced by exactly the same volume of solution. The equilibrium scale reading gives the film pressure, as in (1).

FIGURE 5. PIPET

Surface-active solutes are often present at such great dilutions that no corrections for differences of density are needed, but with more concentrated solutions such corrections should be applied.

In a second type of vertical film balance a slide, hung from one end of the balance beam, is suspended in clean water, and another slide, from the other end of the beam, is suspended in the solution. The details of this method will be given in a separate paper.

HORIZONTAL FLOAT BALANCE. In the horizontal float balance as designed by Langmuir the two surface tensions, γ_0 and γ_f, pull on the two edges of a movable floating barrier, and the difference in the tension is determined by an ordinary balance adapted to measure a horizontal force. The film is kept from flowing around the two ends of the float by two jets of air, which prevent this balance from being an accurate instrument. In a modification devised by Adam the ends of the float are joined to the walls of the trough by very thin strips of gold or platinum, set edgewise in the surface, and these prevent the film from passing.

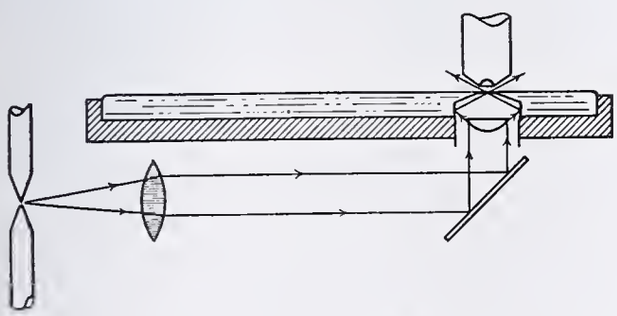


FIGURE 6. SCHEMATIC ARRANGEMENT OF ULTRAMICROSCOPE FOR EXAMINATION OF INSOLUBLE FILMS

FIGURE 7. APPEARANCE OF COLLAPSED MONOMOLECULAR FILM OF EGG ALBUMIN UNDER THE DARK-FIELD ULTRAMICROSCOPE

Trough and Accessories (*9b*)

Figure 3 shows a horizontal type film balance mounted on a trough, in the early form used by Harkins and Freud.

The film is spread between the movable barrier, B, and the float, F, of the torsion balance. The film is compressed by a calibrated screw, H, and the change of area is given by a counter which gives the number of revolutions of the screw. The balance is brought back to a zero reading by turning the rod R. All operations are carried out by controls which lie entirely outside the air thermostat which surrounds the apparatus.

The electrode, E, used to determine the surface potential, is of silver coated with polonium. This is connected through a Compton electrometer to a calomel cell whose liquid arm dips into the solution in the trough. The apparatus for moving the electrode is that used by Harkins and Fischer (*8*) to detect any lack of homogeneity in the film. The electrode is moved across the surface by turning rod R', and the position is read on a scale, S', outside the thermostat. The electrode is raised or lowered at F', and the bearing at D operates very smoothly.

SWEEPING MECHANISM. The surface of the water in the trough is cleaned by sweeping with long square bars, 0.6 cm. (0.25 inch) in cross section, of paraffined stainless steel, glass, or quartz. These are considerably longer than the width of the trough, and four to six clean bars are kept stored on brackets at each end of the trough. Any one of these may be picked up from the brackets, and used to sweep the surface by a mechanism which is operated by controls outside the thermostat (Figure 4).

Spreading the Film

The technique whereby the substance is placed on the surface to form a monomolecular film varies with the nature of the film. Most fats, if they do not contain too high a proportion of polar groups, may be spread from solutions in pentane and hexane mixtures or in benzene. When the polar nature of the molecule becomes so high that the substance is not soluble in pentane-hexane mixtures, the spreading may be effected from other mixed solvents. Mixtures of hydrocarbon and ethyl alcohol, and hydrocarbon and chloroform, have been used. A mixture of water and isoamyl alcohol has proved suitable for the formation of monomolecular films of proteins. Aside from its solvent power for the film-forming substance, the spreading liquid should possess a high

spreading coefficient and a high volatility. Preferably it should be insoluble in the underlying aqueous solution upon which the film is spread.

The accurate determination of the molecular area involves (1) weighing the pure solid or liquid to a sufficient percentage accuracy; (2) careful measurement of the total volume of the solution; and, most difficult, (3) an accurate knowledge of the volume of solution put on the surface of the water.

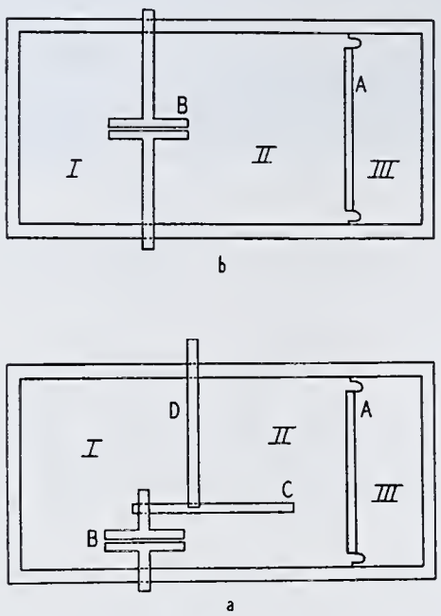


FIGURE 8. DISPOSITION OF VISCOMETER CANAL, PISTON, AND FILM BALANCE ON SURFACE TROUGH

I. Initially a clean water surface
II. Film-covered surface
III. Clean water surface

To accomplish item three a special volumetric pipet designed by Harkins is employed (Figure 5), since this is suitable for the use of an exact volume of a solution in a somewhat volatile solvent.

Dark-Field Ultramicroscope

Whenever there is any doubt as to the monomolecular nature of any film it is examined by a microscope, with a dark-field condenser screwed into the bottom of the trough and focused on the film (Figure 6), according to the technique of Zocher and Stiebel (*15*). The appearance of a partly collapsed film of egg albumin is exhibited in Figure 7. If the film is monomolecular the optical field is empty, except for dust particles which are easy to recognize.

Viscosity and Rigidity of Monolayers

The viscosity of a monolayer is often the property which is the most sensitive of all to a change of phase, and the viscosity and rigidity are among the most important properties of biological films and membranes.

CANAL VISCOMETER. The simplest method for the determination of the viscosity of a monolayer is to allow it to flow through a slit or a canal, B, between two areas, II and I (Figure 8). The difference of pressure between II and I is kept constant by moving barrier B in b, or D in a.

Area III represents the clean surface of water and A is the float of a horizontal type of film balance. It is obvious that the vertical type previously described serves as well. The pressure of film I may be kept constant by the use of a piston oil, but it is much better to use a movable barrier (not shown) which automatically keeps the pressure, π, constant. The barrier is moved by a device actuated by a film balance of the vertical type.

The correct theory of the canal viscometer, as given by Harkins and Kirkwood (*9*), is expressed by the following equation in which η is the viscosity coefficient of the film, η_0 is that of the subphase, a and h are the width and depth of the rectangular canal, α is the film pressure gradient $(f_2 - f_1)/l$, $\pi = 3.14159$ and A is the area flux.

$$\eta = \frac{\alpha a^3}{12A} - \frac{a\eta_0}{\pi}$$

The first term of this equation was developed earlier by Myers and Harkins (*10*), and shows that the power of the a^4 of the Poiseuille law for three-dimensional viscosity is reduced by unity to a^3 when the number of dimensions is reduced by unity. As is self-evident, the second term, which represents the effect of the viscosity of the subphase upon the flow of the film, contains the first power of the width, a, of the canal. The difficult point in the theory was that which gave a most simple result—i. e., that the denominator is π.

A slit may be used instead of a canal, but the theory of its use has not been developed correctly. A slit may be defined as a canal of negligible depth, h. The use of a slit was introduced by Bresler, Talmud, and Talmud (*2*). It has been shown by Nutting and Harkins (*11*) that the greatest difficulty in this method is that involved in the choice of the materials of the walls of the canal or slit, since some materials allow a slip of the film along the walls.

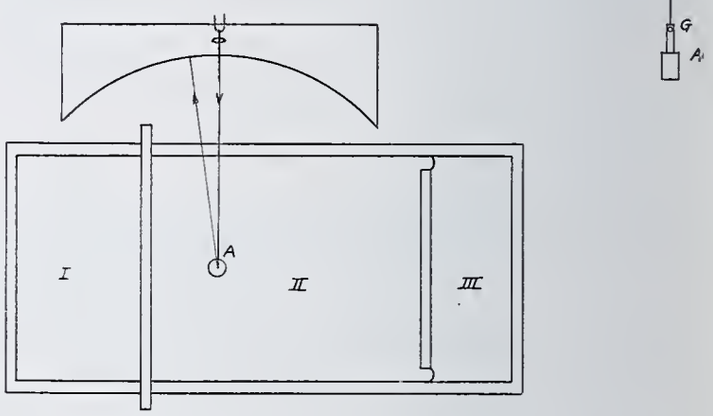


FIGURE 9. DISPOSITION OF TORSION OSCILLATION VISCOMETER

I and III. Clean water surface
II. Film-covered surface
A. Torsion pendulum bob supported by No. 36 phosphor-bronze wire

TORSION OSCILLATION VISCOMETER. An easier but much less sensitive method for determining the viscosity of a film consists of the use of a horizontal circular ring or disk, which merely touches the surface. The ring is supported by a torsion wire which is hung from a support above the film. In Figure 9 the support is shown above the trough, but obviously the figure of the trough is turned through 90°. The ring or disk carries a small mirror which focuses a beam of light on a scale of circular section and two air jets which act on a vane, fastened above the ring, set the system in vibration.

If the pressure on the film is kept constant the amplitude of the oscillation decreases with each successive swing, and the logarithm of the amplitude plotted as a function of the number of swings gives a straight line whose slope gives a viscosity, which is a composite of the viscosity of the film and that of the water associated with it. In the clean surface of water the slope of this straight line is smaller. If λ is the logarithm of the ratio of successive amplitudes and $\Delta\lambda$ gives the difference in the value of the logarithmic decrement between water and water covered by the film, then

$$\eta = \Delta\gamma_{10} \frac{2.3\,I}{2\,\pi\,P}\left(\frac{1}{a^2} - \frac{1}{b^2}\right)$$

where I is the moment of inertia of the ring and rigidly attached material, P is the period, a is the radius of the ring or disk, and b is that of an outer guard ring. It is apparent that the viscosity, η, obtained for the film has a value which depends upon a somewhat arbitrary method for making allowance for the dragging along of the water underneath the film by the motion of the film. Nevertheless the viscosities obtained in this way for liquid films exhibit just the logarithmic relation toward increase of pressure predicted by general theories of the liquid state (*1b*). In other respects η seems to represent almost perfectly the true viscosity of the film, even though it may have a magnitude which is somewhat different from that determined by use of a canal.

Torsion Rotation Viscometer. Fourt and Harkins (*4*) have determined the viscosity of films from the measurement of the torque required to maintain a uniform rate of rotation of the ring or disk described above. The shaft of an electric clock is used as the upper suspension, and the twist between the two ends of the torsion is measured by two beams of light reflected from mirrors mounted on the support of the wire and on the axis of the ring, respectively. These are focused on the same scale and give a single line when the shaft is not in rotation. The torsion constant, τ dynes per radian, may be determined by the method of oscillations. The torque may be obtained by turning the interval, t, between the passage of the two beams of light past a fixed mark.

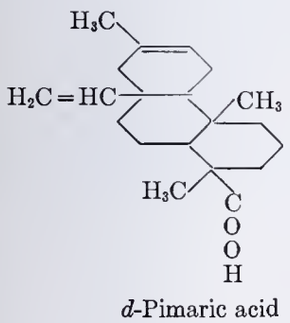
If the viscosity of the film is Newtonian, independent of the rate of shear, the equation for viscosity does not include a term for the rate of rotation of the ring. If Δt is the change in the interval produced by the film, the viscosity is given by

$$\eta = \frac{\tau\,\Delta t}{4\pi}\left(\frac{1}{a^2} - \frac{1}{b^2}\right)$$

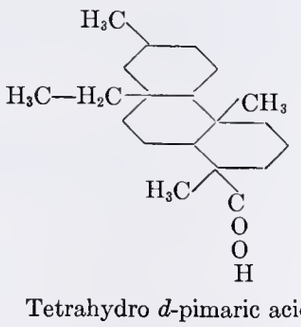
Anomalous viscosity or plasticity gives a dependence upon the rate of shear. In the later work the interval of time Δt was measured by a counter which was started when the first beam of light passed a photocell, and stopped when the second beam fell upon the counter. The method of the rotating ring should be used if the flow of the film is of the plastic type.

Application of Film Research

An illustration of the manner in which film data may be used in decision of molecular structure may be given in the case of *d*-pimaric and tetrahydro-*d*-pimaric acids,



d-Pimaric acid



Tetrahydro *d*-pimaric acid

as compared with cholesterol. In Figure 10 are given the surface pressure area and surface potential relations for each substance. At zero pressure the extrapolated molecular area of the saturated compound is 54 Å.², and of the unsaturated 57 Å.², while at the highest pressure the former is compressed to 47 Å.² and the latter to 43 Å.². Thus at a pressure of 5 dynes per cm.$^{-1}$ the molecular areas are equal (51 Å.²) while the compressibility of the monolayer of the unsaturated is much higher (0.0100) than that of the saturated compound (0.00681). The molecular area of these substances is slightly more than twice that of stearic acid.

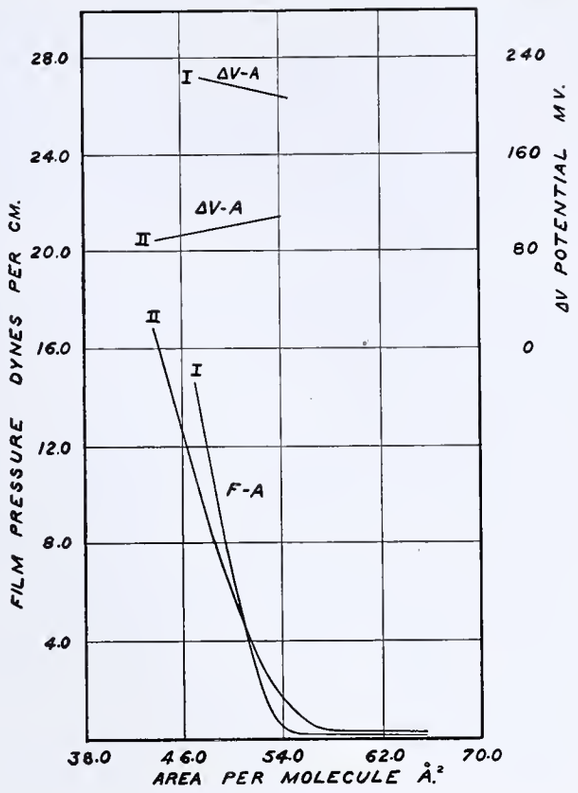


Figure 10. Surface Pressure Area and Surface Potential of Pimeric Acids

The molecule of *d*-pimaric acid contains two double bonds, which occur at or near the top of the molecule when oriented upright in a tightly packed film. The effect of these double bonds on the surface potential is extremely interesting. Since the surface potential of the unsaturated is less than half that of the saturated compound, the dipoles due to unsaturation seem to be opposed to those of the carboxyl group. Furthermore, while the surface potential of the saturated compound rises as the film becomes more tightly packed, that of the unsaturated compound decreases with packing. The former normal effect is due largely to an increase in the number of dipoles with pressure. The latter indicates that increased tightness of packing increases the opposition of the dipoles.

It has been seen that unsaturation at the top of the molecules increases the tightness of packing at the highest pressure and gives a molecular area of 43 Å.². Measurements with molecular models for these compounds gave an area of 42 Å.² for tight packing or a vertically oriented molecule. If the carboxyl group were in position 3 the orientation should be different, and the molecular models for this case gave an area of 37 Å.², which is much less than that which actually was found.

At a low film pressure the unsaturated groups near the top of the molecule change the molecular area in the opposite direction—that is, they cause slight expansion of the film, presumably because the tops of some of the molecules are pulled downward to the water by the dipoles of the unsaturated groups.

Another application of film studies, of possible value, is the determination of the hydrophil content of natural fats and oils.

When it is possible that a considerable variety of oxidation products are present, such as carboxyl acids and alcohols, together with lesser quantities of esters, ketones, aldehydes, lactones, ethers, and unsaturated compounds, the acid number and the iodine number give an incomplete characterization. One important difficulty, however, in the quantitative estimation is that the possibility of interaction of film molecules must not be excluded. Often the assumption must be made that each molecule occupies an area of the same magnitude as when present alone on the water surface. The small amount of experimental work that has been done with mixed films does not seem to permit this assumption. In spite of the technicalities it seems possible that an analytical method could be worked out in many cases.

Another example of the use to which film methods have been put is the interesting work of Gorter and Grendel (*5*) who employed the film balance as a microanalytical technique (less than 0.1 mg. is needed to form a film) in the determination of fat on red blood corpuscles. In this work a quantitative estimation of the total fat content was made on 0.1 to 0.2 cc. of blood. An interesting result of their researches is that many mixed films of biologically interesting compounds such as cholesterol, lecithin, and lipids behave in a simple additive manner. This fact should encourage additional development of research in this field.

An outstanding example of the application of film research to a problem of immediate interest is afforded by the recent work of Stenhagen (*14*) on the structure of phthioic acid, $C_{26}H_{52}O_2$. This material is one of the tubercle acids isolated by Anderson (*1*) and possesses toxic properties. In experimental animals it is observed to cause lesions closely resembling those of tuberculosis.

The study of films of phthioic acid has given noteworthy results in the elucidation of the probable structure of this compound. X-ray reflections from multilayer films of the barium salt showed that the length of the molecule was that of a chain of twelve to fourteen carbon atoms.

The phthioic acid films differ in behavior from those of known fatty acids. On water a highly incompressible film which collapses at an area of 38 Å.^{2} per molecule is formed, in contrast with *n*-decyl-*n*-dodecylacetic acid which forms an expanded film which collapses at about 60 Å.^{2}. The surface dipole of this film was much smaller than that found for phthioic acid.

This evidence suggested to Stenhagen the presence of a small alkyl group in the α-position to carboxyl, because this might account for the observed close packing of the chains. He suggested the formula

$$\begin{array}{ll} CH_3[CH_2]x\diagdown & x \text{ and } y, \text{ about 10 to 12 and different} \\ CH_3[CH_2]y\text{—}C.CO_2H & \\ CH_3[CH_2]z\diagup & z, 0 \text{ or } 1 \end{array}$$

The most probable formula is that of ethyl-*n*-decyl-*n*-dodecylacetic acid. Attempts to synthesize this substance are in progress in the laboratories of Sir Robert Robinson at Oxford.

As a final example the exhaustive researches of Clowes and his co-workers (*3*) on numerous carcinorgenic compounds may be cited. These substances, although not capable of giving coherent films in themselves, react extensively with sterols in stable monomolecular films. In some cases the interaction is sufficiently strong to lead to the formation of association complexes. The association type of interaction, which is especially pronounced when the hydrocarbon-sterol films contain a fat, stearic acid, or a phosphatide as a third component, may possibly provide a mechanism whereby the polycyclic hydrocarbons may be bound into and act as a modifying influence in biological structures.

The subject of two-dimensional solutions of nonpolar solutes in polar solvents is in its infancy. It was discovered by Harkins, Myers, and Fowkes (*9c*) that hydrocarbon oils of high molecular weight, which will not by themselves spread on water, may nevertheless be made to spread as a certain percentage of the material in a monolayer of a polar-nonpolar oil. Numerous two-dimensional solutions of this type were encountered in the work of Clowes.

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Electronic Relays—A Correction

The recent electronic relay circuit described by Redfern (*2*) has been criticized for using high values of grid resistors. Although no trouble has been experienced with the original values, changing R_2 and R_3 to 0.2 and 1 megohm, respectively, will minimize any tendency for the grid to remain positive through secondary emission. For a given time delay, the capacity of condenser C_3 will have to be increased fivefold.

It has been stated that the circuit of Hawes (*1*) utilized cathode bias and therefore the plate current could not be reduced to zero. This circuit uses a combination of bleeder and cathode bias in which the bias for cutoff is supplied by a bleeder across the line, making it possible to reduce the plate current to zero. This biasing method, however, is still subject to the criticism that it reduces the plate voltage available for operating the relay.

Time delay can also be obtained by use of a condenser in the plate circuit, but as the capacitance required is very much larger than the capacitance required in the grid circuit, it appears preferable to produce the delay in the grid circuit, especially with low resistance relays.

In order to eliminate any need for measuring the plate current and to limit the maximum screen current, the following values of R_4 are given: for relay coils with a resistance of 500 to 1000 ohms, 0; 1000 to 3000 ohms, 1000 ohms; 3000 to 7500 ohms, 2000 ohms.

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SUTTON REDFERN

Performance of Some Distillation Columns for the Fractionation of Terpenes

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FRACTIONATING columns of high efficiency are essential in terpene analysis and purification. To be of real value these columns must operate successfully at pressures of 10 to 20 mm. for both viscous and semiviscous liquids, and must have a small operating holdup per theoretical plate. For use in a laboratory devoted to terpene research they must also have a high capacity, in order that analyses may be completed in a relatively short time. Columns that can be efficiently operated when heated by a Nichrome-wound jacket insulated by an outer glass tube are desirable. In addition, an economical and easily repeated type of construction is advantageous.

With these conditions in mind columns packed with Raschig rings, Berl saddles, single-turn glass helices, the concentric glass tube column of Selker, Burk, and Lankelma (*6*), the spiral screen packing of Lecky and Ewell (*3*), the Stedman columns, and the Podbielniak type were considered. The cost of the latter two types made their extensive use impossible. The concentric glass tube type, although having a very low operating holdup and H. E. T. P., apparently does not have a very high capacity and seems to require a more elaborate system of column controls than was feasible. The single-turn glass helices would not operate satisfactorily for terpenes under the conditions described. Preliminary tests made by the authors indicated that columns packed with Raschig rings, Berl saddles, or the spiral screen type could be economically constructed and easily operated with terpene mixtures.

Columns with these three types of packing were then constructed and subjected to performance tests. Each column was equipped with standard taper glass joints and glass heating and insulating jacket. The heating jacket was wound to produce a maximum of 25 watts per 30 cm. (1 foot).

Tests and Measurements

Descriptions of the columns and test data are recorded in Table I. The plate efficiency of all columns was determined by the use of *n*-heptane–methylcyclohexane mixtures, using the data of Beatty and Calingaert (*1*) and Bromiley and Quiggle (*2*). All efficiencies were determined under total reflux and at atmospheric pressure. The operating holdup was measured by the method of Tongberg, Quiggle, and Fenske (*7*) using a benzene–stearic acid mixture. The nondrainable holdup was determined by observing the volume of benzene left in the packing as recommended by Ward (*8*). Pressure drops were measured on columns 4 and 5 by connecting the manometer alternately to the kettle and the head by means of a double diagonal stopcock. When measured in this way the pressure drop in column 4 was 2 mm. at a head reflux of 2.5 ml. per minute, distilling β-pinene at 20 mm. Column 5, when distilling β-pinene at 20-mm. pressure with a head reflux of 0.8 ml. per minute, had a pressure drop of 2.5 mm.

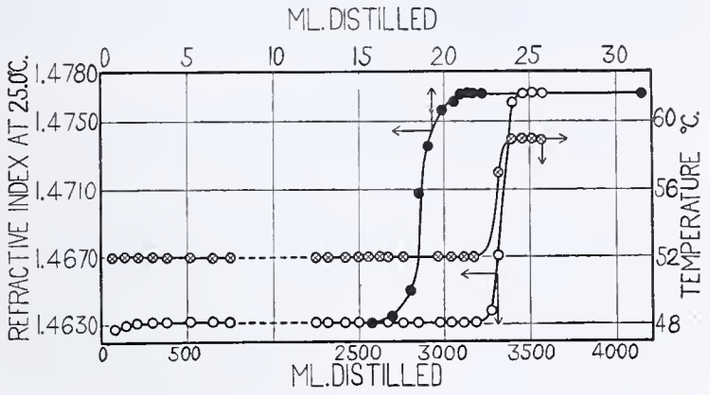

FIGURE 1. SEPARATIONS OF ALPHA- AND BETA-PINENE AT 20 MM.

⊗ Temperature, commercial α-pinene in column 4
○ Refractive index, commercial, α-pinene in column 4
● Refractive index, test mixture in column 5

Construction and Performance

BERL SADDLES AND RASCHIG RINGS. Apparently no data are available for the 4 × 4 mm. Berl saddle packing, but mention has been made of the unsatisfactory results obtained when 4 × 4 mm. Raschig rings are used for analytical columns (*5*). Both these packings give essentially the same H. E. T. P. and the same holdup per plate for a 120-cm. (4-foot) column of 11-mm. (0.43-inch) inside diameter. Because of their high operating holdup these columns could not be used for analysis. When a column of 19-mm. (0.75-inch) inside diameter was filled with these Berl saddles the H. E. T. P. decreased to 3.25 cm. (1.3 inches). This value is comparable with the best values reported for other loose packings (*4*) with the exception of single-turn stainless wire helices (*9*). These data and previous experiments with the distillation of terpenes at 20-mm. pressure using 4 × 4 mm. Berl saddles indicate that this is a satisfactory type of packing for purification of components in the more readily separable terpene mixtures. For some substances Berl saddles should not be used because of the catalytic nature of their unglazed porcelain surface.

SPIRAL SCREEN PACKING. When the directions of Lecky and Ewell (*3*) were followed, using a cupped spiral of 60 × 60 mesh stainless steel screen in a column of 37-mm. (1.45-inch) inside diameter, the H. E. T. P. obtained was nearly twice that reported

TABLE I. COLUMNS AND TEST DATA

Column No.	Column Packing	Column Specifications	Total Plates	H. E. T. P., Inches	Operating Holdup per Plate, Ml.	Nondrainable Holdup, Ml.
1	4 × 4 mm. Raschig rings	I. D. 11 mm. (0.43 inch) Packing height 46 inches	22	2.1	0.5	10
2	4 × 4 mm. Berl saddles	I. D. 11 mm. (0.43 inch) Packing height 48 inches	24	2.0	0.5	8
3	4 × 4 mm. Berl saddles	I. D. 19 mm. (0.75 inch) Packing height 27 inches	21	1.3	0.7	11
4	50 × 50 mesh stainless steel screen spiral	I. D. 37 mm. (1.45 inches) Inner glass tube O. D. 18 mm. (0.71 inch) Packing height 46 inches	70	0.66	0.95	28[a]
5	60 × 60 mesh stainless steel screen spiral	I. D. 10 mm. (0.40 inch) Inner nickel rod O. D. 2.5 mm. (0.10 inch) Packing height 46 inches	64	0.72	0.14	5[b]

[a] Nondrainable holdup, β-pinene, 40 ml.
[b] Nondrainable holdup, β-pinene, 7.5 ml.

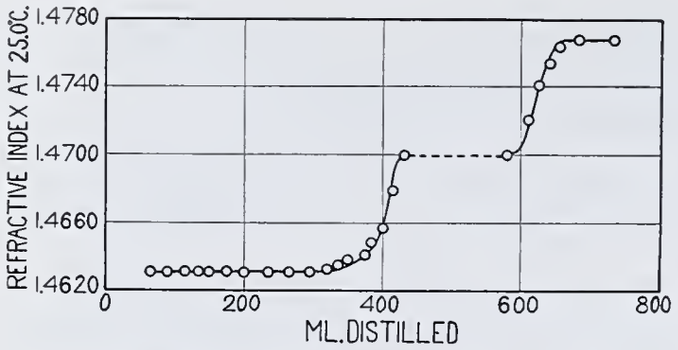

FIGURE 2. FRACTIONATION OF MIXTURE OF ALPHA-PINENE, CAMPHENE, AND BETA-PINENE AT 20 MM.

by them. After experimentation it was found that results comparable with theirs could be obtained if the construction were modified first, by using 50 × 50 mesh screen and, second, after cupping and spot-welding, by grinding the entire packing in the direction of the cup until the outside diameter of the packing was just 0.8 mm. larger than the inside diameter of the glass tube chosen for the column. Column 4 of Table I was constructed in this manner.

The procedure of Lecky and Ewell gave good results when applied to the construction of column 5 of Table I, in which the inside diameter was only 10 mm. (0.40 inch).

Both columns 4 and 5 were made in 120-cm. (4-foot) lengths, which is the standard length of the Pyrex tubing on the market. The tubing was selected only for uniformity of bore, a tolerance of 0.4 mm. being allowed.

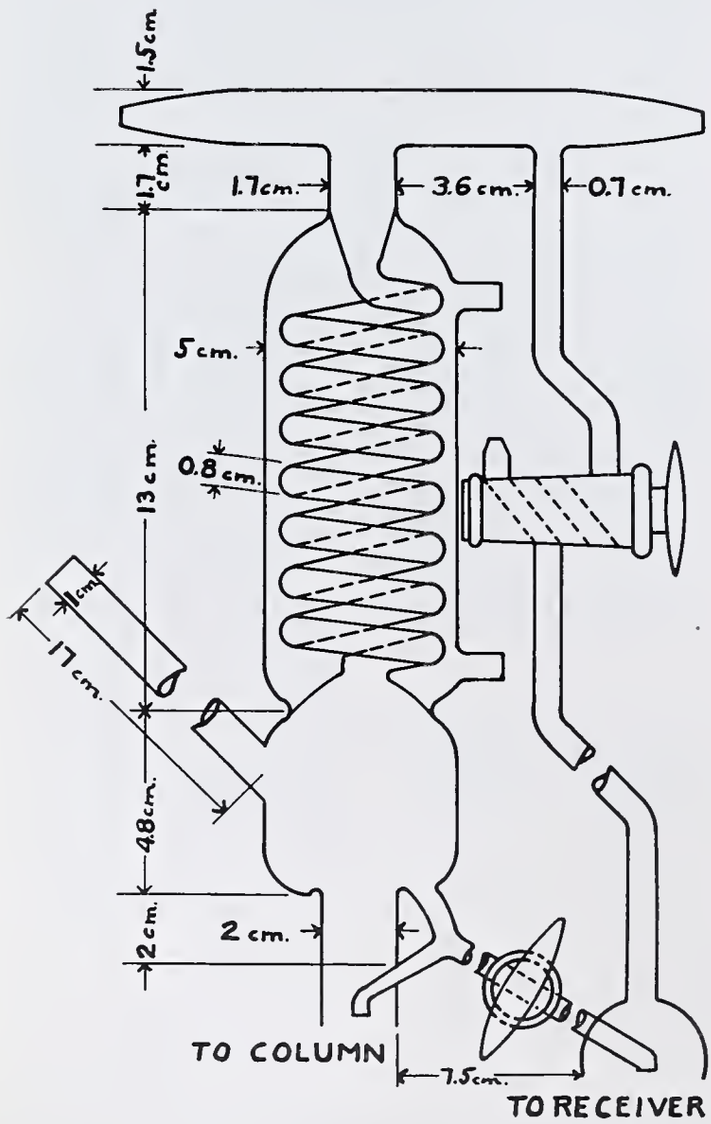

FIGURE 3. TOTAL CONDENSATION–PARTIAL TAKE-OFF HEAD

Costs of Spiral Screen Type

The entire cost of column 4, including all jackets and standard tapers, was $37; of column 5, $35. Labor costs including overhead were calculated at the rate of $1.50 per hour. Two work-hours per foot on column 4 and four work-hours per foot on column 5 were required. These costs do not include the construction of the punches for cutting the spirals, which requires about 15 hours each.

Operation of Spiral Screen Columns with Terpenes

The columns were thoroughly wetted down with the liquid and the column temperature was adjusted to within 2° of the boiling point of the more volatile component. The column and kettle heats were both controlled electrically. The columns were set on total reflux until liquid-vapor equilibrium was attained, at which time continuous fractionation was begun.

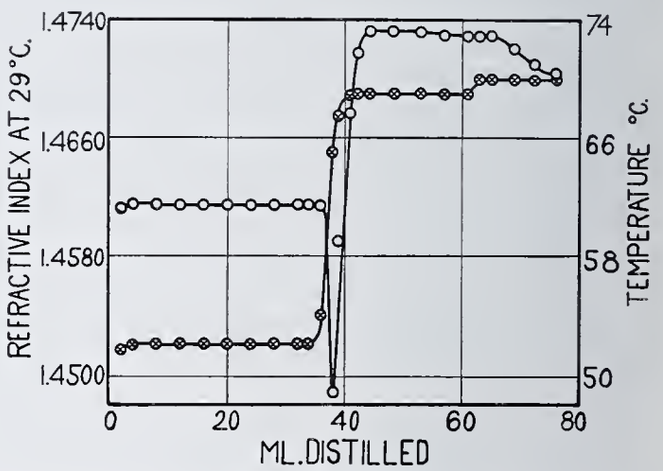

FIGURE 4. FRACTIONATION OF ALPHA-PINENE AND DIPENTENE AT 20 MM.

○ Refractive index
⊗ Temperature

COLUMN 4. Figure 1 contains a refractive index and boiling point plot illustrating how this column functioned at 20-mm. pressure on the separation of α-pinene from the β-pinene in a commercial sample of α-pinene obtained from gum turpentine. This column has also been used successfully to obtain pure β-pinene from commercial material. The column was operated with a head reflux of 2.5 ml. per minute as measured by a calibrated drop counter. No attempt was made to measure reflux at the bottom of the column. A head reflux ratio of 25 to 1 was used.

Figure 2 gives a refractive index plot showing how a 180-cm. (6-foot) length of a column of this size separated a prepared mixture of α-pinene, camphene, and β-pinene at 20 mm. At this pressure camphene boils about equidistant between α- and β-pinene. A head reflux ratio of 40 to 1 used during the collection of pure α-pinene was purposely maintained throughout the transition from α-pinene to camphene. This readily explains the gradual shift toward camphene at the beginning of the transition and the holdup of approximately 110 ml. In the transition from camphene to β-pinene the head reflux ratio was increased to 60 to 1, causing a holdup of about 85 ml. which was 15 ml. less than the operating holdup of the column. One hundred milliliters of the camphene obtained had a solidifying range of 44–45° C. and the remainder solidified at temperatures not lower than 25° C. This distillation of about 800 ml. of mixture was completed in 9 days. This indicates how this column might be used for rapid analysis of large quantities.

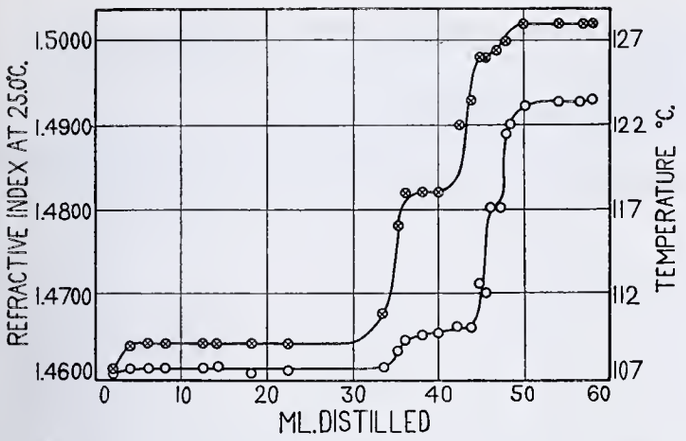

FIGURE 5. FRACTIONATION OF TERPENE ALCOHOLS AT 20 MM.
○ Refractive index
⊗ Temperature

For these distillations a total condensation–partial take-off head was used, as illustrated in Figure 3. The advantages of this modification are its large condensing surface, compactness, and strength.

COLUMN 5. As a preliminary test this column was used to separate a mixture of 35 ml. of carbon tetrachloride and 35 ml. of cyclohexane into approximately 90 mole per cent carbon tetrachloride and 100 mole per cent cyclohexane. The holdup of the column under the conditions of operation was 13 ml. and the separation required 36 hours.

When fractionating terpenes, column 5 was so regulated that the head reflux was about 0.8 ml. per minute. A head reflux ratio of 25 to 1 was used on the plateaus of the distillation curves but usually a higher ratio of about 40 to 1 was used when passing between plateaus.

The low pressure drop of this column combined with its high capacity minimizes any thermal reaction by allowing an analysis to be completed in a short period of time.

In Figure 1 is included a separation of α-pinene from β-pinene made with this column.

Figure 4 gives curves for the separation of a mixture of α-pinene and a dipentene cut of commercial material. The two major fractions were, respectively, α-pinene and dipentene. The small middle fraction on analysis was found to be p-menthane, which the temperature plot fails to detect.

Figure 5 gives curves for the separation of a mixture of terpene alcohols at 20-mm. pressure. Prior to distillation this mixture had a relative viscosity of 31. The operation of the column for a material of this viscosity is nearly as easy as it is for a mixture whose relative viscosity is much less, such as α- and β-pinenes. This ease of operation with such viscous materials makes the spiral screen type of column particularly valuable in the analysis of many terpene mixtures.

Conclusions

For a loose packing the 4 × 4 mm. Berl saddles perform well with terpene materials.

Columns with the spiral screen packing are the most economical and efficiently operative columns for terpene fractionations yet reported.

Acknowledgment

The authors wish to express their gratitude to B. J. Otte, curator of the Chemistry Department, for his efforts in obtaining materials, to Fred Hayes, the machinist, who was so helpful in working out the details of punching and fitting the screen spirals, and to P. J. Thompson, the glassblower, who constructed the glass apparatus.

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A Trap to Prevent Backflow from Suction Pumps

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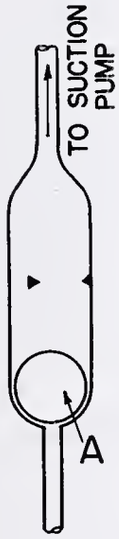
THE backing up of water from suction pumps whenever there is a decrease in water pressure in the supply line is an annoyance known to every chemist. Small metal balls placed in the side tube of the pump are efficient only if the pressure drop is sudden, causing the water to shoot back with a jerk. A gradual drop will permit the water to pass slowly over the ball without moving it, and thus the aperture of the tube will not be closed.

Most commonly used is a glass trap of the type illustrated. The ground-glass ball, *A*, rests on the aperture of the tube that leads to the apparatus to be evacuated, and is pressed down by the air at any reduction of the efficiency of the pump. Since this does not give an absolutely air-tight seal, water slowly backs up into the trap and only when the ground surface is wet is a perfect seal obtained. If the pressure in the water supply returns, the trap contains water through which the air stream must be sucked, causing a corresponding decrease in the vacuum obtainable. Even without water in the trap, the weight of the glass ball must be subtracted from the maximal vacuum obtainable.



This trap can easily be changed into a very satisfactory device by using a hollow glass ball that is lighter than water. In this case the trap is reversed in the position, leaving the connections as indicated by the arrow. The ball rests normally on three indentations in the middle of the trap, where sufficient space is left for the air to pass around. If the water backs up, the ball is lifted from its resting position and pressed against the upper tube outlet, sealing it tightly. With return of the suction, the water is automatically removed from the trap and the ball returns to its previous position. This trap works reliably and gives satisfactory results. In case of sudden release of pressure, the ball will be lifted by the air stream and so close the upper tube without the aid of water. It thus holds a partial vacuum for a considerable time, even when dry.

The trap with the floating ball has been made by the Scientific Glass Apparatus Company, Bloomfield, N. J.

Apparatus for Determining Distillation Ranges at Reduced Pressures

C. E. WATTS[1], JOHN A. RIDDICK, AND FRED SHEA, Commercial Solvents Corporation, Terre Haute, Ind.

LOW- and medium-boiling liquids and solvents are frequently evaluated by subjecting them to the A. S. T. M. method for distillation range. Both the apparatus (which is supplied by all the chemical supply houses) and the procedure (A. S. T. M. D268-41) are recognized as standard by the important producers of solvents, and are incorporated as requisites in many commercial specifications.

The advent of a number of new solvents whose distillation ranges cannot be easily or readily obtained at atmospheric pressure has necessitated the development of apparatus for determining distillation ranges at predetermined and uniformly maintained reduced pressures. Such an apparatus has been developed in this laboratory and has given very satisfactory results over a period of several years for control work in the manufacture of high-boiling liquids.

TABLE I. DISTILLATION RANGES

Volume Distilled	2-Mm. Pressure 1	2-Mm. Pressure 2	10-Mm. Pressure 1	10-Mm. Pressure 2	50-Mm. Pressure 1	50-Mm. Pressure 2
	Temperatures					
Ml.	° *C.*	° *C.*	° *C.*	° *C.*	° *C.*	° *C.*
First drop	119.0	119.0	188.0	187.0	224.0	225.0
1	135.0	135.0	190.0	188.0	226.0	228.0
5	164.2	165.0	192.0	192.0	230.0	231.0
10	175.5	174.0	194.0	194.0	233.0	234.0
20	185.0	183.0	197.0	196.5	236.0	236.0
30	188.2	188.0	198.2	198.0	237.0	237.0
40	191.0	190.0	200.0	199.2	239.0	239.0
50	192.0	190.1	201.0	200.0	239.0	240.0
60	192.8	192.0	201.5	201.0	240.0	240.0
70	192.8	192.2	202.5	201.5	241.0	241.0
80	193.0	193.0	203.0	202.5	242.0	242.0
90	193.0	193.2	204.0	203.0	244.0	244.0
95	195.0	194.0	206.0	205.0	244.2	244.2
98 (dry)	195.0	194.0	206.0	206.0	248.0	249.0

APPARATUS. A 250-ml. distilling flask with glass tube sealed in as shown in Figure 1. This tube may be fitted with a fine glass capillary for the control of bumping or flooding. The authors have used a capillary with 0.175-mm. opening delivering about 1200 ml. of air per hour. This air intake can be reduced or eliminated entirely by applying a rubber tubing with a screw clamp when desirable in any particular distillation.

An all-glass condenser (Figure 1) with the same size of condensing tube as the A. S. T. M. distillation apparatus.

A vacuum receiver which consists of a 100-ml. graduated cylinder modified by the addition of a side tube, as shown in Figure 1.

A vacuum control which utilizes a vacuum tube to reduce the current at the mercury contact to 0.01 milliampere. The unit is housed in a white-pine box with a front panel 30 cm. (12 inches) high and 22.5 cm. (9 inches) wide. The electrical equipment is mounted on a 4.7-mm. (0.1875-inch) piece of Masonite which fits into the box on supports giving a 6.88-cm. (2.75-inch) clearance from the bottom. The following components are connected as shown in Figure 2:

One BSBF8G2 Dunco relay; coil resistance, 3500 ohms (Struthers Dunn, Inc., 139 North Juniper St., Philadelphia, Penna.)
One No. 43 radio tube and socket
One 40-watt lamp and receptacle (approximate resistance, 250 ohms)
One 500,000-ohm, 0.5-watt resistor
One 50-ohm, 10-watt resistor
One 2-microfarad, 450-volt paper condenser
One 6-volt door-bell transformer
One 6-volt, 2-coil door bell or buzzer

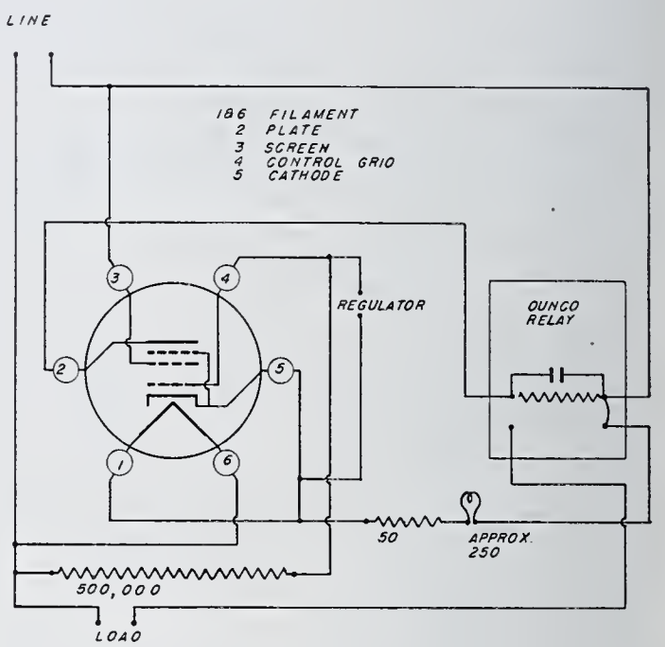


FIGURE 2

[1] Present address, 3525 Pope St., S. E., Washington, D. C.

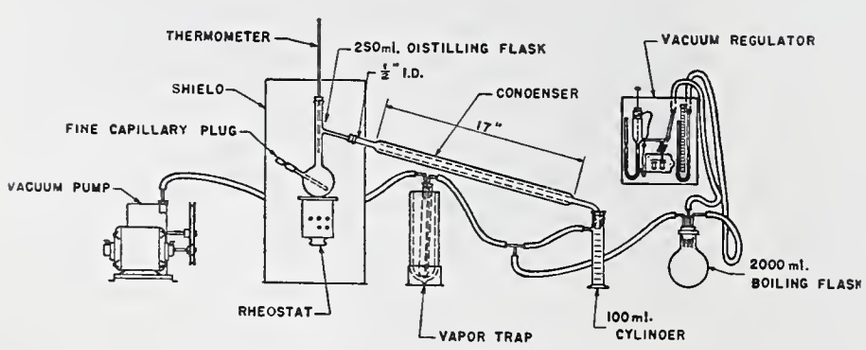


FIGURE 1. DIAGRAM OF APPARATUS

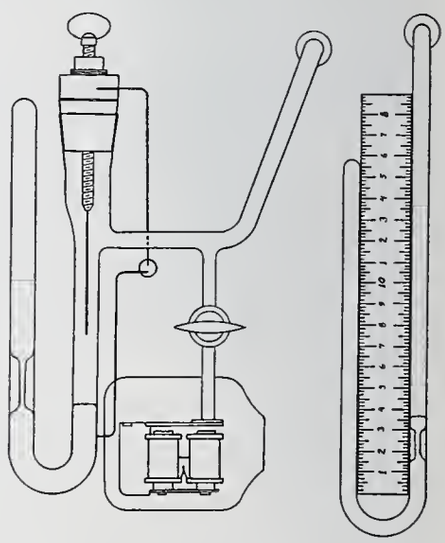


FIGURE 3

The load leads are connected to the primary of the transformer, and the secondary is connected directly to the coils of the bell. The bell and clapper are cut off, the interrupter contacts are removed, and a piece of smooth rubber is cemented to the armature, as shown in Figure 3. The pressure-adjustment tube and the manometer are of convenient size, and are made, mounted, and connected as shown in Figure 3.

A dry-ice trap is used to collect vapors and prevent them from entering the vacuum pump.

Determinations are carried out in the same manner as in the standard A. S. T. M. distillation procedure, except that the system is under reduced pressure. Uniform and reproducible pressures as low as 2 mm. can be obtained, as shown by Table I. Readings were taken during the distillation of 100-ml. portions of a large sample of methyl oleate at three different pressures.

A Study of the Electric Hygrometer

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IN A previous paper (*5*) on the determination of water in insulating oil, use was made of the electric hygrometer developed by Dunmore (*3*). This device appeared promising as a rapid field method for determining water in oil, and was likely to have other general applications wherever small amounts of water were a vital factor in industrial or laboratory operations. It seemed important, therefore, to study the mechanism by which the electric hygrometer functioned. The effects of such factors as time of approach to equilibrium, temperature, inert gas pressure, mass of water present, mass of hygroscopic salt on the unit, and resistance of the unit were studied. During the course of the study, a modified design of the hygrometer unit was developed and has been used throughout in the experimental results given below.

Modified Hygrometer

The hygrometer unit described by Dunmore consisted of No. 38 AWG palladium wire wound on an aluminum cylinder coated with polystyrene and with a polyvinylacetate film containing different percentages of lithium chloride. A similar unit was constructed in this laboratory and tried out in the determination of water in oil, without further investigation of its characteristics. It was calibrated by employing oils of a known water content as determined by the combustion method (*4*).

After several weeks of successful operation, the behavior of the coil became erratic. It was felt that the movement of the wire in the film due to the solvent action on the polyvinyl acetate by the oil vapors, especially by the chlorinated hydrocarbon type of insulating oil, may have contributed to this phenomenon.

Accordingly, a new hygrometer was constructed. A No. 20 S. T. Pyrex male ground joint with 5-cm. tubulations on both ends was selected. The closed tubulation from the smaller end of the ground surface was dipped in paraffin and threadlike bifilar markings in the paraffin exposing the glass were made in a lathe. The exposed glass was etched for 30 minutes in a sulfuric-hydrofluoric acid mixture. After removal of the paraffin, the entire glass cylinder with its spirally etched grooves was coated with commercial platinizing solution and heated to a dull red appearance. After cooling, the cylinder was sandpapered, whereupon the platinum was removed from the ridges, resulting in two spiral threads of platinum baked on the glass 0.062 cm. apart and 90 cm. long. The finished coil had a diameter of 1.5 cm. and was 2.5 cm. long.

A substitute for glass sealed lead-in wires was effected by painting two stripes on the ground surface of the male joint and extending them to the end of the other tubulation (Figure 1). At these points copper wires were soldered on with ordinary soft solder. The hygroscopic film was put on by merely immersing the coil in an alcoholic solution of the desired salt, allowing the alcohol to evaporate in the air. The removal of an impaired film could thus very simply be accomplished by immersion in water. Before application of the salt film, the coil was subjected to 100 volts direct current to reveal any possible electrical bridges or other imperfections. Measurements were carried out with 3 volts alternating current on the coil. Later it was found that almost all the conduction took place in the first quarter of the coil. Therefore, the recommended length of the coil is one fourth that given above.

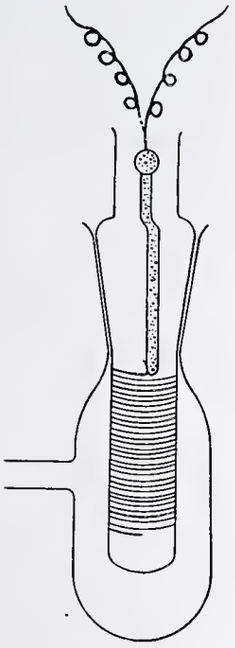

FIGURE 1. MODIFIED ELECTRIC HYGROMETER

Relation between Resistance and Water Vapor Pressure

A knowledge of the relation between the water vapor pressure and resistance of the coil was obtained through a study of the behavior of the coil under three experimental conditions—namely, an approximate mass of water vapor, the pressure of which was varied by changing the volume with a mercury piston; the humidity of saturated salt solutions; and the formation of water from the explosion of a known volume of a hydrogen-oxygen mixture.

In Figure 2, a schematic diagram of the combined apparatus is shown.

In buret *B* a prepared known mixture of hydrogen and oxygen is stored and introduced into the explosion pipet, *A*. The combination manometer-buret containing mercury had an inside diameter of 15 mm. in order to reduce to a minimum the sticking of the mercury column which was read with a cathetometer. The water vapor volume was changed by evacuating or applying pressure to the reservoir at the base. The entire apparatus was evacuated to a pressure of less than one micron at the start of an experiment and the hygrometer unit chamber at *D* was isolated by means of the three-way stopcock. The following data on a single quantity of water were obtained: (1) mass of water from the known hydrogen-oxygen mixture and a check of this value with that obtained from the product *PV;* (2) distribution of the water from (1) between the vapor space and the lithium chloride film; (3) partial pressure of water in equilibrium with the hygrometer unit; and (4) resistance of the hygrometer unit.

In the changing volume method, a suitable quantity of water vapor was introduced into the buret and, after the pressure and volume were read, was expanded into the previously evacuated hygrometer cell chamber. The difference between the calculated reduced pressure and the measured reduced pressure enabled one to estimate the micrograms of water absorbed on the coil. The results are shown in Figure 3 for two hygroscopic salts, lithium chloride and potassium acetate. The observed water vapor pressure where the sharp rise in the curve occurred corresponds to that of a saturated solution of the particular salt as given in International Critical Tables (*6*). Thus, in the vertical section of

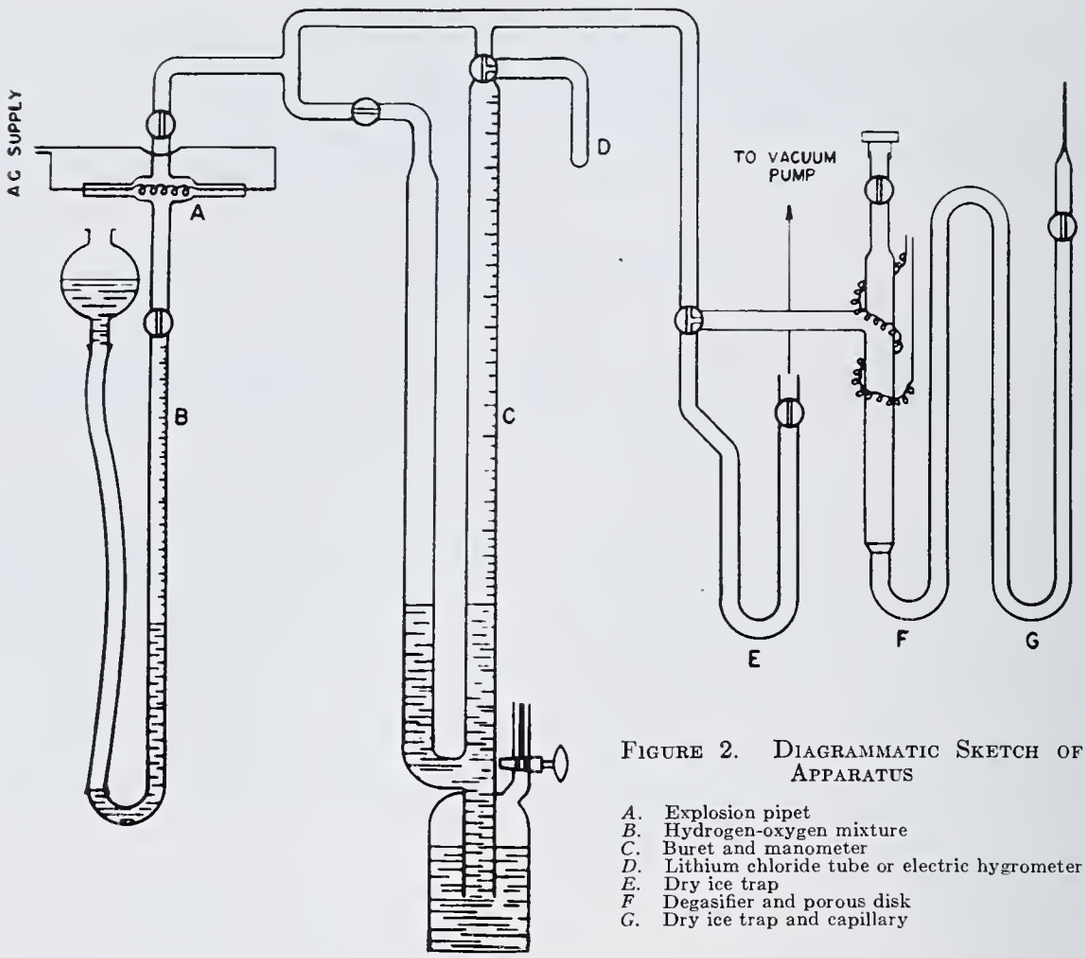

FIGURE 2. DIAGRAMMATIC SKETCH OF APPARATUS

A. Explosion pipet
B. Hydrogen-oxygen mixture
C. Buret and manometer
D. Lithium chloride tube or electric hygrometer
E. Dry ice trap
F Degasifier and porous disk
G. Dry ice trap and capillary

the curve there is present on the hygroscopic film a mixture of salt hydrate and saturated solution. In order that the water vapor pressure may remain constant (or nearly so) in this region on increasing the volume, water immediately passes from the coil into the surrounding space as is illustrated in the lower curves—i. e., micrograms on coil *vs.* pressure. The attending increase in resistance may be caused by a reduction in the thickness of the saturated liquid film or by the formation of isolated patches of liquid.

In the more nearly horizontal section of the curve, no solid phase is present and the film is an aqueous solution of electrolyte. The change in resistance in this section is caused by a change in the number of ions per unit volume. Support of this contention follows from a knowledge of the temperature coefficient of conductance, which was found to be approximately that of electrolytic solutions. Furthermore, the resistance readings became steady in a relatively short time in this region, whereas there was always a drift observed in the constant-pressure section.

Alcoholic solutions containing 0.1 per cent lithium chloride gave resistances above one megohm which were too high to be accurately measured on the alternating current bridge. There seemed to be no point in investigating solutions above 1 per cent, since it was desirable to maintain a minimum of water on the coil while operating in the variable-pressure zone. Approximately twice as much water was observed on the 1 per cent lithium chloride coil as on the 0.5 per cent lithium chloride coil.

SATURATED SALT SOLUTIONS. In these experiments a small amount of solid salt was introduced into a side arm of the cell chamber, followed by the addition of 1 ml. of saturated salt solution. The assembly was evacuated and placed in a constant-temperature bath. In Figure 4 the results are shown at a temperature of 20 ± 0.02° C. It was hoped that this procedure would yield a rapid and accurate means of calibrating and checking the coil. However, in a closed system containing two liquids of different vapor pressure—i. e., the film and the saturated salt solution—the transfer of water through the intervening vapor space requires a long time before the resistance changes become inappreciable. The

TABLE I. RELATION BETWEEN PARTIAL PRESSURE AND WEIGHT OF WATER AND RESISTANCE OF HYGROMETER

Volume of $H_2 + O_2$ *Ml.*	H_2 %	Temperature ° *C.*	$P^a_{initial}$ *Mm.*	P^b_{final} *Mm.*	Volume *Ml.*	Water $H_2 + O_2$ *Mg.*	PV	Resistance *Ohms.*
			Hygrometer 4					
2.51	52.0	27.0	15.4	6.5	63.7	950	945	6460
2.42	52.5	27.8	14.8	6.7	63.6	925	900	5680
2.58	52.5	28.0	15.3	7.1	63.6	990	935	5560
2.53	52.5	28.2	15.4	7.6	63.6	970	945	5365
After 16 hours		27.8		7.8				5240
			Hygrometer 12					
2.51	52.0	26.5	15.2	6.1	63.7	955	935	4195
2.46	52.0	27.2	14.9	6.8	63.7	935	915	4100
After 16 hours		27.0		5.9				4680

[a] $P_{initial}$ = partial pressure of water before exposure to lithium chloride of hygrometer unit.
[b] P_{final} = partial pressure of water in equilibrium with lithium chloride corresponding to the measured resistance.

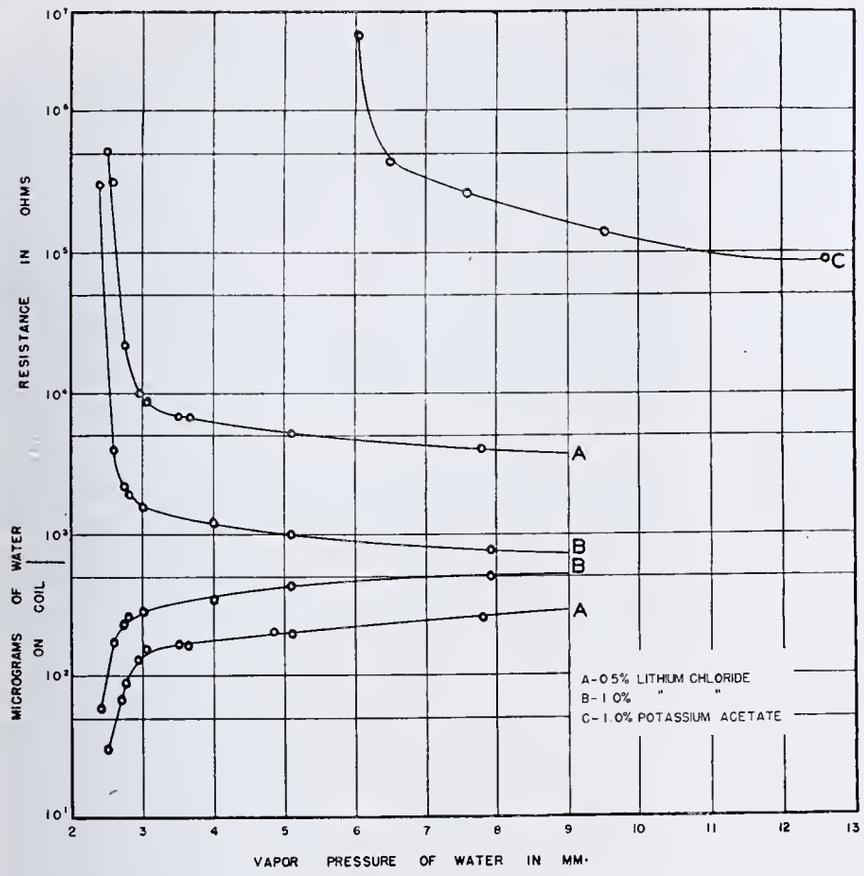

FIGURE 3. RELATION BETWEEN SALT CONCENTRATION, WATER VAPOR PRESSURE, AND RESISTANCE OF COIL

Temperature, 27 ± 1° C.

supercooling of the saturated salt solution on evacuation, as well as the long time required for steady resistance readings, made it desirable to change to the third method of producing a known weight of water vapor. In Figure 4 (different ordinate scale) as in Figure 3 the sharp rise in the curve was about to take place at a pressure corresponding to that of a saturated solution of lithium chloride.

EXPLOSION OF HYDROGEN-OXYGEN MIXTURES. In the determination of water in oil one is chiefly concerned with number of micrograms of water in a definite quantity of oil. A measured volume of a known mixture of hydrogen and oxygen should on explosion give a rapid and accurate method for obtaining small quantities of water. In this manner a very small weight—for example, 10 micrograms of water—may be easily obtained, since a gas mixture may be accurately prepared containing a small percentage of hydrogen (1 ml. of a 1 per cent hydrogen in oxygen mixture yields 7.5 micrograms on combustion).

While at times there appeared to be some regularity between resistance and micrograms of water, erratic results were frequently obtained. In all cases the partial pressure of water fell on a smooth curve when plotted against resistance, but this was not true when the micrograms of water were substituted for the partial pressure. The data given in Table I illustrate these conclusions, as well as the correlation between the water measured by exploding a known mixture of hydrogen and oxygen and measured by the product of pressure and volume. Although the differences between the two measurements were always in the same direction, the values are not known with sufficient accuracy to compute the quantity of water which is adsorbed on the glass surface. It was concluded that the hysteresis of the etched surface with its lithium chloride aqueous film was responsible for this anomalous effect. The past history of the film apparently determined the equilibrium between the water adsorbed and the partial pressure of water. Thus a different partial pressure would result on the addition of a fixed weight of water, depending on whether the previous experiment was one involving a larger or smaller weight of water.

Hysteresis phenomena involving water vapor have been recorded in the literature for many substances. The partial pressure of water vapor in equilibrium with cellulose is pro-

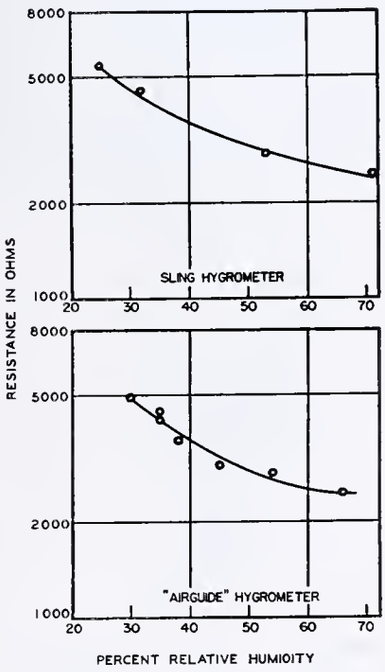

FIGURE 5. RELATION BETWEEN MEASURED HUMIDITY OF ROOM AND RESISTANCE OF COIL

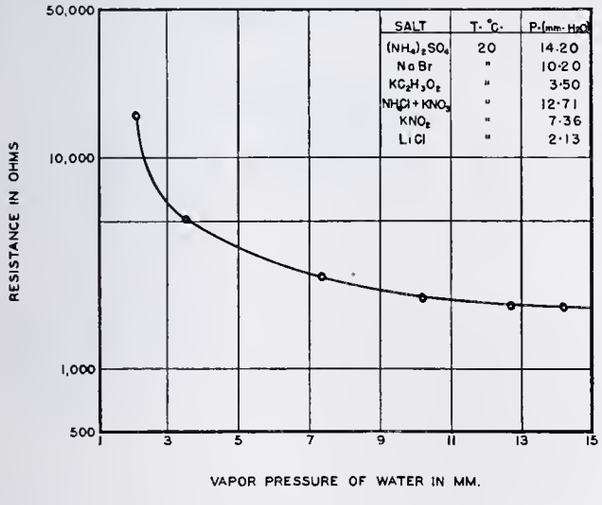
SALT | T. °C. | P. (mm. H2O)
(NH4)2SO4 | 20 | 14.20
NaBr | " | 10.20
KC2H3O2 | " | 3.50
NH4Cl + KNO3 | " | 12.71
KNO3 | " | 7.36
LiCl | " | 2.13
RESISTANCE IN OHMS
VAPOR PRESSURE OF WATER IN MM.

FIGURE 4. RESISTANCE–WATER VAPOR PRESSURE RELATION AT 20° C.

Water vapor pressure controlled by saturated salt solutions

nouncedly different on the adsorption curve than on the desorption curve. Recently, the hysteresis phenomenon connected with quartz and water vapor was described by Barrett, Birnie, and Cohen (*2*), and was shown to consist of a rapid reversible adsorption and a slow non-reversible sorption.

Unfortunately, the hygrometer could not be heated to a sufficient temperature to restore the surface each time to zero water content because of the probable dislocation of the conductors. However, if, as has been demonstrated, the resistance of the coil depended on the partial pressure of water, for an unlimited supply of water vapor, disregarding sudden changes in humidity, the hygrometer unit should behave satisfactorily as a humidity indicator. In Figure 5 is shown a comparison between the readings of a commercial spring hygrometer and a sling hygrometer and the resistance of the electric hygrometer at room temperature over a 2-week period. In some cases a discrepancy of as high as 6 per cent in relative humidity was observed between the sling hygrometer and the "Airguide" hygrometer.

Temperature and Inert Gas Pressure

A field instrument which is not greatly influenced by moderate changes in temperature would have many advantages over a temperature-compensated device. For this reason the major portion of the work has been carried out in the variable-pressure zone of the coil. In this region, the temperature coefficient of resistance was relatively small (cf. Figure 6). In the constant-pressure zone, the effect of temperature was often confused by the previous history of the coil and was very much greater in magnitude.

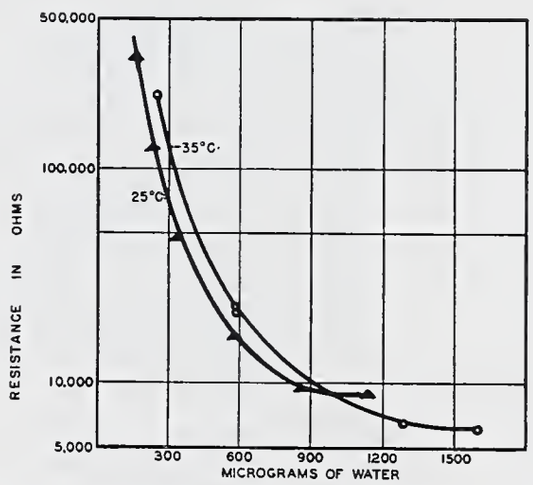

FIGURE 6. EFFECT OF TEMPERATURE ON RESISTANCE OF COIL

The curves at the two different temperatures tend to become parallel to the abscissa as the water vapor pressure of the film approaches that of pure water. The curves also intersect, which indicates that there are two opposing effects at play. First, the increase of temperature brings about a decrease of resistance of electrolytic solutions, and secondly, at the higher temperature, the film possesses a higher vapor pressure and therefore water must pass from the coil to the surrounding space with attending increase in resistance.

The effect of inert gas pressure appears to be one of delay of the attainment of equilibrium. Diffusion of water vapor to and from the coil is inversely proportional to the pressure. At 1-micron inert gas pressure there is no measurable delay. At 2-cm. inert gas pressure, the time required for equilibrium is approximately 20 minutes.

Electrical Resistance Bridges

The laboratory resistance measurements were carried out on either a direct or alternating current bridge, depending on the magnitude of the resistance. An alternating current measurement is preferred, in order to reduce polarization effects to a minimum. The direct current bridge was a modified General Radio megohm bridge (*1*) with an external direct current galvanometer, 1-mm. deflection of which was equivalent to 0.01 microampere. An off-balance of 0.5 per cent can be detected in the range 1 to 100 megohms and progressively lower sensitivity at higher resistances. The alternating current bridge was a typical Jones and Joseph bridge (*7*) with a cathode ray tube used conveniently as a null detector with three stages of amplification. All measurements were made at 1000 cycles. An off-balance deflection of one part in 100,000 could be detected. A bridge suitable for use in the field should present no problem.

Other Hygroscopic Compounds

Unless otherwise stated, all the work reported in this paper was carried out using lithium chloride as the hygroscopic salt. It is apparent that for humidities lower than that corresponding to the vapor pressure of the saturated salt solution, a different and unreliable relation exists between resistance and water vapor pressure. The continuous drifts in resistance observed at very low water vapor pressures were caused by the time required for the water to pass into the vapor space. In addition to potassium acetate (Figure 3), some work was carried out on 0.1 per cent aqueous phosphoric acid solution, but the hydrates of phosphoric acid are none too definite and it was feared that any neutralization of the acid would cause a continued variation in resistance. A definite hydrate whose saturated solution has a very low vapor pressure was desired in attempting to estimate the water content of an oil sample by measuring the aqueous partial pressure above the oil sample. However, two additional factors—namely, the slowing up of the attainment of equilibrium by the dissolved air in the oil and the effect of small temperature changes on the oil–water vapor equilibrium—must be considered.

Conclusion

It has been shown experimentally that the resistance of the electric hygrometer, if without hysteresis, may be expected to follow the water vapor pressure in a regular manner except at a water vapor pressure below that of the saturated solution of the salt used as the electrolyte. By selecting the desired saturated solution, the electric hygrometer or a device of similar principle could be adapted to the control of humidity in industrial operations. Because of hysteresis, there is little possibility that the electric hygrometer can be used for water-in-oil determinations where one is interested in the mass of water in a closed space and not the vapor pressure of water.

Acknowledgment

The electrical bridges employed in this work were constructed in this laboratory under the direction of W. F. Davidson, director of research.

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(1) Balsbaugh, IND. ENG. CHEM., **31**, 318 (1939).
(2) Barrett, Birnie, and Cohen, *J. Am. Chem. Soc.*, **62**, 2839 (1940).
(3) Dunmore, F. W., *Bur. Standards J. Research*, **23**, 701 (1939).
(4) Evans, Davenport, and Revukas, IND. ENG. CHEM., ANAL. ED., **11**, 553 (1939).
(5) *Ibid.*, **13**, 589 (1941).
(6) Intern. Critical Tables, Vol 3.
(7) Luder, *J. Am. Chem. Soc.*, **62**, 89 (1940).

PRESENTED before the Conference on Electrical Insulation, Division of Engineering and Industrial Research, National Research Council, at Williamsburg, Va.

Adapters for Collection of Distillation Fractions under Vacuum

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RECEIVERS are usually changed during low-pressure distillation: (1) by the use of a series of stopcocks which permit the complete removal and replacement of the receiver, or (2) by the use of glass adapters, sometimes known as "cows", which lead the distillate into receivers already connected to the vacuum system. Since either method involves special equipment, which may not be available, two adapters are described in this paper which can be made at low cost and without a mastery of the technique of glass blowing.

The design of the first model (Figure 1) is adapted from Bruhl (*1*), uses only commonly available materials, and is very useful in working with small quantities of liquids. Although the dimensions are not critical, those of the apparatus in use in this laboratory are given as an example.

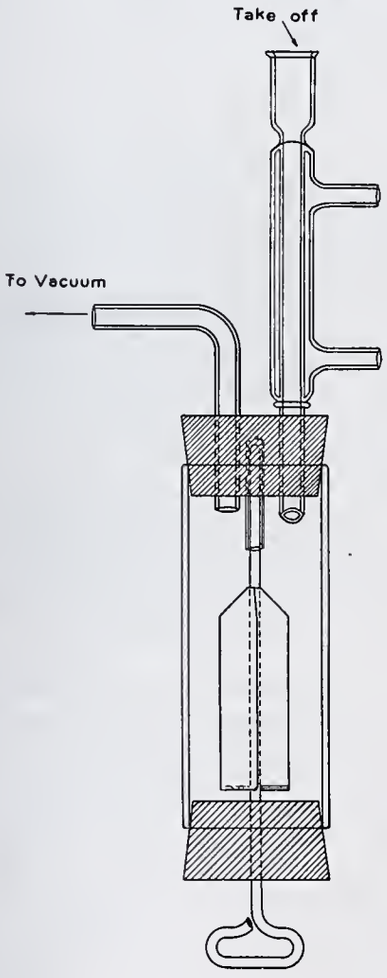

FIGURE 1

The outer jacket is made from a 12-cm. length of 48-mm. tubing with No. 10 rubber stoppers fitted in the ends. Passing through the lower, and extending to a glass bearing in the upper stopper is a 3-mm. wire, which is bent at its free end to form a handle. To this wire are soldered, at right angles to each other, four metal vanes with the lower ends bent horizontally to form a platform. The upper stopper is fitted with a condenser (optional) and a bent tube leading to a vacuum pump. In operation, four 80 × 15 mm. vials are placed in the corners produced by the vanes and are held in position by rubber bands. By means of the wire handle it is possible to rotate the metal frame and place the desired receiver under the drip point of the condenser.

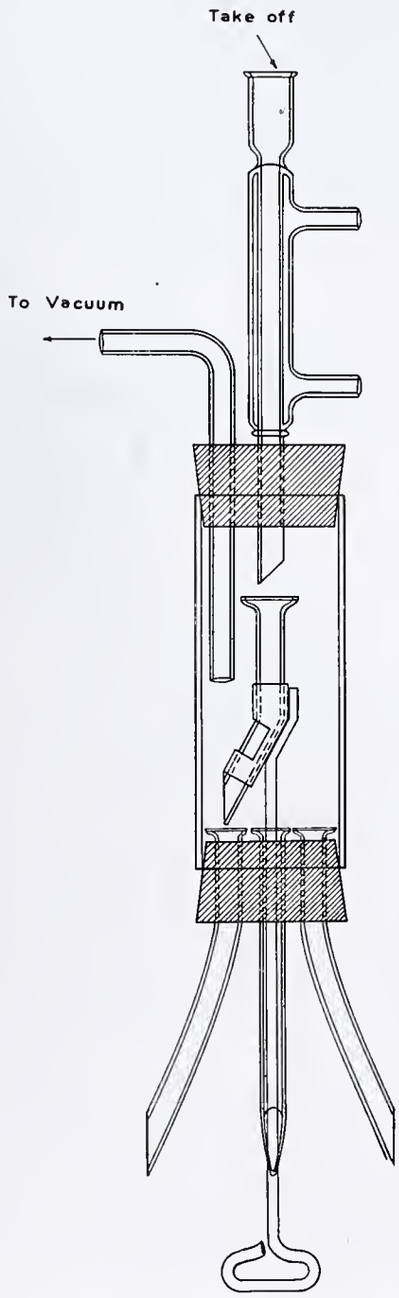

FIGURE 2

By modifying the first design slightly it is possible to obtain a second model (Figure 2) more suitable for the collection of larger fractions.

The outer jacket is the same, but the stoppers are fitted with different attachments. In this case it is necessary to center the drip point of the condenser so that the falling distillate will be caught by the rotating adapter, which consists of a short length of 10-mm. tubing, flanged at one end and ground to a drip point at the other. Since the proper functioning of this rotating adapter requires that the upper end be practically free from lateral

motion, it is supported by a 3-mm. wire handle extending through the lower stopper and bent so that the wire and upper portion of the glass tube have the same axis. The tube is held to the wire by a piece of thin metal sheeting soldered to the wire and bent around the glass at two points. In the lower stopper are also placed four slightly bent 8-mm. glass tubes, which are flanged at the upper end and placed so that the centers are equidistant from the wire. When in operation, four receivers are attached to these delivery tubes by means of rubber stoppers, and then as a drop of distillate leaves the condenser it can be directed by means of the rotating adapter to any one of the delivery tubes and thus to the receiver.

Adapters of the type described are free from the usual susceptibility to damage caused by thermal and mechanical strains. The rubber stoppers, do not lower the efficiency of operation, since pressures as low as 1 mm. have been obtained, nor do they affect the purity of product, since at no time is the distillate in contact with any material other than glass. Moreover, the individual adapters offer certain advantages. The first model is designed for small fractions, but the capacity can easily be varied by changing the length of the whole system. The use of fragile, preweighed vials as direct receivers is an important convenience in working with small quantities where losses due to transfer are appreciable. The second suggested design is of such a nature that it is not necessary to change the position of the receivers when changing fractions. Thus, receivers can be held firmly and may even be large and heavy, since changing fractions merely requires twisting the wire handle.

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(1) Bruhl, *Ber.*, **21**, 3339 (1888).

Ferrocyanide Method for Separation of Hafnium from Zirconium

WALTER C. SCHUMB AND FRANK K. PITTMAN

Massachusetts Institute of Technology, Cambridge, Mass.

MOST of the methods for the separation of hafnium from zirconium depend upon the repeated fractional crystallization or precipitation of such compounds as the oxychloride, phosphate, oxalate, fluohafnate, etc., and the number of fractionations required in general is large. In 1932 Prandtl (*2*) described a procedure by which the separation of these elements may be effected in comparatively few operations by precipitation of the ferrocyanides, yielding a product containing 90 per cent hafnium oxide and 10 per cent zirconium oxide starting with a material containing about 1 per cent hafnium oxide. Of all the methods reported thus far, this would appear to be the fastest and most efficient.

In certain details the procedure described by Prandtl is somewhat indefinite, such as the actual concentration of sulfuric acid which should be present in the solution prior to precipitation with sodium ferrocyanide and the proper quantity of sodium ferrocyanide to be used in the precipitations. Since the authors' preliminary experiments with the method gave results somewhat at variance with those reported by Prandtl, it was decided to examine more closely the conditions under which separation by this procedure may be accomplished.

The method described by Prandtl depends upon the fractional precipitation of the ferrocyanides of zirconium and hafnium from a solution containing oxalate and sulfate ion, the purpose of which is to form complex ions of different stability. The hafnium is enriched in the precipitate.

PROCEDURE. Zirconium (and hafnium) hydroxide is precipitated from a solution of the oxychloride by means of ammonium hydroxide. The precipitate is filtered and treated with just enough dilute sulfuric acid to cause it to dissolve. This takes several hours, because the reaction must be carried out at room temperature. An amount of ammonium sulfate at least equal to the weight of oxide in the solution is added to the clear solution, which is warmed for several hours. If no precipitate forms, a solution of oxalic acid saturated in the cold is added in the ratio of 200 cc. of oxalic acid for each 100 grams of oxide present. To this solution, still warm, is now added with stirring a solution of sodium ferrocyanide containing a weight of $Na_4Fe(CN)_6.10H_20$ equal to that of the oxide in solution.

(Some ambiguity exists in the original description at this point, which is as follows: "ich .... eine Lösung von soviel ... Natriumferrocyanid zusetzte, als dem Gewicht des in Lösung vorhandenen Zirkonium-Hafniumoxydes entsprach." That this implies an amount of ferrocyanide equal to the weight of the mixed oxides is assumed, since if it were to mean equivalent to, the precipitation of both zirconium and hafnium would be complete and no separation effected. Furthermore, in referring to the addition of ammonium sulfate, in the same paragraph, an almost identical construction is used which leaves no doubt as to its meaning: "Zu dieser Lösung setzt man mindestens so viel Ammoniumsulfat hinzu, als das Gewicht des in der Lösung vorhandenen Zirkonium-Hafniumoxydes beträgt.")

The mixture is allowed to stand with stirring at room temperature for several hours. The resulting yellow precipitate is then filtered and converted to the hydroxide by digestion with excess concentrated sodium hydroxide. (The precipitate will be greenish-yellow if iron is present, but this does not affect the efficiency of the separation.) The process may then be repeated with this mixture of zirconium and hafnium hydroxides.

In illustrative data quoted by Prandtl, the addition of 500 grams of sodium ferrocyanide to 1 kg. of zirconium oxide was said to give 40 grams of enriched zirconium oxide. The concentration of the starting material was given as about 1.3 per cent hafnium oxide and that of the product as 20 per cent hafnium oxide. There appears to be a discrepancy between the body of the report, which calls for a 1 to 1 ratio of ferrocyanide to oxide, and these data which call for a 1 to 2 ratio.

In repeating the experiment just referred to, using the 1 to 2 ratio, the authors obtained the following results (calculated to the same basis as Prandtl's): The addition of 500 grams of sodium ferrocyanide to 1 kg. of zirconium oxide (12 per cent hafnium oxide) yielded 200 grams of zirconium oxide (18 per cent hafnium oxide). These results differed from those of Prandtl in the amount of material precipitated, the increase in the ratio of hafnium to zirconium, and the efficiency of the enrichment of the hafnium oxide. It was felt that the cause of these discrepancies could be found in the ambiguities in the original paper above referred to. This led to the undertaking of a detailed study of the factors affecting the separation.

Starting Material

The source of the zirconyl chloride containing hafnium used throughout this work was the mineral cyrtolite, an altered zircon found in fairly large quantities near Bedford, N. Y. The extraction of the oxychloride from the mineral was accomplished by two methods, sodium carbonate fusion and sulfuric acid extraction.

In the case of the fusion, the hafnium and zirconium in the mineral were converted to water-insoluble sodium hafnate and sodium zirconate, which were dissolved in sulfuric acid. The hydroxides of zirconium and hafnium were then precipitated from this acid solution, washed and dissolved in concentrated hydrochloric acid, and the oxychlorides were crystallized by evaporation. In the case of the sulfuric acid extraction, the mineral was treated directly with concentrated sulfuric acid and the oxychloride prepared from the acid solution by way of the hydroxide as stated above. The second method was found to be much simpler and much more efficient on large batches than the fusion.

By a combination of these two methods, a total of about 3600 grams of zirconyl chloride was obtained from 18 pounds of the mineral. This oxychloride was freed from all other substances by several recrystallizations from concentrated hydrochloric acid solutions, after each of which the crystals were washed with a 1 to 1 mixture of concentrated hydrochloric acid and reagent alcohol. The material thus obtained could be converted to an oxide which contained about 12 per cent hafnium oxide and 88 per cent zirconium oxide.

Method of Analysis

Since there is no available simple chemical process for the separation of hafnium from zirconium, any analytical method for determining the percentage of hafnium must depend either upon physical measurements or upon the conversion of one definite compound into another. By determining the density of the oxide mixture, one is able to calculate the percentage of hafnium oxide present, because the molecular volumes of the two oxides are very nearly the same. However, the determination of the density of the finely powdered oxide is attended with a certain amount of difficulty, due to adsorption of gases by the finely divided material, and the accuracy of the results is always open to question, because of uncertainty concerning the density of pure hafnium oxide. For this reason, density determinations were discarded as a method of analysis after extended trials.

The method of analysis used was based, with slight modifications, on the procedure worked out in detail by Claassen (*1*), which depends upon the fact that zirconium and hafnium form selenites of constant and definite composition which can be converted quantitatively to the oxides (*4*).

Approximately 1 gram of air-dried oxychloride was dissolved in about 200 cc. of distilled water, and to this solution were added 50 cc. of a 10 per cent solution of selenious acid. A white, flocculent precipitate of the basic selenites of zirconium and hafnium was formed. The mixture was placed on a steam bath and allowed to digest until the flocculent basic selenite was converted to the crystalline normal selenite. This process took between 12 and 15 hours, and precautions were taken to prevent evaporation of the solution. The finely powdered crystalline selenite was then washed by decantation with about 2 liters of boiling distilled water to remove all excess selenious acid. The washed powder was transferred to an evaporating dish and placed in a drying oven at a temperature of 125° to 140° C. for 10 to 12 hours.

Approximately 0.5 gram of this dried powder was accurately weighed into a tared porcelain crucible. The crucible was then heated very carefully with a Bunsen burner until no further evolution of selenium oxide could be seen, after which the burner was replaced by an air-blast Méker burner, and the crucible and oxide were ignited to constant weight. The weight of the empty crucible was checked after the ignition. From the weight of the selenite and the weight of the oxide, the percentage of hafnium oxide could be readily calculated by means of the formula

$$\% \, HfO_2 = \frac{374.86 \text{ [wt. of oxides} - 0.35702 \text{ (wt. of selenites)]}}{\text{wt. of oxides}}$$

The method is accurate to within 0.5 per cent when the amount of hafnium oxide is about 25 per cent.

Factors Influencing Separation

Effect of Hydrolysis. Because zirconium hydroxide is a somewhat weaker base than hafnium hydroxide, the hydrolysis of salts of these two elements would tend to result in a precipitate richer in zirconium than the original solution. This tendency, if not overcome, would cause a decrease in the amount of separation brought about by the ferrocyanide precipitation, because the precipitate in this case is enriched in hafnium.

The extent of hydrolysis is affected by changes in the concentrations of both the hydrolyzable substance and the hydrogen ion. Preliminary experiments indicated that these factors had negligible influence upon the degree of separation achieved. However, until a detailed study of the effect of dilution had been made, the sodium ferrocyanide was added in powdered form in order to obviate any possible harmful influence of increased dilution.

The results of these preliminary experiments also suggested that it might be unnecessary first to convert the oxychloride to the hydroxide, and several comparative series of experiments showed this to be the case.

Table I. Effect of Variation of Amount of Ammonium Sulfate on Separation

$(NH_4)_2SO_4$ Added per 100 Grams of Oxychloride	Oxychloride from Ferrocyanide Precipitate	HfO_2 in Oxide from First Precipitate	Oxychloride from Second Precipitate	HfO_2 in Oxide from Second Precipitate
Grams	*Grams*	*%*	*Grams*	*%*
0	15	12.2	0	..
30	22	15.8	47	11.0
40	14	14.7	17	10.4
60	14	13.8	0	..
80	13	12.5	0	..
95	15	12.0	0	..
120	14	12.0	0	..

Effect of Variation of Ammonium Sulfate Of the two complex-forming constituents, oxalate and sulfate ions, the former is the more powerful, and it is reasonable to expect that in its presence the ammonium sulfate would have relatively little effect. The following series of experiments was carried out, in order to determine definitely what effect, if any, the sulfate did have in the absence of the oxalic acid.

A series of solutions was made up containing known ratios of oxychloride to ammonium sulfate. To each of these solutions were added 15 grams of solid sodium ferrocyanide per 100 grams of oxychloride, as preliminary experiments had indicated this to be the optimum amount, and the precipitate thus formed was converted to the oxychloride and analyzed. In some cases, a second precipitate formed in the solutions from which the ferrocyanide precipitate had been removed. These precipitates were also converted to the oxychloride and analyzed.

The results of this series of runs are shown in Table I.

The table shows that there is a certain dependency of separation on the amount of ammonium sulfate present, but the magnitude of this effect is small. It also indicates the optimum amount of ammonium sulfate to be 30 grams for each 100 grams of oxychloride; and this would be true, were it not for an additional factor—namely, the formation of the second precipitate. The nature of this second precipitate can be understood when one remembers that a permanent precipitate will be formed when zirconyl and sulfate ions are mixed together in a molar ratio of 1 to 1 (*3*). Referring to Table I, when between 30 and 40 grams of ammonium sulfate have been added, the molar ratio becomes 1 to 1, and the precipitate forms. This precipitate is but slightly soluble, and its presence in the solution would interfere with the separation brought about by the ferrocyanide precipitation. Thus 30 grams of ammonium sulfate per 100 grams of oxy-

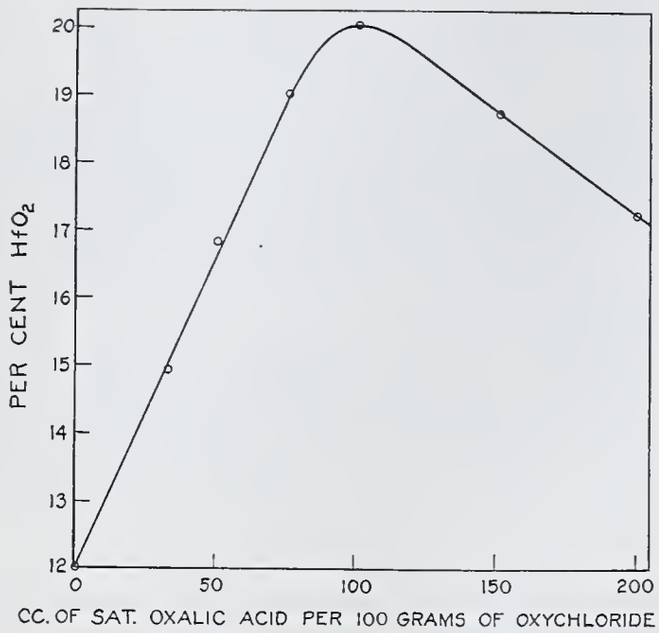

FIGURE 1. EFFECT OF OXALATE-ION CONCENTRATION

chloride cannot be recommended as the optimum amount. It was found that the best amount to use was between 80 and 100 grams. The exact amount added is not of critical importance, but a ferrocyanide precipitate which filters somewhat more easily is formed in the presence of about 100 grams of ammonium sulfate.

TABLE II. EFFECT OF SODIUM FERROCYANIDE

$Na_4Fe(CN)_6.10H_2O$ Added per 100 Grams of Oxychloride	HfO_2 in Oxide Prepared from Precipitate
Grams	%
5	18.4
10	19.7
15	20.0
20	18.8
30	15.3
45	13.3
60	12.0

EFFECT OF OXALATE-ION VARIATION. In an attempt to study the effect of the oxalate ion concentration on the separation, 10 and 20 grams, respectively, of ammonium oxalate were added to solutions of 100 grams of the oxychloride in 300 cc. of water, and the resulting solutions were treated with 15 grams of sodium ferrocyanide. The ferrocyanide precipitates thus formed were very slimy, and could not be filtered even with the use of filter aids. It was thought that the colloidal nature of this precipitate could be overcome by the presence of more electrolyte in the solution from which the precipitate was formed, and hence ammonium sulfate was added to the solution before the ferrocyanide was precipitated. However, when 10 grams of ammonium oxalate and 80 grams of ammonium sulfate were added to the oxychloride solution, a white precipitate formed which would not dissolve on dilution, but which increased in size as water was added. In order to prevent the formation of this precipitate, it was found necessary to have the solution acid, either with oxalic or with sulfuric acid.

The solutions finally used to determine the dependency of the increase in hafnium content on the amount of oxalate ion were made up to contain 100 grams of oxychloride, 30 cc. of concentrated sulfuric acid, 100 grams of ammonium sulfate, and definite known amounts of a solution of oxalic acid, saturated at room temperature, in 300 cc. of water. To each of these solutions were added 15 grams of solid sodium ferrocyanide. The precipitates thus formed were filtered, converted to the oxychloride, and analyzed. The results of this series of runs are shown in Figure 1. An examination of this curve shows that, although the optimum amount of oxalic acid solution is approximately 100 cc. of an oxalic acid solution, saturated at room temperature, for each 100 grams of oxychloride, it is not necessary to regulate this amount too closely. Any volume between 90 and 110 cc. of the oxalic acid solution is satisfactory.

EFFECT OF VARYING AMOUNT OF SODIUM FERROCYANIDE ADDED. A series of solutions was made up containing 100 grams of oxychloride, 30 cc. of concentrated sulfuric acid, 100 grams of ammonium sulfate, and 100 cc. of saturated oxalic acid solution, in 300 cc. of water. To each of these solutions was added a different known amount of solid sodium ferrocyanide, and the precipitate thus formed was filtered, converted to the oxychloride, and analyzed. The results of this series of experiments, shown in Table II, confirmed the previously drawn conclusion that the optimum amount of sodium ferrocyanide is 15 grams for every 100 grams of oxychloride in solution.

A detailed study of the effects of acid concentration and dilution was next carried out, using the optimum amounts of oxalic acid and sodium ferrocyanide and ammonium sulfate, as previously determined.

EFFECT OF ACID CONCENTRATION. A series of solutions was made up containing 100 grams of oxychloride, 100 grams of ammonium sulfate, 100 cc. of oxalic acid solution saturated at room temperature, and different known volumes of concentrated sulfuric acid in 300 cc. of water. Each solution was then treated with 15 grams of solid sodium ferrocyanide, and the precipitate thus formed was filtered, converted to the oxychloride, and analyzed. The results are shown in Table III.

These results clearly indicate, as was expected from preliminary experiments, that the concentration of free acid in the solution may vary from 0 to about 5 *N* with no noticeable effect on the separation, and that large quantities of sulfate ion in the presence of oxalate ion have no effect, which confirms the work previously described on the effect of ammonium sulfate.

It would appear that the addition of sulfuric acid is unnecessary, but, if it is not added, the ferrocyanide precipitate is difficult to filter, until about 30 cc. of acid has been added, after which no further difficulty is encountered. For this reason, the recommended amount of acid is about 30 cc. of concentrated sulfuric acid for each 100 grams of oxychloride.

TABLE III. EFFECT OF ACID CONCENTRATION

H_2SO_4 Added per 100 Grams of Oxychloride	Oxychloride Recovered from Ferrocyanide Ppt.	HfO_2 in Oxide from Ferrocyanide Ppt.	Sulfate Present per 100 Grams of Oxychloride	*N* (Approx.) with Respect to H_2SO_4
Cc.	*Grams*	%	*Moles*	
0	16	19.5	0.7	...
15	15	20.0	1.0	1.3
30	15	20.0	1.3	2.6
60	15	20.2	1.8	5.2

EFFECT OF DILUTION. In order to determine in more detail the effect of dilution, a series of precipitations was made from solutions, the total volumes of which were 300, 400, 500, 800, 1000, and 1500 cc. In no case was there any difference in the amount of separation realized, thus confirming the conclusions drawn from preliminary experiments. However, in the more dilute solutions, the ferrocyanide precipitate was much more difficult to filter, and for this reason it was found advisable always to precipitate from a solution having a total volume of between 400 and 600 cc. per 100 grams of oxychloride.

EFFECT OF TIME INTERVAL BETWEEN PRECIPITATION AND FILTRATION. Since there was a possibility that the composition of the ferrocyanide precipitate might change on standing in contact with the solution from which it was formed, a

series of experiments was made in which the time before filtration was varied. The shortest time interval between precipitation and filtration was 1 hour, the longest was 2 weeks. In some cases stirring was provided, in others the mixture was not stirred. In no case was there any difference in the amount of separation, but the precipitates which had stood for some time were much easier to filter. The best results were obtained when the precipitate was allowed to stand overnight before filtration. Longer standing tended to cause a certain amount of decomposition of the ferrocyanide, and since this introduced iron into the solution, it was objectionable.

Effect of Successive Ferrocyanide Precipitations. All the previous discussions have dealt with a single ferrocyanide precipitation; in each case the solution remaining still contained a major portion of the zirconium and hafnium originally present. It seemed reasonable to expect that further precipitations could be made from this same solution, and that the subsequent precipitates should be of varying hafnium content. A series of experiments was made in which the solution from the first precipitation was treated with successive portions of sodium ferrocyanide, until five precipitations in all had been made from the initial solution. Each of the precipitates was analyzed (Figure 2).

This curve shows that, by starting with a material containing about 12 per cent hafnium oxide, five fractions could be obtained in which the composition varied from 19 per cent to 3.8 per cent hafnium oxide. The amounts of each of these fractions were approximately the same, about 15 grams per 100 grams of oxychloride initially used.

Recommended Procedure

As a result of the previous detailed studies of the conditions for the separation of hafnium from zirconium by the ferrocyanide method, the following procedure is recommended as giving the maximum separation:

The oxychloride is dissolved in 300 cc. of water for each 100 grams of the oxychloride. To this solution are then added 30 cc. of concentrated sulfuric acid, 100 grams of ammonium sulfate, and 100 cc. of oxalic acid solution, saturated at room temperature, for each 100 grams of oxychloride. The solution at this point should be perfectly clear. If there is a white precipitate, an extra 50 cc. of water should be added, and the solution left standing until it is clear. To this is added, for each 100 grams of oxychloride, a solution containing 15 grams of sodium ferrocyanide in about 50 cc. of water—a nearly saturated solution. The ferrocyanide solution is added dropwise from a buret over a period of several hours, while the zirconium-hafnium solution is being stirred vigorously.

The yellow precipitate formed by the addition of the sodium ferrocyanide to the zirconium-hafnium solution should be allowed to stand for at least 1 hour, and preferably for 10 or 12 hours before filtration. Suction is used for the filtration, and the precipitate is drained as dry as possible but is not washed. The precipitate is then transferred from the funnel to a large beaker, where it is suspended in water. To this suspension is added ammonium hydroxide, which converts the zirconium and hafnium ferrocyanides to the hydroxides. The hydroxide precipitate, after being washed by decantation until all the ferrocyanide has been removed, is dissolved in concentrated hydrochloric acid, from which solution the oxychlorides may be obtained by evaporation.

The filtrate from the ferrocyanide precipitation, which still contains a major portion of the zirconium and hafnium, may be treated with further portions of sodium ferrocyanide and thus separated into several fractions of varying hafnium-zirconium content. In each case the ferrocyanide precipitate is treated as outlined above. The final filtrate after the desired number of ferrocyanide precipitations have been carried out will, in most cases, still contain some hafnium and zirconium which must be recovered. This is done by precipitating the hydroxides, washing them, dissolving them in concentrated hydrochloric acid, and evaporating to obtain the oxychlorides.

Each of the fractions thus obtained can be further fractionated in the same manner, and by combining fractions having the same hafnium-zirconium ratio, and continuing the precipitations, the zirconium and hafnium can gradually be separated from each other in any desired state of purity.

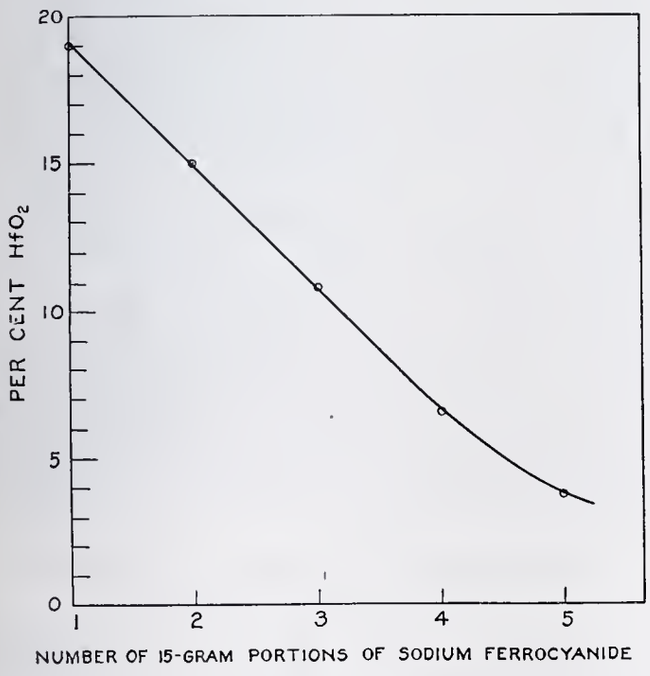

Figure 2. Effect of Successive Ferrocyanide Additions

Using the procedure outlined above, in four successive ferrocyanide precipitations the hafnium oxide content of an oxide mixture was enriched as follows: 12 to 20 per cent; 20 to 36 per cent; 36 to 62 per cent; and 62 to 80 per cent.

Summary

A systematic study of the factors involved in the separation of hafnium from zirconium by the ferrocyanide method, proposed by Prandtl, has resulted in modifications of the procedure to give the maximum degree of separation.

The factors studied include the dilution and acid concentration, each of which affect the extent of hydrolysis of the zirconium and hafnium ferrocyanides precipitated, the amounts of ammonium sulfate, oxalic acid, and sodium ferrocyanide added, the time interval allowed between precipitation and filtration, and the effect of successive ferrocyanide precipitations carried out upon the same initial solution. The optimum conditions for each of these factors have been determined.

By the procedure described an oxide mixture containing initially 12 per cent hafnium oxide and 88 per cent zirconium oxide was converted in four operations into an oxide mixture containing 80 per cent hafnium oxide and 20 per cent zirconium oxide.

The Claassen method for the analysis of zirconium-hafnium mixtures has been found entirely satisfactory as to accuracy and reproducibility of results. Certain minor modifications have been made in the technique to adapt it to the present problem.

Large quantities of hafnium-high zirconyl chloride can be easily extracted from Bedford (N. Y.) cyrtolite by means of concentrated sulfuric acid.

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Determination of Arsenic in Organic Compounds

An Iodometric Semimicroprocedure

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IN THE method described the substance is decomposed by action of sulfuric and nitric acids, and the arsenic is precipitated by hypophosphorous acid. The arsenic is collected on a filter and is then dissolved by action of excess bromine (from Koppeschaar's bromate-bromide solution) in measured amount. The excess bromine is determined iodometrically. This procedure is well suited to the analysis of relatively small (semimicro) samples, for the reason that in the oxidation involved ($As^0 \rightarrow As^5$) the equivalent weight of arsenic is one fifth the atomic weight. In familiar methods involving trivalent and pentavalent arsenic the equivalent weight is one half the atomic weight.

Analytical Reduction of Arsenic to the Elementary State

Available methods include reduction by stannous chloride and concentrated hydrochloric acid (Bettendorff reaction, *7*), by hypophosphorous acid (*35*), by chromous sulfate (*33*), or by titanous chloride in the presence of Rochelle salt (*22, 32*). The last two were not submitted to trial, as they require inconvenient precautions and are relatively expensive.

The Bettendorff reaction, familiar as a qualitative test (*7, 11*), was applied quantitatively by Andrews and Farr (*3*), who introduced tartaric acid into the reagent to prevent contamination of the precipitated arsenic by tin. The separated arsenic is determined by dissolving in a measured volume of standard iodine, excess of which is finally titrated. This method has been used for the determination of arsenic in nonferrous alloys (*20*), in metallurgical agglomerates (*31*), and in steels (*27, 30*).

Reduction by hypophosphorous acid (*12*) was recommended by Thiele (*35*) for detection of arsenic, and was applied quantitatively by Engel and Bernard (*13*). The solution of sodium hypophosphite and hydrochloric acid used for the reduction has been called Thiele's reagent (*5, 29, 35*) and also Bougault's reagent (*8*). Reduction by hypophosphorous acid has been applied to the determination of arsenic in metals and ores (*9, 14–17, 37*). In these methods, as in those involving the Bettendorff reaction, the filtered and washed element is determined volumetrically by means of excess standard iodine solution. Excess iodine is best titrated with standard arsenite solution (*14*) in buffered alkaline solution. Titration with thiosulfate is inadmissible whether conducted in alkaline buffered solution (*1, 6, 36*) or in acid solution (*25*). In the microprocedure of Kolthoff and Amdur (*23*) the elementary arsenic is dissolved in standard ceric sulfate solutions, excess of which is titrated with standard arsenious acid solution.

The hypophosphorous acid reagent was found by Grippa (*19*) to be more sensitive than the Bettendorff-Andrews-Farr reagent. This was confirmed in the present study; comparative trials showed that hypophosphorous acid caused more complete and much more rapid separation of arsenic than did stannous chloride. Hypophosphorous acid is preferable also because it does not introduce the necessity for use of an acid wash liquid, as when stannous chloride is used. For these reasons reduction by hypophosphorous acid was selected for incorporation into the procedure under development.

Thiele (*35*) reported the sensitivity of the hypophosphorous acid test for arsenic to be increased by presence of iodide. In trials to test this claim, solutions containing arsenious acid, sulfuric acid, and potassium iodide were treated with sodium hypophosphite and hydrochloric acid. On heating the liquid a yellowish solid volatilized from the flask, and an odor like that of hydrogen sulfide was noticed. Following this abnormal behavior the results obtained for arsenic were too high. These phenomena were observed only when reduction was attempted in the presence of iodide. An explanation has not been sought, for the reason that no interference from this cause occurs in the method to be described, the initial decomposition serving to free the mixture of any iodine present in the substance analyzed. It is suggested that the iodide (as hydriodic acid) may reduce some sulfuric acid to sulfurous acid, and that the latter may be reduced to sulfur by hypophosphorous acid, as shown by Maquenne (*28*). The behavior described recalls the comment made by Andrews (*2*): "At times, under conditions which were not more closely investigated, arsenic precipitated by hypophosphorous acid contains small amounts of phosphorus, whether in the free state or in combination with hydrogen or with arsenic it is difficult to say."

Thorough washing of the precipitated arsenic is essential. Preliminary experiments yielded high results, eventually traced to incomplete removal of hypophosphorous acid from the arsenic on the filter. It was found that if the precipitate was washed with hot recently boiled water until the washings were free of chloride, the arsenic retained no interfering substance. The use of air-free water for washing is a precaution against possible air oxidation of arsenic, a matter which has been the subject of disagreement. Thiele (*35*) reported air oxidation of freshly precipitated arsenic to be too rapid to permit the isolation of the element in a quantitative method. Evans (*15*) recommended a solution of ammonium chloride as a wash liquid which, for reasons not stated, would protect the arsenic from oxidation by air. Cooke (*10*) reported that oxidation of precipitated arsenic during washing with freshly boiled hot water is negligible. In the present study qualitative tests of both filtrates and washings showed presence of no appreciable amounts of arsenic which had escaped reduction by hypophosphorous acid or which had dissolved in the wash liquid as a result of oxidation on the filter.

Iodometric Determination of Arsenic after Removal as the Element

Unsatisfactory features of methods previously described are the relatively slow rate of solution of arsenic (especially if densely granulated) in dilute iodine solution, and the difficulty in detecting presence of undissolved particles of arsenic

in the liquid deeply colored by iodine and containing flocculent masses of filter paper pulp. Both difficulties are excluded by use of bromine (from Koppeschaar's bromate-bromide solution, *26*) instead of iodine for bringing the arsenic into solution, an innovation by which the procedure is much improved at this point.

To complete the analysis potassium iodide is added, the solution is buffered, and the iodine (representing excess bromine) is titrated with standard sodium arsenite solution. For the necessary buffering of the acid solution sodium carbonate was judged not desirable because of possible loss of iodine with escaping carbon dioxide. The use of borax led to undesirable increase in the volume of the liquid, owing to the relatively low solubility of this salt in cold water. Disodium phosphate, recommended by Washburn (*38*), was found to be satisfactory in every respect.

Decomposition of Organic Compounds for Analysis

The use of sulfuric acid and potassium persulfate, previously found effective in the analysis of organic mercury compounds (*18, 34*), caused insufficiently rapid decompositions of several arsenic compounds, even in the presence of copper added as a catalyst. Comparative trials of decomposition by concentrated sulfuric acid in conjunction with powdered potassium permanganate, potassium nitrate, or nitric acid (Neumann's method), showed the last mentioned to be the most rapid and satisfactory. Undecomposed nitric acid is removed by evaporating the liquid to fumes of sulfuric acid, and traces of nitrogen oxides which may persist (and which might interfere later) are eliminated by addition of ammonium sulfate to the hot digestion mixture. During the decomposition of arsenic compounds in the presence of halogen (chlorine, bromine) no loss of arsenic occurred by escape of volatile halide.

Analytical Procedure

Apparatus. The all-glass apparatus recommended (*34*) for use in the determination of mercury in organic compounds is suitable also for the analysis of organic arsenic compounds as described below.

Decinormal Sodium Arsenite Solution. Dissolve rapidly about 5 grams of pure arsenic trioxide (accurately weighed) in water containing 10 grams of sodium hydroxide, and at once add dilute sulfuric acid until the solution is just acid to litmus (*24*). If the solution is diluted to a liter in a calibrated flask the normality may be calculated. An approximately decinormal solution may be standardized as follows, using potassium iodate as a primary standard:

Transfer to a 250-ml. glass-stoppered Erlenmeyer flask 25 ml. of 0.1 *N* potassium iodate solution, or an accurately weighed portion (about 0.1 gram) of the pure salt, and add water to 50 ml. Add 5 ml. of 20 per cent potassium iodide solution and 2 ml. of 6 *N* sulfuric or hydrochloric acid, stopper the flask, mix the contents, and allow to stand one minute. Remove the stopper, rinse it with water, add 30 ml. of phosphate solution (350 grams of $Na_2HPO_4.12H_2O$ per liter of water), and titrate the liberated iodine with the sodium arsenite solution, adding 3 ml. of 0.5 per cent soluble starch solution just before reaching the end point.

Procedure. Weigh the sample (of size to yield approximately 15 mg. of arsenic) on a piece of cigaret paper or in other convenient manner and transfer to the flask. Introduce 2 ml. of concentrated sulfuric acid and warm the mixture gently, while agitating the flask, until the cigaret paper is consumed and the sample is completely dispersed in the acid. Support the flask in an inclined position in the hood and from a pipet introduce in single drops 1 ml. of concentrated nitric acid. When the initial vigorous reaction has subsided, apply to the lower tip of the flask the small flame of a "micro" burner. Regulate the heating so that the nitric acid slowly evaporates but without active ebullition of the liquid. When the removal of nitric acid is complete and fumes of sulfuric acid appear, the digestion mixture is usually not darker than a light straw color. If the liquid is dark in color at this point, allow the flask and contents to cool somewhat, and repeat the treatment with 1 ml. of concentrated nitric acid. Remove excess nitric acid as before and when light fumes of sulfuric acid appear allow the mixture to cool slightly and add cautiously 1 gram of powdered ammonium sulfate. When gas evolution ceases, rotate the flask and heat gently. The liquid should be colorless while still hot; a light yellow color indicates presence of nitrogen oxides. The decomposition ordinarily requires 15 to 30 minutes.

Table I. Determination of Arsenic in Pure Arsenic Trioxide

(By method described with omission of decomposition procedure)

Arsenic Taken Mg.	Arsenic Found Mg.	Error Mg.	Error %
25.52	25.28	−0.26	−0.9
25.52	25.25	−0.27	−1.0
25.52	25.84	+0.32	+1.2
10.23	10.31	+0.08	+0.8
10.23	10.27	+0.04	+0.4
10.23	10.22	−0.01	−0.1
10.23	10.23	±0.00	±0.0

Allow the flask to cool, wash the mouth and walls with water, and mix the contents (volume about 15 ml.). Add 3 grams of sodium hypophosphite ($NaH_2PO_2.H_2O$) and at once agitate the flask so as to dissolve the salt rapidly. If the sodium hypophosphite is allowed to stand in the liquid it quickly forms a hard cake and then dissolves slowly. Dilute the mixture to about 30 ml., add 35 ml. of concentrated hydrochloric acid, and mix. Ignore any solid which separates at this point, as it redissolves during the subsequent heating. Attach the condenser and heat the flask gently with a small flame. Precipitation of elementary arsenic begins in about 2 minutes and is apparently complete in about 5 minutes, as is indicated by the fact that no further darkening of the contents of the flask occurs. Regulate the heating so that the liquid just begins to boil after about 10 minutes, and then continue gentle boiling for 2 minutes. Wash the inner wall of the condenser by water introduced at the top, and again boil the liquid gently for one minute. Prolonged boiling is to be avoided, as it was found to yield an arsenic precipitate of such character that effective washing was difficult. Allow the flask and its contents to cool somewhat, rinse the condenser, and then withdraw it and set it aside.

Decant the solution through a retentive 11-cm. filter paper (Whatman No. 44 was used) retaining in the flask as much of the precipitate as possible. Wash the precipitate in the flask three or four times by decantation with freshly boiled hot water. Finally, wash the filter with hot water until the washings show no more than a trace of chloride when tested with silver nitrate solution. Transfer the filter paper and contained precipitate to the flask after using the folded filter to pick up any of the arsenic which may have crept onto the funnel. Wipe the flange and lip of the flask with a fragment of moistened filter paper and add this to the contents of the flask.

Introduce about 25 ml. of water, and then add from a pipet 15 ml. of Koppeschaar's solution (2.8 grams of potassium bromate and 50 grams of potassium bromide per liter). While holding the flask quiet add rapidly 2 ml. of 6 *N* hydrochloric acid and at once insert the ground-glass stopper. Shake the flask vigorously until the filter paper is completely disintegrated and no dark particles of arsenic are visible; this should require less than one minute. Immerse the flask in ice water to produce subnormal pressure within, and after a minute or two pour 5 ml. of 20 per cent potassium iodide into the gutter of the flask, and loosen the stopper slightly to allow the potassium iodide to pass into the flask. Shake the tightly stoppered flask to absorb in the liquid, the vapors of bromine, then remove the stopper and rinse it and the mouth of the flask with water. Add to the flask 40 ml. of phosphate buffer solution (350 grams of crystallized disodium hydrogen phosphate, $Na_2HPO_4.12H_2O$, per liter of water) and mix the contents well.

Titrate the iodine with the 0.1 *N* sodium arsenite solution. When the iodine color is faint, stopper the flask and shake well to dislodge traces of iodine adherent to the paper. Then add 3 ml. of 0.5 per cent solution of soluble starch and complete the titration.

Determine the effective strength of the bromate-bromide solution in a blank titration conducted exactly as described above, introducing a filter paper to compensate any effect due to the filter paper present in the analysis. Calculate the arsenic present from the difference between the two titrations (1 ml. of 0.1000 *N* arsenic represents 1.4982 mg. of arsenic).

Experimental Results

The accuracy of the procedure described, exclusive of the decomposition of organic material, was tested by determinations of arsenic in aliquot portions of a solution prepared from Bureau of Standards arsenic trioxide in the same manner as was the standard arsenite solution. The results, shown in Table I, indicate that accuracy is higher when relatively small quantities of arsenic are present. In this case also the small size of the precipitate of arsenic shortens the operation of washing.

In order to determine whether or not arsenic is lost during decomposition of organic matter, samples of Bureau of Standards arsenic trioxide were weighed on cigaret paper and carried through all the steps of the procedure described above. The results shown in Table II indicate loss of arsenic during the procedure to be relatively small.

Table II. Determination of Arsenic in Arsenic Trioxide by Complete Procedure

Arsenic Taken	Arsenic Found	Error	
Mg.	*Mg.*	*Mg.*	%
13.92	13.82	−0.10	−0.7
13.70	13.66	−0.04	−0.3

Analytical Results for Organic Compounds of Arsenic

Results obtained in the analyses of organic arsenic compounds are presented in Table III.

The compounds (except neoarsphenamine) were obtained from the Eastman Kodak Company, and were purified by crystallization prior to use. Neoarsphenamine was obtained from the Mallinckrodt Chemical Works; samples were withdrawn from freshly opened ampoules and weighed rapidly. Most of these compounds are arsonic or arsinic acids, some of which do not have well-defined melting points. In such cases the purity of each compound was judged from the determined neutralization equivalent. The monobasic disubstituted arsinic acids can readily be titrated with standard alkali, using phenolphthalein as indicator. The dibasic primary arsonic acids cannot be titrated to a sharp end point corresponding to either one or both acid hydrogens by the usual procedures. King and Rutterford (*21*) found that the neutralization equivalents of primary arsonic acids can be determined by titrating them with standard alkali, using thymolphthalein indicator in a solution which is half-saturated with sodium chloride. This procedure was used for determining the neutralization equivalents of the primary arsonic acids analyzed.

The sample (about 0.2 gram) is transferred to a 50-ml. flask, two drops of 0.1 per cent thymolphthalein in alcohol are added, and then 0.1 *N* sodium hydroxide (standardized against Bureau of Standards acid potassium phthalate) is run in until all the sample is dissolved and the solution assumes a definite blue color. The volume of the contents of the flask is approximately doubled by addition of saturated sodium chloride, neutral to thymolphthalein, and the bleached solution is titrated with the alkali to a definite blue end point.

The neutralization equivalents determined in this way are shown in Table III.

That the method for arsenic described is applicable to halogen-containing compounds was shown by analysis of organic arsenic compounds in the presence of added organic compounds containing chlorine or iodine (Table III). The results show that the presence of halogens causes no loss of arsenic during decomposition and does not interfere with the subsequent procedure.

The results in Table III show the method to be satisfactory with respect to accuracy and precision. The time required for a single analysis is about 2 hours. If several flasks are available, time can be saved by running analyses on an overlapping schedule.

Table III. Determination of Arsenic in Organic Compounds

Compound	Neutralization Equivalent Found	Neutralization Equivalent Calculated	Sample	Arsenic Found	Arsenic Calculated
			Mg.	%	%
Diphenylarsinic acid, Eastman No. 2208, m. p. 174°	261.0 262.5 Av. 261.8	261.9	34.27 31.74 33.43 45.72	28.46 28.56 28.85 28.48 Av. 28.59	28.60
Phenylarsonic acid, Eastman No. 2020	100.5 102.1 Av. 101.3	101.0	41.94 43.13	37.22 37.13 Av. 37.18	37.10
m - Nitrophenylarsonic acid, Eastman No. 4469	123.1 122.9 Av. 123.0	123.4	41.34 47.48	30.65 30.46 Av. 30.56	30.34
Arsanilic acid, Eastman No. 1369	107.0 107.3 Av. 107.2	108.4	47.56 47.30	34.59 34.58 Av. 34.58	34.53
Diphenylarsinic acid with 50 mg. of iodobenzoic acid	(261.8)	261.9	52.71 50.64	28.70 28.61 Av. 28.66	28.60
Diphenylarsinic acid with 50 mg. of chlorobenzoic acid	(261.8)	261.9	50.80 51.14	28.54 28.50 Av. 28.52	28.60
p - Hydroxyphenylarsonic acid[a], Eastman No. 4836, m. p. 171°			42.50 42.77	34.24 34.30 Av. 34.27	34.37
n-Butylarsonic acid, Eastman No. 1892, m. p. 160°	93.3 92.9 Av. 93.1[b]	91.0	40.43 41.22	40.65 40.81 Av. 40.73	41.18[b]
p-Benzarsonic acid, Eastman No. 2082	82.6 82.6 Av. 82.6[c]	82.0	53.62 55.25	29.90 30.00 Av. 29.95	30.46[c]
Neoarsphenamine, U. S. P. XI, Mallinckrodt No. 3676			41.21 46.20	19.62 19.78 Av. 19.70	19.37[d] 19.53[d] Av. 19.45[d]

[a] Neutralization equivalent could not be determined, probably because dissociation constant for phenolic hydrogen is close to that of indicator (*21*).

[b] Neutralization equivalent found indicates a purity of 97.7%; if impurity is an inert substance arsenic content should be 40.3%. If impurity is an acid or a base, whether arsenical or not, it will have a different effect upon neutralization equivalent than upon arsenic content.

[c] Neutralization equivalent found indicates a purity of 99.3%; if impurity is an inert substance arsenic content should be 30.2%; see [b].

[d] Values represent analyses made by an umpire method, involving distillation of arsenic trichloride and titration with potassium bromate standardized against arsenic trioxide. Procedure is essentially that of Association of Official Agricultural Chemists for determination of arsenic in iron-arsenic tablets (*4*).

Summary

A semimicromethod is described for the determination of arsenic in organic compounds. It involves decomposition of the sample by action of hot concentrated sulfuric and nitric acids, precipitation of elementary arsenic by hypophosphorous acid, solution of the filtered and washed arsenic in excess bromine (acidified Koppeschaar solution), and iodometric determination of excess halogen by titration with standard sodium arsenite in a solution buffered with disodium phosphate. Results indicate the accuracy and range of applicability of the method. The procedure is convenient, moderately rapid, and applicable in presence of halogens.

Acknowledgment

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A New Procedure for Detecting Acidity[1]

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A RECENT paper (*1*) from this laboratory described in detail a sensitive procedure for detecting "basicity" in both soluble and slightly soluble materials, such as hydroxides, oxides, phosphates, fluorides, organic bases, and the salts of weak organic acids. The reagents for this delicate test are prepared by treating neutral solutions of copper or nickel sulfate, chloride, or nitrate with a deficit of dimethylglyoxime, thionalide (β-aminonaphthalide), or cupron (benzoinoxime) and then filtering. A typical instance, nickel solution treated with dimethylglyoxime (=DH_2), can be represented:

$$Ni^{++} + 2DH_2 = Ni(DH)_2 + 2H^+$$

The filtrates from these incomplete precipitations are slightly acid equilibrium solutions; each has a characteristic pH value. If a solution of this type is brought into contact with a material that decreases the effective hydrogen-ion concentration of the reagent, a highly visible precipitate of the inner-complex salt is produced at once: nickel dimethylglyoxime (red), nickel thionalide (black-brown), or copper benzoinoxime (green). This procedure has proved useful in a variety of practical cases, and has also found microchemical application in connection with spot tests.

The successful analytical use of a procedure based on disturbing an established equilibrium in solutions of complex salts naturally led to attempts to extend this principle to other systems of this nature. Such solutions always present an equilibrium between the complex compound (or its ions) and the materials that have united to form the complex. The stability of the complex (or its ions) determines the position of the equilibrium.

Equilibrium solutions of silver-ammine chromate were studied with a view to applying them in this new type of test. It was found that they can be used to detect materials that consume acid, ammonia, or ethylenediamine.

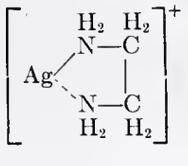
Silver chromate dissolves readily in excess ammonia water. The yellow solution (CrO_4^{--} ion) contains the complex salt $[Ag(NH_3)_2]_2CrO_4$. Therefore, if silver chromate is digested with a quantity of ammonia water insufficient to bring about complete solution, there will be obtained, on filtration, a yellow solution that smells of ammonia. In this there have been established the equilibria

$$Ag_2CrO_4 + 4NH_3 \rightleftarrows [Ag(NH_3)_2]_2\,CrO_4 \rightleftarrows 2Ag(NH_3)_2^+ + CrO_4^{--}$$

and any removal of ammonia will lead to the precipitation of the highly colored silver chromate. Consequently, this type of ammoniacal solution of silver chromate can serve to reveal the presence of materials that consume ammonia by uniting with it to form an ammonium salt, react with it to form an ammoniate, or consume ammonia in the precipitation of a hydroxide.

The ammoniacal solution of silver chromate has the practical defect that it loses ammonia unless the reagent is stored in a tightly stoppered vessel. This loss results in the formation of a precipitate, even in the absence of ammonia-consuming materials. If a drop of the solution is placed on filter paper a brown spot of silver chromate appears in a few minutes. On a spot plate the solution decomposes more slowly, and tiny crystals of black-brown silver chromate separate on the surface of the drop. Nonetheless, the ammoniacal solution can be used for the present purpose because the silver chromate produced by the slow breakdown of the solution differs in form and color from that arising immediately as the result of chemical action.

A stable silver chromate equilibrium solution can be prepared by dissolving the salt in aqueous ethylenediamine (=en). Since this base boils at 118° C., its vapor pressure in a silver-en chromate solution is so slight that the reagent can even be kept at water-bath temperature for as long as 5 minutes without danger of decomposition. The silver-en chromate is far more stable than the analogous silver ammonia chromate because coordination of the en-molecule on the silver ion produces a five-membered ring



[1] Translated by Ralph E. Oesper, University of Cincinnati.

It is well established that such auxiliary valence rings contribute markedly to the stability of the respective complex ions. In line with this is the fact that silver-en chromate can be isolated by crystallization, quite in contrast with the unstable ammonia compound. Solutions of the en-salt present the equilibrium

$$2[\text{Ag-en}]^+ + CrO_4^{--} \rightleftarrows 2\text{en} + Ag_2CrO_4$$

The equilibria in these ammoniacal and ethylenediamine-silver chromate solutions will be shifted above all by materials that furnish hydrogen ions which combine with ammonia or ethylenediamine

$$NH_3 + H^+ \rightarrow NH_4^+ \text{ or en} + H^+ \rightarrow \text{enH}^+$$

and so withdraw these basic materials from the complex. This causes a precipitation of silver chromate. All water-soluble acids, acid salts, and salts whose solutions react acid because of hydrolysis, affect these reagents for this reason.

It is worthy of special note that anhydrides which are not soluble or only slightly soluble in water react positively with silver chromate equilibrium solutions. Examples are tungstic anhydride, molybdic anhydride, and silica. In such cases it is difficult to decide whether this result is due to withdrawal of ammonia by the acid anhydride to form an ammonium salt, for instance

$$MoO_3 + 2NH_3 + H_2O \rightarrow (NH_4)_2MoO_4$$

or whether the ammonia is adsorbed on the surface of an acid adsorbent and thus immobilized. The latter explanation appears the more likely in the case of silica.

A positive reaction with these reagents is also shown by solutions of salts that form ammoniates: cobalt, nickel, and cadmium sulfates, for instance.

The shifting of equilibria in these solutions has provided a sensitive means of solving certain analytical problems. Special emphasis needs to be placed on the necessity of excluding halides in these tests, because they will disturb the equilibrium by forming silver halide.

Experimental

Reagents. Silver-Ammonia Chromate. Silver chromate, produced by the interaction of silver nitrate and potassium chromate, is well washed, and small portions of the moist salt are vigorously swirled with 6 *N* ammonia until considerable quantities of the solid remain undissolved. The suspension is allowed to stand one hour, with occasional swirling, and then filtered into a bottle that can be tightly stoppered. If used repeatedly, small amounts of silver chromate are deposited because of the loss of ammonia, but the reagent can be restored to usefulness by merely filtering.

Silver-Ethylenediamine Chromate. Freshly precipitated, moist silver chromate is shaken continuously with an aqueous solution of ethylenediamine kept at 60° C. Additional portions of the salt are introduced until considerable quantities remain undissolved. The suspension is filtered, and the filtrate allowed to stand 12 hours at room temperature. Large crystals of $[\text{Ag-en}]_2CrO_4$ are deposited; these are collected on a filter, washed once with ice-cold water, and then dissolved in warm water. The solution is stable for weeks.

Procedure

One drop of the unknown solution or a few milligrams of the solid to be tested are placed on a white spot plate and treated with 1 to 2 drops of one of the reagents. If an acid or a base-consuming compound is present in the sample an immediate precipitation occurs; the quantity of red-brown silver chromate indicates roughly the proportion of active material in the sample. If the ammoniacal reagent is employed, fine black crystals will always appear on the surface of the liquid after a few minutes because of the loss of ammonia. This should not lead to unwarranted conclusions.

Applications

The silver chromate equilibrium solutions react positively with all soluble and almost all slightly soluble compounds of acid character, with salts whose solutions are acid, and with those metal ions that can form the more stable ammine ions. The present discussion is limited to applications that are new and interesting to the analytical chemist.

Behavior toward Silica. Hydrous silica was prepared from solutions of alkali silicates by repeated evaporations with hydrochloric acid on the water bath. The residue was washed with hot water until all traces of acid were removed. A deep red-brown appeared at once when this hydrated silica was tested on a spot plate with 1 to 2 drops of reagent. Prompt reaction was shown also by samples of this product that had been exposed to the air for several days, or that were partially dehydrated (aged) at 140° C. Another portion of the hydrous silica was ignited to constant weight in platinum; during the ignition samples were removed from time to time and tested. The reaction was positive in all cases. A sample that had been brought to constant weight was ignited for 4 hours longer. It produced a color that was less intense than that brought forth by the unignited material, or by the portions that had not been blasted so long.

Finely ground sand and quartz—that is, naturally occurring crystalline silica—showed no reaction, even after long contact with the stable silver-en chromate equilibrium solution. Some natural silicates (certain zeolites, kaolin, opal, garnierite) gave definite positive results, while others (amazonite, talc, muscovite) showed no reaction whatsoever.

The dissimilar behavior of different forms of silica and natural silicates is noteworthy and requires comment. Precipitated and ignited silica is resistant to alkalies at ordinary temperatures; partial solution occurs only on warming with concentrated lyes. Therefore the immediate precipitation of silver chromate from the equilibrium solutions by silica is certainly not due to the formation of soluble ammonium, or ethylenediamine silicate. Since silica cannot be credited with being a source of hydrogen ions, the assumption is compelled that some forms of silica have the ability to adsorb ammonia or ethylenediamine. This surface adsorption of the base has the same effect as reaction with hydrogen ions—namely, inactivation—the equilibrium is disturbed, and silver chromate comes out of solution.

It appears that only amorphous silica has the ability to affect silver chromate equilibrium solutions; the crystalline varieties are without this power. Not enough cases have been studied to warrant the general statement that all forms of amorphous silica are reactive in this sense. This property may possibly be possessed only by those varieties that are characterized by exceptionally great surface development. The diminution of activity with prolonged ignition indicates that the free surface plays a decisive role. The varying behavior of silica toward these reagents may be useful in petrography with reference to characterizing certain silicate rocks.

It seems likely that the gangue of ores consists, in part at least, of amorphous silica. The acid-free washed residues of magnesites, limestones, and pyrolusites from various sources gave distinct positive reactions. The silica obtained by dissolving a number of commercial irons in acid also responded to this test. This method of detecting silicic acid can be employed in organic qualitative analysis to test "acid-insoluble residues" for silica.

Behavior toward Metal-Acid Anhydrides. Tungstic and molybdic anhydrides are not soluble in water, but they react with silver chromate equilibrium solutions. A few grains of the powdered oxide immediately turn red-brown if spot-tested with 1 to 2 drops of the reagent.

If an alkali tungstate is evaporated with hydrochloric acid, and the resulting yellow tungstic acid is thoroughly washed to remove any adherent hydrochloric acid, the product gives a strikingly strong positive reaction. Minute quantities of tungstic acid are sharply revealed if moistened on a filter paper with silver-en chromate reagent. If the tungstic acid is ignited for several hours in platinum, the reactivity toward the equilibrium solution gradually diminishes but never disappears entirely.

The anhydride of vanadic acid gives an immediate reaction, but this is not seen so distinctly as in the case of molybdic and tungstic anhydride, because the brown vanadium pentoxide masks the result to some extent.

The behavior of niobic and tantalic anhydride is remarkable. Two milligrams of the commercial oxides were tested on a spot plate with 2 drops of silver-en chromate solution. A distinct reaction was observed at once with niobic pentoxide. In contrast, tantalic anhydride gave no color; even after standing in contact with the reagent for an hour, nor when the test mixture was warmed briefly by a current of hot air. Since the positive reaction with the niobic anhydride might have been due to silica in the specimen, 2 grams of the oxide were heated in a platinum crucible with hydrofluoric and sulfuric acid and fumed to dryness, and the residue was ignited for 2 minutes. This silica-free niobic anhydride reacted about as definitely as freshly precipitated silicic acid. Tantalic anhydride subjected to this same treatment gave a weak but detectable reaction. These purified oxides were then ignited for an hour, pulverized, and retested. In both cases the result was now negative. These experiments were repeated several times; the findings were always the same. Consequently niobic anhydride can be definitely distinguished from tantalic anhydride by the silver-en chromate test, provided the samples have not been ignited too long. A spectrographic examination of the niobic oxide before and after ignition might reveal traces of some other more volatile, more reactive oxide that is removed by the ignition.

The activity of the water-insoluble metal acid anhydrides toward the equilibrium solutions is, in all likelihood, to be ascribed not to the formation of an ammonium salt, but rather to adsorption of ammonia or ethylenediamine. This hypothesis is particularly applicable in the cases of tungstic and niobic anhydrides, whose activity falls off distinctly or even vanishes on prolonged, intense heating.

Behavior toward Hydrolyzable Salts. Salts derived from weak bases and strong acids hydrolyze in aqueous solution. These solutions exhibit an acid reaction toward the usual indicators and cause an immediate precipitation of silver chromate from the reagents. Since the hydrolysis of the salt comes to equilibrium these hydrolyzed solutions are likewise equilibrium solutions, and consequently their reaction with the silver-en chromate reagent presents the interesting case of the mutual interaction of two equilibrium solutions.

Aqueous solutions of ferric, chromic, titanic, bismuth, and zirconium sulfate react at once with silver-en chromate solution. Likewise, basic salts, such as the evaporation and ignition residue of alum, give an immediate color when spot-tested with this reagent.

The high sensitivity of this test for aluminum is noteworthy. On a white spot plate as little as 1 microgram of aluminum can be plainly detected in 1 drop (0.15 ml.) of aluminum sulfate solution. The test is still more sensitive if one drop of the aluminum solution is evaporated to dryness on a porcelain crucible lid and the residue is then spot-tested. In this way it is possible to detect as little as 0.4 microgram of aluminum.

A striking demonstration is provided by the following comparative procedure. Two 3-ml. portions of a 1 to 250,000 aluminum sulfate solution are put into test tubes. One specimen is treated with several drops of ammonia water, the other with about 0.1 ml. of silver-en chromate solution. No precipitate will appear in the first portion even after warming, while a distinct turbidity of silver chromate immediately will be seen in the portion that has been treated with the equilibrium solution.

Behavior toward Ammine Salts. Those metal ions whose complexes with ammonia and ethylenediamine, respectively, are more stable than the corresponding silver complexes will react positively with the silver chromate equilibrium solutions. Solutions of the sulfates and nitrates of copper, cadmium, zinc, cobalt, and nickel produce immediate precipitation of silver chromate.

The detection limits (in micrograms) when 1 drop (0.05 ml.) of a sulfate solution was brought into reaction with 1 drop of silver-en reagent were found to be: copper, 2.5; cadmium, 7; cobalt, 2; nickel, 2.5; zinc, 2.5. The difference in these figures shows that the reactivity of the ammine salt-formers with silver chromate equilibrium solution is dependent on the degree of tenacity with which the ethylenediamine is held in the respective complex ions.

The findings discussed above are summarized in Table I.

Table I. Reaction toward Silver Chromate Equilibrium Solutions

Material Tested	Reaction
Silica	
Freshly precipitated	Strongly positive
Partially dehydrated at room temperature or at 140° C.	Prompt
Ignited to constant weight	Slow decrease in intensity but always positive
Quartz, sand	Negative
Zeolites, kaolin, opal, garnierite	Positive
Amazonite, talc, muscovite	Negative
MoO_3	Strongly positive
WO_3	Strongly positive
After ignition	Decreased intensity, but always positve
V_2O_5	Immediate, somewhat masked by color of V_2O_5
Nb_2O_5	Immediate
After treatment with H_2F_2 and H_2SO_4	Immediate
Treated as before and then ignited	Negative
Ta_2O_5	Negative
After treatment with H_2F_2 and H_2SO_4	Weakly positive
Treated as before and then ignited	Negative
Ferric, chromic, titanic, zirconium, and bismuth sulfate	Strongly positive
Basic aluminum sulfate	Strongly positive
Copper, zinc, cadmium, cobalt, and nickel sulfate	Strongly positive

A useful preliminary test to establish the possible presence of metal ions that are capable of forming ammine complexes is easily carried out.

The solution of the sample must be freed of halide by fuming with sulfuric or nitric acid. After proper dilution, the solution is treated with excess ammonia water, warmed, and filtered. A few drops of the clear filtrate are evaporated to dryness in a small porcelain crucible, and the residue is heated to drive off the ammonium salts. After cooling, the contents of the crucible are spot-tested with 1 to 2 drops of the silver-en reagent. If silver chromate appears, the presence of a metal ion capable of forming an ammine complex is established.

This procedure can be used in the ordinary qualitative scheme to furnish a reliable indication of the presence of zinc, which will be found in the ammoniacal filtrate obtained from the precipitation of the hydrous aluminum oxide.

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A Mercury Azotometer

For Determination of Organic Nitrogen by the Micro-Dumas Method

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PREGL'S volumetric azotometer for Dumas nitrogen microdeterminations has been used almost without modification since the introduction of the method (*1*, *2*, *4*), although there are certain inherent, but fortunately relatively constant, errors introduced by such an apparatus (*5*). The purpose of the present paper is to describe a weight azotometer which is rugged, less costly, easily constructed, and easily repaired. In addition, the errors from caustic adsorption and corrosion are completely eliminated, since the beginning and end of a determination find the absorbing solutions at the same level in the apparatus, and at no time does the caustic come in contact with a stopcock.

The apparatus developed in this laboratory depends upon the measurement of the amount of nitrogen by the displacement of mercury. As 0.013 gram of mercury corresponds to 0.001 ml. of nitrogen, the weighings of the mercury azotometer need not be more accurate than ±0.01 gram. The authors have obtained satisfactory results with an old chainomatic balance worthless for exact weighing.

Apparatus

The apparatus (Figure 1) consists of three units, a carbon dioxide absorption tower, *D*, the mercury azotometer proper, *A*, and a reservoir, *B*, to receive displaced mercury and facilitate the adjustment of liquid levels.

All units are constructed from Pyrex 12-mm. standard tubing and 2-mm. capillary tubing. Standard taper 2-mm. bore stopcocks and standard taper $^{7}/_{25}$ ground joints are used in the apparatus. A $^{7}/_{25}$ glass stopper is used to prevent loss of mercury from the female joint of azotometer *A* during the weighing.

The capillary tube leading from *D* for connection with the combustion tube should be set into the tower wall as pictured to ensure a minimum diameter of the carbon dioxide bubbles entering the absorption liquid (*3*). Such a scheme prevents the formation of gas bubbles too large to be absorbed efficiently. A ring, *L*, should be engraved on the absorption tower's exit capillary to facilitate level adjustments.

Mercury fills the absorption tower to a point halfway between the leveling bulb outlet and the gas entrance tube. Dirty mercury should be used to prevent clinging of gas bubbles to the mercury–potassium hydroxide interface. Enough 50 per cent potassium hydroxide solution is placed in the system to allow the absorption tower to be filled to mark *L* by raising the leveling bulb. *A* and *B* should each be half filled with clean, dry mercury. In practice the combustion of the sample is carried out with the stopcock of the absorption tower, 1, closed and the remainder of the azotometer system disconnected. At the completion of the combustion the weighed azotometer about half filled with mercury is coupled into the system and the liquid in the absorption tower is raised to its original level, *L*, by adjusting the height of *B*. The nitrogen collected forces mercury out of the azotometer into the leveling tube, so that when the final weighing is made the weight loss of the azotometer is the weight of the volume of mercury equal to the volume of nitrogen produced in the combustion.

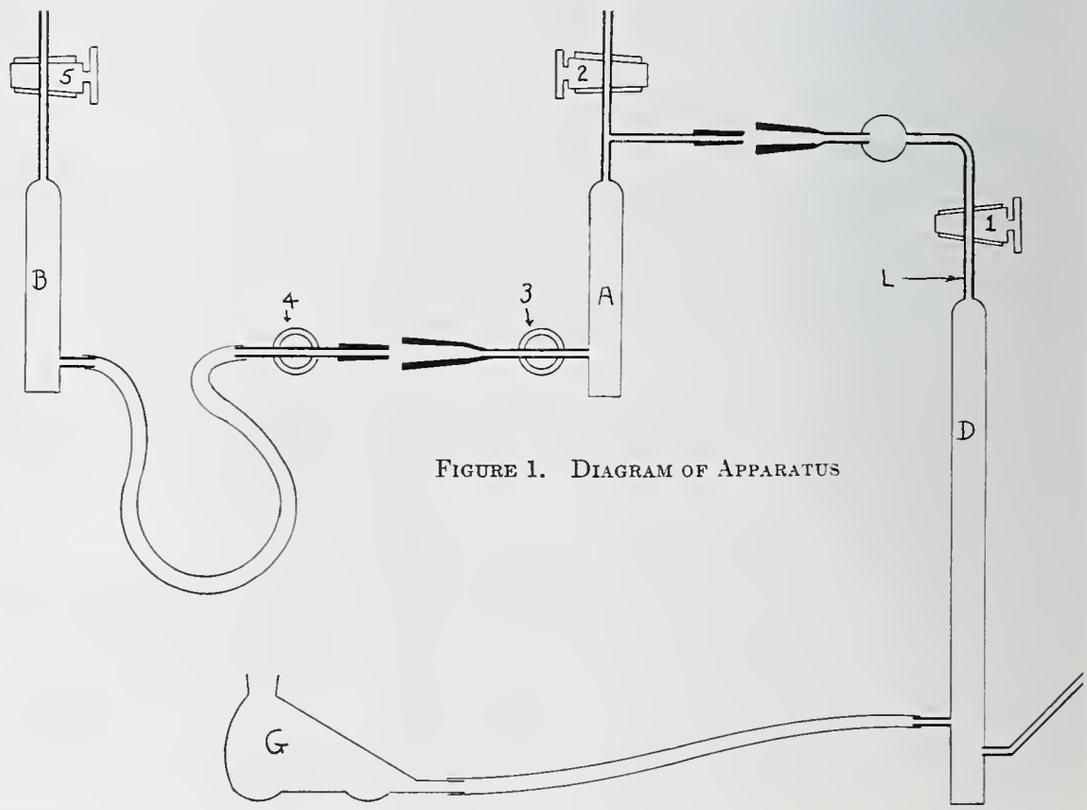

FIGURE 1. DIAGRAM OF APPARATUS

Procedure

The preparation of the sample and sweeping out of the apparatus are carried out in the usual manner. The 3-way stopcock (not pictured) is turned to the closed position and *D* is freed of gaseous carbon dioxide, washing three or four times with air by raising and lowering the leveling bulb, *G*. The level of the 50 per cent potassium hydroxide solution is adjusted to *L* on the stem of the tower, the leveling bulb is clamped in a ring at such a height as to maintain this level in the tower, and stopcock 1 is turned to the closed position.

A and *B* are coupled into the train by means of the ground joints, and with stopcocks 2, 3, 4, and 5 open, *B* is raised and lowered until any air bubbles have been driven out of the mercury-filled connection. Stopcocks 3 and 4 are then closed, and *A* is removed from the train and weighed to the nearest centigram. It is then replaced in the train and stopcock 2 is closed. After the combustion and washing out have been completed, stopcocks 1, 3, and 4 are opened and the level of the potassium hydroxide solution is restored to *L* by adjusting the position of *B*. Inasmuch as the surface of potassium hydroxide solution in the leveling bulb and in the absorption tower will rest at the same height, *L*, when atmospheric pressure is reached within the apparatus, the adjustment of *B* by raising and lowering will cause realignment of levels throughout the apparatus until, with the potassium hydroxide solution in *D* at *L*, the system will be at atmospheric pressure. In this manner the adjustment of level is made to the aqueous solution; adjustment to mercury levels could be made, but there is greater likelihood of error. After the levels have been adjusted, stopcocks 3 and 4 are closed, *A* is separated from the system, the stopper is placed in the female joint, and the azotometer is again weighed to the nearest centigram. (The authors found no significant difference in results with and without lubrication of the ground joints. If desired, a lubricant can be used without danger of errors due to variation in the amount of grease on the portion of the apparatus to be weighed, as lubrication with more than 0.01 gram of grease is highly improbable.)

The calculations are similar to the standard calculations. The temperature of the apparatus and the barometric pressure are determined. The air error of the apparatus is deter-

Table I. Analysis of Acetanilide

(Calcd. N = 10.37%)

Series I	Series II	Series III
%	%	%
10.27	10.25	10.33
10.28	10.24	10.34
10.26	...	10.32
	Av. 10.29	

mined in grams of mercury and is subtracted from the weight of displaced mercury (the loss in weight of A). The percentage of nitrogen is given by the formula:

$$\% \text{ N} = \frac{100 \times (\text{wt. of Hg displaced} - \text{wt. of blank}) \times \text{N}_2 \text{ reduction factor}}{\text{density of Hg} \times \text{wt. of sample}}$$

Results

Acetanilide is the only substance whose analysis is reported in this paper, inasmuch as the method deals only with the collection of nitrogen produced by a standard combustion procedure. If the combustion method is valid for any substance when a volumetric azotometer is used, it should be equally acceptable in the present scheme. Research samples of materials of established structure have been analyzed using the weight azotometer, with satisfactory results. The sample of acetanilide was Mallinckrodt's U. S. P. powder recrystallized twice from water. Its melting point was 114.20°, and a cooling curve proved it to be homogeneous. A series of ten Kjeldahl determinations with the acetanilide averaged 10.35 per cent, and the same number of determinations by the Dumas method using an old and somewhat corroded Pregl azotometer averaged 10.45 per cent.

All the results given in Table I are for analyses by the junior author, and represent the valid results of three series of four determinations each.

Acknowledgment

The authors wish to express their thanks to the Atwater Research Fund for financial assistance and to the Corning Glass Works for construction of the apparatus.

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Determination of Ethyl Alcohol by Microdiffusion

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LEVINE and Bodansky (*6*) have pointed out that most current methods for the determination of ethyl alcohol in tissue and body fluids employ a solution of potassium dichromate in sulfuric acid for oxidation of the alcohol to acetic acid, and that these methods differ only in the means of separating the alcohol from the tissue or fluid, and in measuring the partial reduction of the dichromate.

In addition to distillation and aeration methods, several procedures based upon desiccation of the sample have been developed. These are modifications of Widmark's alcohol method (*8*), which employs a 50-ml. glass-stoppered flask with a small cup suspended from the bottom of the stopper. The alcohol passes by gaseous diffusion from the sample contained in the cup into a solution of dichromate in sulfuric acid in the bottom of the flask. The quantity of alcohol oxidized is determined by reducing the excess dichromate with potassium iodide, and titrating the liberated iodine with standard thiosulfate solution. In Cavett's modification of this method (*1*), the excess dichromate is titrated with ferrous sulfate solution containing methyl orange.

The present method substitutes the Conway microdiffusion unit (*2*) for the Widmark flask. The alcohol diffuses from a blood or urine sample placed in the outer chamber of the unit into a solution of potassium dichromate in sulfuric acid in the central chamber. The excess dichromate is determined iodometrically, as in the method of Widmark. The apparatus is inexpensive and simple to operate, so that a large number of determinations may be run simultaneously. In addition, the Conway unit has the advantage of being applicable to a variety of biochemical determinations (*2, 9*).

Experimental

Sizes of Samples for Analysis. Analyses are performed on blood or urine samples containing approximately 1 to 1.5 mg. of ethyl alcohol. Since the concentration of alcohol in blood and urine is roughly proportional to the degree of intoxication (*4*), the size of the sample taken for analysis is varied according to the apparent condition of the subject as follows: 1 ml. for mild or doubtful, 0.5 ml. for moderate, and 0.25 ml. for severe intoxication.

Collection and Measurement of Samples for Analysis. Blood and freshly voided urine specimens are collected in tubes which are tightly fitted with rubber stoppers. The tubes for blood samples contain oxalate or citrate. The author has confirmed Cavett's observation (*1*) that the blood or urine can stand for 24 hours in the ice box without appreciable change in alcohol concentration.

The 0.25 ml. pipet is made from capillary tubing of about 1-mm. bore, and is calibrated for content. In measuring samples with this pipet, about 0.1 ml. of water is drawn up first, leaving a small air space at the tip, and then the pipet is filled to the calibration mark with blood or urine. The air space between the two columns prevents mixing of the fluids. When the pipet is emptied, the water washes out the film of blood or urine left along the inner wall of the pipet.

Microdiffusion Procedure. The glass cover plate is suitably smeared with "cello-seal" (Fisher Scientific Co.), a lubricant which does not liquefy at the temperatures employed (25° to 50°).

Approximately 0.5 ml. of 0.4 N potassium dichromate in 10 N sulfuric acid is delivered with a volumetric pipet into the central chamber of the unit. A constant time of drainage is employed. The exact volume or concentration of the dichromate solution need not be known, since blank analyses are run with each series.

The blood or urine sample is pipetted quickly into the outer chamber, and the vessel is sealed immediately with the greased glass cover plate. The unit is rotated to spread the fluids over the floors of the chambers. After the unit has stood for 2 hours at 50°, 6 hours at 37°, or 10 hours at 25° (or for longer times), the cover plate is removed and the dichromate solution is diluted with approximately 1 ml. of water. Then about 0.5 ml. of 3 M potassium iodide solution is added with stirring to the central solution, and the liberated iodine is titrated at once with standard 0.1 N thiosulfate solution. (The large excess of potassium iodide reduces the loss of iodine vapor from the solution before addition of thiosulfate. Tests showed that 0.4 to 0.5 per cent of the iodine is lost per minute if the central solution is allowed to stand after addition of the potassium iodide. Accordingly,

Table I. Recovery of Ethyl Alcohol Added to Blood and Urine

Time of Reaction	Alcohol in Blood: 65.5 mg. per 100 ml., 37°	Alcohol in Blood: 155 mg. per 100 ml., 25°	Alcohol in Blood: 155 mg. per 100 ml., 37°	Alcohol in Blood: 155 mg. per 100 ml., 50°	Alcohol in Blood: 341 mg. per 100 ml., 50°	Alcohol in Urine: 144 mg. per 100 ml., 24–25°	Alcohol in Urine: 144 mg. per 100 ml., 37°	Alcohol in Urine: 144 mg. per 100 ml., 50°	Alcohol in Urine: 374 mg. per 100 ml., 37°
Hours	%	%	%	%	%	%	%	%	%
0.5	26.5	...	30	47	41	..	...	37.5	...
1.0	57	27.5	60.5	79.5	70	..	44.5	72	53
2.0	80	48	84	98	103	48	82	99	77
4.0	92	79.5	96	..	...	66.5	92.5	..	91
6.0	97.5	86.5	99	..	...	94.5	100.5	..	103
8.0	..	93	..	..	...	97.5	...	..	...
10.0	..	102	..	..	...	101	...	..	...

if most of the thiosulfate is added less than a minute after the liberation of the iodine, the amount of iodine lost is negligible.)

The thiosulfate is added rapidly with efficient stirring, until nearly all of the iodine is reduced, and then dropwise until the color of the solution is light yellow-green. A drop of 1 per cent starch solution is added, and the titration is completed to the point at which the deep blue starch-iodine color is replaced by the light blue-green color of the chromic ion. Owing to the small volume of the solution being titrated, this color change is very abrupt.

About 2 ml. of thiosulfate solution are required in the blank analyses, so that the final volume of fluid in the central chamber is never greater than 4 ml. The central chambers of the units used in this study have capacities of 4.5 to 5 ml.

Blank Analysis. An analysis is performed with water in place of blood or urine in the outer chamber. In the author's experience, blank analyses usually agreed to within 0.01 ml. (0.5 per cent).

Calculation. The volume of thiosulfate solution which corresponds to the quantity of dichromate reduced, and to the quantity of alcohol oxidized to acetic acid, is given by the difference between the volumes of thiosulfate required in the blank analysis, V_B, and in the alcohol determination, V_A. Since 1 ml. of 0.1 N thiosulfate is equivalent to 1.152 mg. of alcohol, the milligrams of alcohol per 100 ml. of blood or urine equal

$$\frac{(V_B - V_A) \times 1.152 \times 100}{\text{Volume of sample analyzed}}$$

Recovery of Ethyl Alcohol Added to Blood and Urine. Standard solutions were prepared by adding weighed quantities of 97.5 per cent ethyl alcohol (percentage determined by specific gravity measurement) to urine and to a 1 per cent sodium chloride solution. The alcohol was weighed in sealed glass bulbs, which were broken beneath the surfaces of the solutions, so that no alcohol vapor could escape. Three standard solutions, containing 65.5, 155, and 341 mg. of alcohol per 100 ml. of blood, were prepared by diluting blood (found previously to contain no alcohol) with suitable volumes of the sodium chloride solutions, which contained 1705 mg. of alcohol per 100 ml.

Series of determinations with varying reaction times were made at three different temperatures on aliquots of the blood and urine solutions.

Table I shows that 97.5 to 103 per cent of the added alcohol was recovered from the blood or urine after reaction periods of 2 hours at 50°, 6 hours at 37°, or 10 hours at 24–25°. The analyses were performed on 1-ml. portions of the solutions, except for the blood containing 341 mg. and the urine containing 374 mg. of alcohol per 100 ml.; 0.25-ml. samples were analyzed in these two series.

Duplicate determinations usually agreed to within about 2 per cent. Approximately two-thirds of the dichromate solution was reduced by the alcohol in most cases.

Interfering Substances. Of the volatile compounds which may be encountered in body fluids, acetone need not be considered, since it is stable even in boiling dichromate solution.

A number of organic compounds, important in industry, are known to produce conditions resembling ethyl alcohol intoxication when sufficient amounts of their vapors are inhaled. Tests were made with some of these substances to determine whether they could be distinguished from ethyl alcohol in the present method. Chloroform and diethyl ether, two common anesthetics which may linger in small amounts in the blood and urine of persons following anesthesia, were also tested.

Analyses were performed at 37° on 1-ml. portions of solutions (or dispersions) containing 50 to 100 mg. of the organic compound to be tested per 100 ml. of water.

No appreciable quantity of dichromate in the central chamber was reduced when solutions of the following substances were placed in the outer chamber: benzene, chloroform, carbon tetrachloride, naphtha, and trichloroethylene.

Ten to 20 per cent of the dichromate was reduced when methyl alcohol, isopropyl alcohol, amyl alcohol, octyl alcohol, isoamyl acetate, acetaldehyde, and propionaldehyde were tested, indicating that the vapors of these substances were readily oxidized by the dichromate solution. Accordingly, the drunkenness caused by these compounds cannot be differentiated readily from ethyl alcohol intoxication by the present method, as is possible in the case of the preceding group.

Diethyl ether was oxidized slowly by the dichromate. This compound is not likely to interfere seriously with the ethyl alcohol determinations, since ethyl alcohol is present at a relatively much higher level during intoxication and is more rapidly oxidized by the dichromate.

Table II. Concentration of Ethyl Alcohol in Blood and Urine of Intoxicated Persons

Apparent Degree of Intoxication	Alcohol Found: Blood	Alcohol Found: Urine
	Mg./100 ml.	*Mg./100 ml.*
Mild to moderate	132, 135	185, 188
	159, 163	260, 261
	215, 224	276, 289
Moderate to severe	162, 170	170, 176
	188, 190	269, 276
	236, 254	267, 278
	245, 260	313, 316
	304, 310	442, 452
	380, 387	468, 480
Very severe, "dead drunk"	520, 547	.....

Blood and Urine Levels

Normal. The blood and urine of six individuals who had taken no alcoholic beverages for several days contained no measurable amounts of alcohol. When 1-ml. samples were analyzed, the thiosulfate titrations ranged from 1.905 to 1.94 ml., as compared with 1.925 ml. for the blank titration. Cavett (*1*) and Gibson and Blotner (*3*) found traces of alcohol, 0 to 6 mg. per 100 ml. of blood, and 0 to 2 mg. per 100 ml. of urine, in persons who had taken no alcohol. These levels are probably close to the limits of sensitivity of these methods, and insignificant compared to the values encountered in intoxication.

Alcohol Levels in Blood and Urine during Intoxication. The results in Table II conform fairly well to the general classification of intoxication in terms of ethyl alcohol concentrations (*4*). The subjects were persons brought into the Emergency Service of the Detroit Receiving Hospital. In most cases the duplicate values agree to within about 3 per cent. The urinary levels of alcohol are considerably higher than the corresponding blood concentrations in the cases studied. Jetter (*5*) has made this same observation, and states that this is particularly true for cases in which the bladder was previously emptied.

Rate of Disappearance of Alcohol from Blood. Newman, Lehman, and Cutting (*7*) showed that the alcohol concentration in blood falls in a linear fashion with increasing time, following the intravenous administration of ethyl alco-

Table III. Rate of Disappearance of Ethyl Alcohol from Blood of Intoxicated Person

Time after Taking First Blood Sample	Alcohol in Blood	Condition of Subject
Hours	*Mg./100 ml*	
0	304, 310	Moderate to severe intoxication, speaks with difficulty
3	244, 256	Uncooperative, fighting restraints
6.5	164, 168	Quiet and sleepy
9	95, 97	Fairly rational
12	32, 38	Rational

hol to dogs. It was thought of interest to make a series of similar measurements with a human subject, to illustrate further the use of the present method over a wide range of alcohol concentration. Table III gives the results obtained for a 12-hour period with a patient who is classified in the "moderate to severe" range of intoxication in Table II. If the alcohol values are plotted against the corresponding times, the points fall along a straight line.

Summary

The Conway microdiffusion unit has been adapted to the determination of ethyl alcohol in blood and urine. The alcohol diffuses from the sample in the outer chamber of the unit into the central chamber, where it is oxidized by a solution of potassium dichromate. The excess dichromate is determined iodometrically.

Some illustrative alcohol concentrations in blood and urine during intoxication are reported.

A number of industrially important organic compounds whose vapors can cause drunkenness resembling ethyl alcohol intoxication were tested for possible interference with the method.

Acknowledgment

The writer appreciates the advice and assistance given him by C. G. Johnston and I. B. Taylor, relative to the clinical phase of this study. He wishes also to thank C. P. McCord and G. C. Harrold, of the Industrial Hygiene Department, Chrysler Corporation, Detroit, for supplying some of the organic compounds tested, and for helpful information.

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Semimicrodetermination of Carbon

Using the Van Slyke-Folch Oxidation Mixture

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Van Slyke and Folch (*4*) pointed out that all the wet carbon combustion methods hitherto employed were unsatisfactory because the oxidizing mixtures used did not give quantitative results with the more difficultly combustible compounds. For this reason these methods did not find a place in the organic laboratory.

Wet combustion methods were tried in this laboratory employing either an iodic acid (*3*) or chromic acid (*2*) oxidizing mixture, but the results were unsatisfactory. While theoretical results could be obtained with many organic compounds, certain substances such as sugar acetates and some organic acids invariably gave low results. The Van Slyke-Folch (*4*) manometric method, in which an oxidizing mixture, consisting of fuming sulfuric, phosphoric, chromic, and iodic acids was used, gave excellent results with all the compounds tried. Equally satisfactory results were obtained using the Van Slyke-Folch oxidizing mixture, and a simple apparatus, whereby the carbon dioxide evolved was absorbed in alkali and weighed. Inasmuch as the Van Slyke manometric apparatus is not available in many laboratories, the following procedure, requiring simple manipulation and inexpensive equipment, is described.

Reagents

Van Slyke-Folch combustion mixture: 25 grams of chromium trioxide, 5 grams of potassium iodate, 167 ml. of sirupy phosphoric acid (specific gravity 1.7); and 333 ml. of fuming sulfuric acid (20 per cent free sulfur trioxide) are placed in a 1-liter Pyrex Erlenmeyer flask provided with a ground-glass stopper. The open flask containing the mixture is heated over a wire gauze with a flame, until the temperature reaches 140° to 150° C. During heating, the flask is occasionally rotated to assist in the solution of the chromic anhydride. When a temperature of 150° C. has been reached, the flame is removed, the flask covered with an inverted beaker, and the mixture allowed to cool to room temperature. The glass stopper is then inserted, and an inverted beaker is kept over the stopper to prevent the solution from being contaminated with dust.

Potassium iodate, reagent grade, pulverized.

Dehydrated phosphoric acid, prepared from 85 per cent acid by boiling.

Apparatus

The apparatus is shown in Figure 1. Tubes *A A* are filled with soda lime and serve to obtain carbon dioxide–free air. The reaction flask, *B*, has a 15-ml. capacity and is connected to a reflux condenser, *C*, with a 14/35 standard taper glass joint. The construction of an Allihn condenser is modified (Figure 2) to contain a cold-water column inserted into its center. This modification greatly increases the efficiency of the condenser. A 5-ml. capacity cup, *D*, with a stopcock, *b*, forming part of the condenser, serves to introduce the oxidizing mixture into the reaction flask. Stopcock *b* is greased with viscous dehydrated phosphoric acid. The rest of the stopcocks are greased with ordinary stopcock grease.

E is a bubble counter containing 0.5 ml. of concentrated sulfuric acid. A small glass-wool plug is inserted into each of its arms to trap any sulfuric acid which might splash over to tube *F* or condenser *C*. *F* is filled with granular zinc (30-mesh) to trap any volatile acids which form in the determination. The moisture-absorption tube, *G*, is filled with Anhydrone (magnesium perchlorate) leaving 1-cm. space at each end for a glass-wool plug. Previous to use in the apparatus, a slow stream of

carbon dioxide is passed through *G* for 1 hour, then allowed to stand for 2 hours in contact with the gas, and finally dry carbon dioxide–free air is drawn through to displace the unabsorbed carbon dioxide. The carbon dioxide absorption tube, *H*, is filled with Ascarite (asbestos impregnated with sodium hydroxide, obtained from Arthur H. Thomas Company) and a 1-cm. layer of Anhydrone at each end of the U-tube. Tube *I* contains granular calcium chloride and is used to prevent backward diffusion of moisture and air. The parts of the apparatus are joined with heavy-walled rubber tubing.

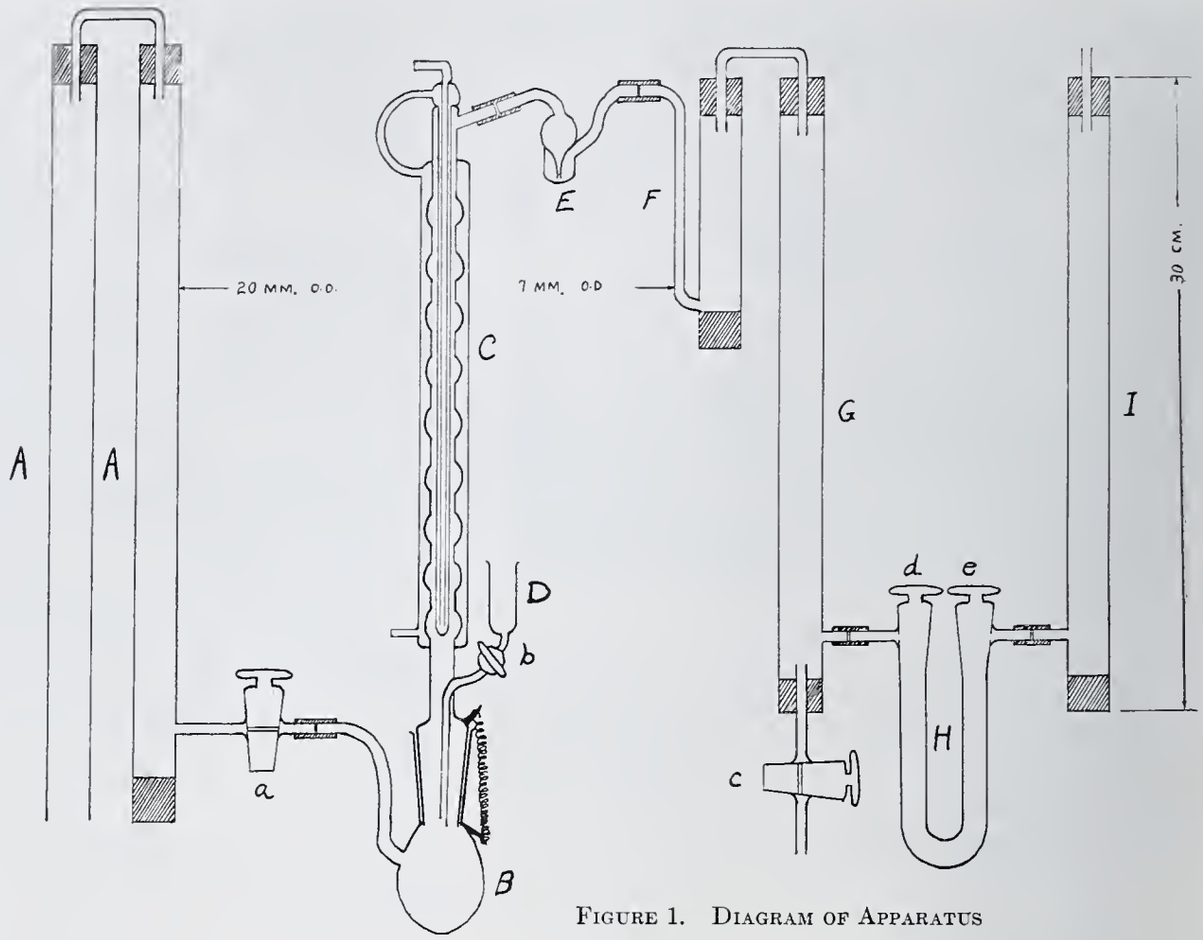

FIGURE 1. DIAGRAM OF APPARATUS

Determination

The sample (5 to 30 mg., depending upon the carbon content) is weighed on a semimicro-balance to an accuracy of 0.02 mg. A less sensitive analytical balance may be used, if the method of weighing recommended by Benedetti-Pichler is used (*1*). The sample is then placed in the dry combustion flask. If the sample is aqueous and is nonvolatile, an aliquot, preferably not more than 2 ml., is introduced with a pipet, acidified with a drop of C. P. hydrochloric acid, and evaporated to dryness. Three hundred milligrams of potassium iodate are added, and the flask is joined to the condenser, the ground joint being greased with dehydrated phosphoric acid, and held in place by a coiled spring.

With stopcocks *a*, *b*, and *d* closed, the apparatus is evacuated by applying suction through stopcock *c* (about 60 mm. of mercury pressure is sufficient) until bubbles cease to appear in the bubble counter. Stopcock *c* is closed and disconnected from suction. The apparatus is maintained under partial vacuum during the combustion. Four milliliters of the Van Slyke-Folch mixture are poured into cup *D*, and introduced into the reaction flask by opening stopcock *b*, care being taken to prevent air from entering the apparatus. The combustion flask is heated with a microburner at such a rate that its contents will come to a boil within about 2 minutes, and the boiling is then continued for 1 minute. When the combustion is completed, stopcock *a* is slightly opened (so that no more than one bubble per second passes through the bubble counter) and carbon dioxide–free air is let into the apparatus until normal pressure is established. Stopcocks *d* and *e* are then opened and the carbon dioxide is swept into the absorption tube, *H*, by connecting the upper part of the calcium chloride tube to suction, and sucking carbon dioxide–free air at the rate of one bubble per second for 15 minutes. Stopcocks *d* and *e* are then closed, and the absorption tube is disconnected, carefully wiped with a clean towel, and weighed. The amount of carbon in the sample is obtained by multiplying the weight of carbon dioxide by a factor of 0.2727.

TABLE I. DETERMINATION OF CARBON

Substance	Sample	CO_2	Carbon Found	Carbon Calculated
	Mg.	*Mg.*	%	%
Glucose	22.82	33.40	39.91	40.00
	11.22	16.50	40.11	
2,3,4,6-Tetramethyl-β-methyl-glucoside	15.30	29.47	52.53	52.78
	10.65	20.50	52.49	
Starch acetate	24.00	44.20	50.22	50.00
	6.48	11.85	49.87	
Palmitic acid	7.87	21.61	74.88	74.94
	14.40	49.58	74.95	
Phenylhydrazine hydrochloride	27.34	49.76	49.63	49.82
	12.90	23.48	49.65	
Benzoic acid	13.30	33.40	68.51	68.85
	11.18	28.20	68.78	

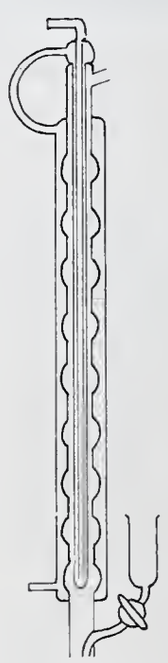

FIGURE 2

The results of carbon analysis of six compounds are given in Table I.

Summary

A simple apparatus for the semimicro-determination of carbon in organic compounds, using the Van Slyke-Folch oxidation mixture, is described. This wet-oxidation method gave theoretical yields with all the organic substances tried, and the accuracy of the procedure is equal to that of dry combustions. In routine work single determinations require about 30 minutes.

Acknowledgment

The authors wish to thank T. C. Broyer for his criticism and suggestions.

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Field Testing of Mold-Resistant Properties of Interior Oil Paints

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THE object of the present paper is to discuss the problems involved in testing mold-resistant properties of interior oil paints in the field and to describe the author's experience in such work.

The previously reported (*12*) laboratory method for determining mold resistance of oil paints affords a rapid and convenient way for evaluating the relative effectiveness of toxicants under the readily reproducible standardized conditions. However, such effectiveness against fungi is only the first prerequisite for a good paint preservative. In addition, it should be stable, not affect the physical or chemical properties of the paint, and be effective under the conditions of actual use over a fairly long period of time (*2, 3, 14*). In other words, after a quick elimination of ineffective preparations by the laboratory method, the selected compounds should be subjected to a further testing "in the field".

The mold resistance of outside oil paints tested by exposing wooden panels in localities where the prevalent natural conditions are conducive to mold growth (such as certain parts of Florida, Louisiana, Cuba, and the Panama Canal Zone) has been previously reported by various investigators (*1, 4, 5, 6*). However, no systematic study of the mold-resistant properties of interior oil paints under conditions similar to those of actual use could be found in the literature.

As a preliminary step for the investigation, a survey of the occurrence of mold growth on paint at different types of industrial plants was made in the midwestern and eastern states. Results of this survey, supplemented by subsequent experience during the actual testing of fungicidal paints, gave the following picture of the occurrence and cause of mold growth on interior paints:

1. Generally speaking, the prevalence of mold growth on interior painted surfaces (walls, ceilings, window sash, etc.) in the various industrial plants examined was much greater than was originally anticipated. Occurrence of mildew was particularly common in industries where, owing to the nature of the processes involved, a high relative humidity was maintained.
2. In many of the food-processing and packing establishments, such as malt houses, breweries, distilleries, meat and vegetable packing houses, bakeries, and dairies, and in cheese, butter, margarine, pickle, and similar plants, in addition to having high relative humidity, the air also frequently carries dust and water spray and vapors containing organic matter. The latter materials when deposited or adsorbed on the walls and other surfaces provide most favorable conditions for the development of mildew (*9, 13, 14*).

FIGURE 1. TYPICAL MOLD GROWTHS

Left. Unusually heavy growth on wall and pipes repainted less than a year prior to time picture was taken
Right. Interior oil paint, showing fuzzy circular colonies

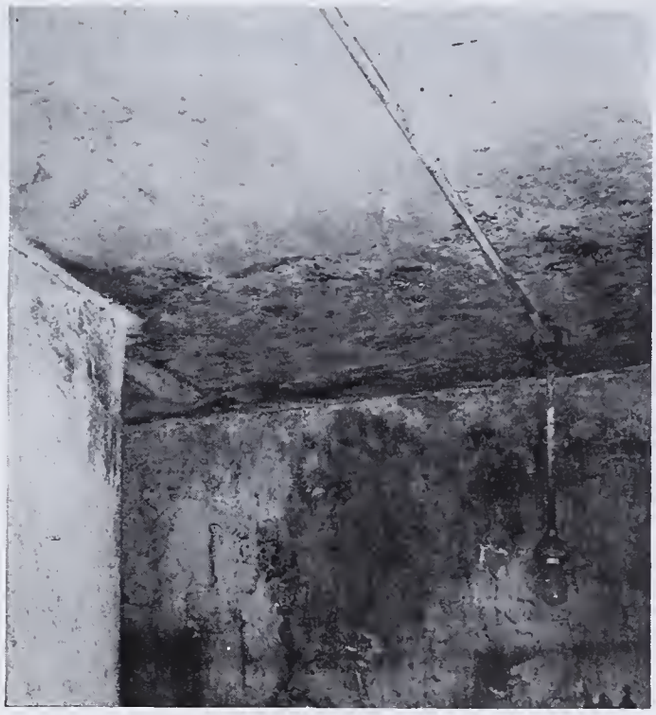

FIGURE 2. DIFFERENCE IN MOLD INFESTATION

Outside wall at back, interior partition at left. Significant gradation in intensity of mold growth on ceiling

3. Mold growth on painted interior surfaces usually manifests itself as black and less frequently as green or red discoloration (*1, 11*). Although this discoloration is sometimes mistaken by the layman for dirt, the mold growth can be readily distinguished from dirt by the fact that it is usually patchy or blotchy and consists of circular, spreading, and somewhat fuzzy spots (Figure 1).

4. Mold infestation of the painted surfaces is seldom uniform and depends on the local variations in two important factors—moisture content of the paint film and relative humidity of the atmosphere. The following examples illustrate this:

The inner side of a cold outside wall of a building on which moisture is condensing is more apt to have mildew than the inner side of a warmer wall or the interior partitions (Figure 2). For this reason heavier mold growth can be expected during the cold winter months.

For the same reason (moisture condensation), ceilings and portions of the walls near the ceiling have, as a rule, a much heavier mold growth than the lower parts of the walls (Figure 3).

Probably because of the difference in air circulation, corners of the rooms, usually, have more mold growth than the adjacent open wall space.

In selecting a location for testing mold resistance of interior paints, all the above factors should be taken into consideration. Since in indoor testing the conditions conducive to mildew do not, as a rule, vary much from year to year in any one given place, the intensity and uniformity of mold growth on paint from the previous year are one of the best guides in choosing the area for the test.

The uniformity of the conditions throughout the test area is particularly important, since usually only the relative values, such as a comparison between the treated and the untreated paint, are desired. As a further precaution, in a series of test panels every fourth or fifth panel should be painted with an untreated paint. The absence of mold growth on fungicidal paint is considered as a proof of its mold resistance only when the untreated paint panels in the same series show a fair amount of molding. The test paints are best applied to the selected areas during the season when the surfaces to be painted are driest.

From what has been said on the importance of moisture condensation on the surface, it is apparent that the test paint should be applied directly to the walls and ceiling of the building rather than to the separate panels. This fact has been verified by the parallel use of wooden panels and direct painting on ceiling and walls. In all cases, molding of the corresponding untreated or insufficiently preserved paints was less severe on the wooden panels than on the walls.

A typical experiment in testing mold-resistant properties of interior oil paints treated with a selected group of fungicidal agents under actual conditions of usage was as follows:

The 6 × 30 foot test area selected was on the interior of the northern brick wall in a relatively old grain-processing plant in the Middle West. It was located under the fresh-air intake, and at the time of selection had the greatest amount of mildew found anywhere in the plant. For many years previously the walls and ceiling of the building had been whitewashed during the annual summer clean-up period, but in the past 4 years the practice was changed to painting the walls with oil paints and the ceiling with a water-cement paint. The test area was prepared for painting

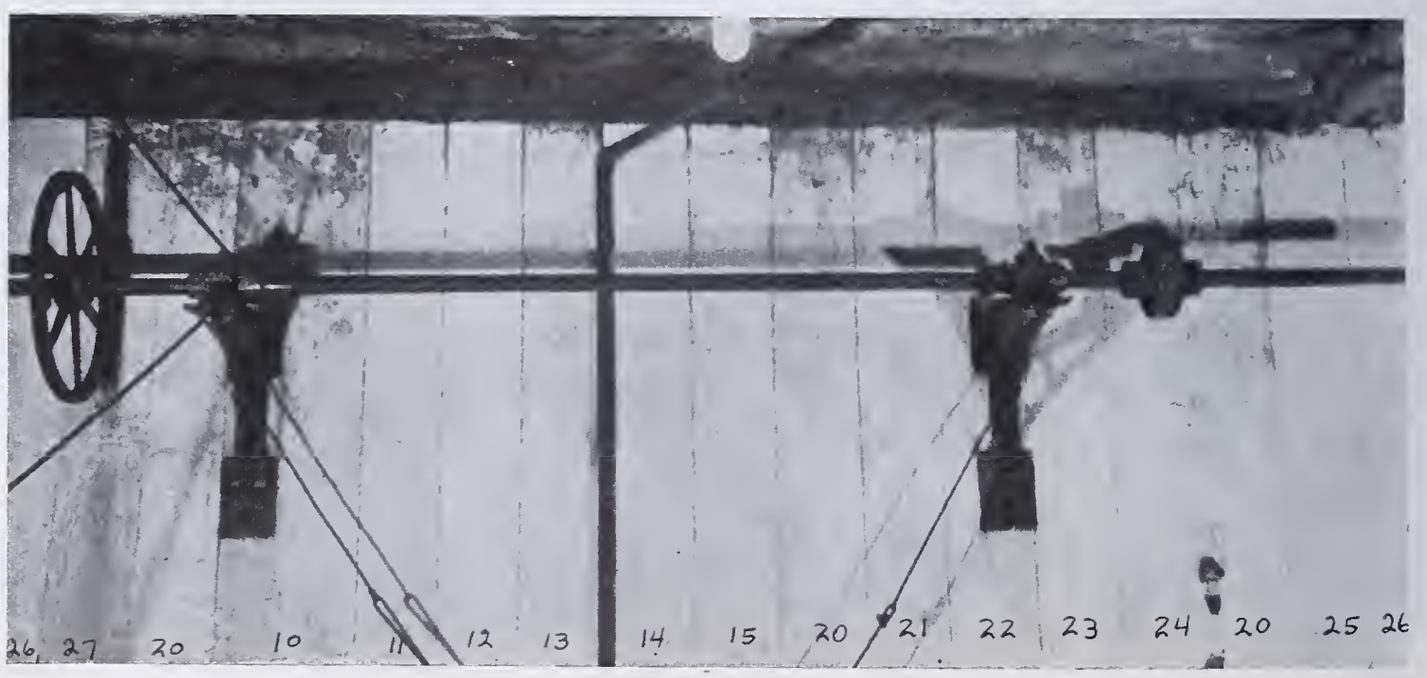

FIGURE 3. GENERAL VIEW OF TEST AREA AFTER EIGHT MONTHS

Note gradation in intensity of mold growth on No. 10, untreated paint, and complete absence of mildew on panels 12, 22, and 26.

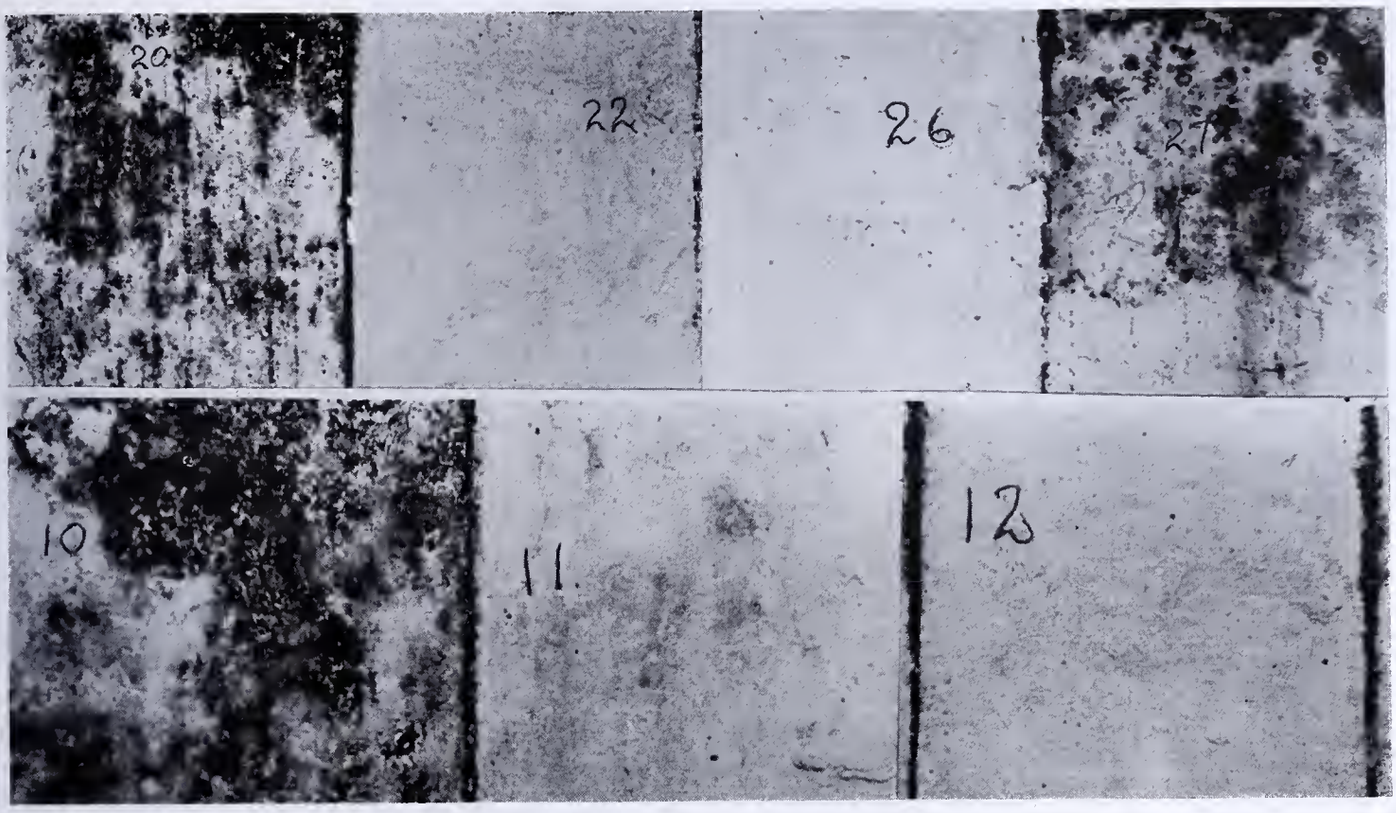

FIGURE 4. CLOSE-UP OF TYPICAL SECTIONS AFTER EIGHT MONTHS' EXPOSURE

10, 11, 12. Painted with mill white paint
10. Untreated paint
11. 1.5 per cent tetrachlorophenol
12. 3 per cent tetrachlorophenol
20, 22, 26, 27. Painted with gloss paint
20. Untreated paint
22. 3 per cent tetrachlorophenol
26. 3 per cent zinc tetrachlorophenate
27. 0.63 per cent calomel

in the usual way—that is, it was scrubbed with soap and phosphate-softened water using a stiff brush, rinsed with fresh water, and the old loose paint was scraped off with a putty knife. The test area was divided into 25 sections, each approximately 12 inches wide and extending 76 inches downward from the ceiling; two coats of test paint were applied. The painting was done in August, 1939, during the summer shut-down when the walls were dry; the plant started operation 2 weeks later.

The paints used were as follows:

Paints 10 to 15 were made up from a widely used flat mill white paint with heat-bodied linseed oil vehicle.

Paints 20 to 27 were made up from a typical interior gloss white paint of a cold-cut Damar resin type.

These paints were treated with preservatives as follows (all percentages are on the original paint weight basis):

Nos. 10 and 20, controls, without preservatives
Nos. 11 and 21, with 1.5 per cent tetrachlorophenol
Nos. 12 and 22, with 3 per cent tetrachlorophenol
Nos. 13 and 23, with 1.5 per cent lead tetrachlorophenate
Nos. 14 and 24, with 3 per cent lead tetrachlorophenate
No. 25, with 1.5 per cent zinc tetrachlorophenate
No. 15, with 2.5 per cent zinc tetrachlorophenate
No. 26, with 3 per cent zinc tetrachlorophenate
No. 27, with 6.3 volume per cent (manufacturer's recommendation) of a commercial antimildew paste containing 8 per cent (by weight) of calomel, giving a total of 0.63 per cent calomel by weight of the total paint, or 3.5 per cent calomel by weight of the nonvolatile portion of the vehicle.

Periodic examination of the test area showed practically complete absence of mildew during the first 3 months. However, with the advent of cold weather, when moisture condensation on the wall kept the paint surface continuously wet, the mold growth began to make its appearance.

The original untreated paints, 10 and 20, and the calomel-containing paint, 27, were the first to show mold growth. Paints 13 and 23 with 1.5 per cent of lead tetrachlorophenate were next, and 8 months later, on April 3, 1940, when photographs shown in Figures 3 and 4 were taken, a small amount of mold growth was also noted on paint 21 (gloss white with 1.5 per cent tetrachlorophenol). At the same time, all paints containing 3 per cent of toxicants and the mill white paint containing 1.5 per cent tetrachlorophenol were still mold-free.

In all cases the mold growth started on the upper end (near the ceiling) of the test sections and with time gradually spread downward (Figure 3). The same photograph gives the general view of the setup and the appearance of a part of the test area and the surroundings at that time. Note particularly the severity of mold infestation of the ceiling (painted with a cement paint) and the very interesting gradation in the intensity of mold growth on section 10. Close-ups of the upper portions of the two sets of typical test sections are given in Figure 4.

The next set of photographs was taken on August 26, 1940, after one year of exposure. By that time the plant had been shut down for cleaning and redecoration; thus, the walls had already been repainted with an oil paint and the ceiling sprayed with a cement-water paint. These operations resulted in some damage to the test area, both from the water during cleaning and from the spraying of the cement paint. In addition to this, dirt accumulation from April to August was rather severe. The general view of the test area, photographed after a light washing with water to remove surface dirt, is shown in Figure 5. The paints containing tetrachlorophenol (except for the upper portions of the sections splattered with the cement paint) washed practically clean, indicating that their original dark color was due primarily either to the surface dirt or to the loose readily washable mold growth on the dirt film. In contrast to this, the mold growth on the untreated paint, No. 10, could not be washed off with the same effort, indicating that in this case the mildew had grown into the paint itself.

The above-described test area was allowed to remain undisturbed for another year. During the second season dirt deposition on the walls was again heavy. The general view of the test area, taken July 15, 1941, after a light washing,

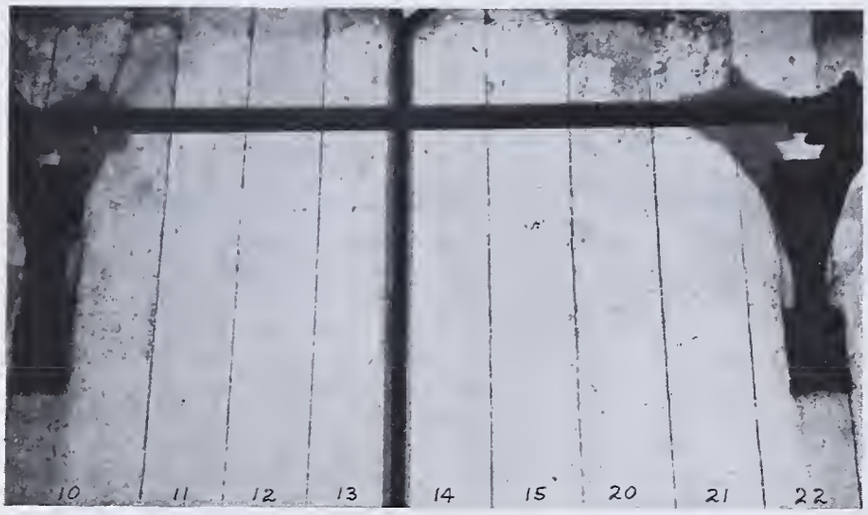

FIGURE 5. GENERAL VIEW OF PART OF TEST SECTION (ONE-YEAR EXPOSURE) AFTER WASHING

(Figure 6, above), shows that after two years' exposure the general distribution of mold growth was about the same as after the first year. The sections painted with flat mill white paint washed almost to their original whiteness, while the gloss paint, both treated and untreated, acquired a gray cast, making it impossible to wash it clean.

In May, 1939, two sets of 12 × 24 × 0.5 inch white pine panels were prepared in the laboratory, in which one half (6 × 24 inch areas) of each panel was painted with the untreated paints, No. 10 or 20, and the other half of each with treated paints (same as previously listed). Two coats of each paint were applied at the laboratory, and in addition one set of panels received a third coat of paint applied on the location just before the panels were exposed. The wooden panels were hung on the wall near the ceiling in the same room and just east of the test area described above.

After one season's exposure, in contrast with the wall tests, only a small amount of mold growth could be seen on any of the wooden panels. However, after the second year, panel sections painted with untreated flat mill white paint developed considerable mold growth, while on the treated paints the growth was small or absent. Photograph of the back side (toward the wall) of these panels is given in Figure 6 (below). The front side of the panels (toward the room) accumulated too much dirt for the observation to be of value. The 2-year results with the flat mill white paints on wooden panels were in good agreement with those obtained with the wall tests The gloss paint on the wooden panels of the same set molded but lightly except in places where dirt accumulation was considerable. There was no difference in mold growth between the two-coat and the three-coat panels (the third coat of which was applied on the location).

The difference in the results obtained with the wooden panels and the painted wall area, after one season's exposure, is accounted for by the difference in moisture condensation

FIGURE 6. PANELS AFTER TWO-YEAR EXPOSURE

Above. General view of test area after washing. Note clearness of Nos. 11 and 12, containing tetrachlorophenol in mill white.
Below. Back side of "half and half" wooden panels. Left-hand portion of each was painted with untreated paint, No. 10.

on the surfaces. Another contributing factor might be that the wood used in the panels was new, while the painted wall area had a previous mold growth. This mold growth, although partially scraped and washed off in preparing the surface for repainting, nevertheless provided a ready-made source of mold infection for the new paint. A considerable volume of evidence is now at hand which suggests that under favorable conditions certain types of mold growth, when painted over, can and will come through a nontoxic paint film. This has also been pointed out by Findlay (*1*).

For the above reason, cleaning and disinfection of old moldy surfaces prior to repainting are important and should be made a part of the repainting procedure (*1*, *7*, *8*, *10*). This contention is confirmed by actual tests in which identical paints were applied to a contaminated area, one half of which was first washed with a disinfectant solution while the other half was not.

Summary and Conclusions

Mold growth on interior oil paints is common in industrial plants where a high relative humidity is maintained.

The mold growth is never uniform even in any one room, but is heaviest on surfaces where moisture condensation takes place, such as the interior side of cold outside walls, locations near refrigeration pipes, ceilings, etc.

Organic matter, vapors, and dust are conducive to mold growth but interfere somewhat with the testing of the mold-resistant properties of paints. However, where mold growth develops on the dust deposited over an oil paint, a preserved paint can be relatively easily washed clean, while on a nonfungistatic paint the mold growth becomes established in the paint itself and cannot be washed off.

Mold resistance of interior paints should be tested by applying the test paints to carefully selected sections of walls and ceiling on which water condensation is continuously taking place and which are found to be the most heavily contaminated areas in the plant selected. The use of wooden panels, even when most advantageously exposed, may give erroneous results due to the difference in conditions between them and the painted surfaces of the building.

In preparing moldy surfaces for repainting, disinfection of the cleaned surface is beneficial, since some molds can grow through a paint film.

Without an adequate preservative the paint with a cold-cut resin type of vehicle molded as readily as the paint with a vegetable oil type of vehicle.

Tetrachlorophenol and zinc tetrachlorophenate were found to be the most effective paint preservatives among those tested.

Under extremely severe conditions of testing 3 per cent of tetrachlorophenol and 3 per cent of zinc tetrachlorophenate preserved both an oil and a cold-cut resin type of interior paint for 2 years.

The field test confirmed earlier conclusions on the relative effectiveness of fungicides in oil paints as determined by the rapid laboratory method.

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Determination of Small Amounts of Benzene in the Presence of Cyclohexane

And of Toluene in the Presence of Methylcyclohexane

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THE method described in this paper consists in measuring the temperature rise (ΔT) caused by the interaction of benzene or toluene with nitrating acid under definite conditions, and reading the percentage of aromatic from a curve relating ΔT with hydrocarbon composition. Benzene, up to 12 per cent, can be determined by this empirical method without diluting the sample. For higher concentrations the sample must be diluted with cyclohexane, so that ΔT will not exceed 20° C.

This thermometric method, which depends specifically upon the heat of reaction of benzene with nitrating acid, is especially suitable for the analysis of benzene-cyclohexane mixtures resulting from the hydrogenation of benzene. Analysis by refractive index or density is unreliable, owing to the fact that cyclohexane is usually contaminated with methylcyclopentane (*1*) (C_6H_{12}: n_D^{20} 1.4264, d_4^{20} 0.7781; $CH_3C_5H_9$: n_D^{20} 1.4099, d_4^{20} 0.7488). Also, open-chain paraffins often contaminate benzene and the cyclohexane produced from it. The thermometric method gives results which are at least as accurate as those obtained from freezing point data. In the analysis of a series of synthetic benzene-cyclohexane mixtures, in which the benzene concentrations varied from 0 to 12 per cent, the average deviation from the mean was 0.06 per cent.

The thermometric method works equally well with toluene-methylcyclohexane mixtures; but in the case of xylene-dimethylcyclohexane mixtures ΔT is dependent upon the isomeric composition of the xylene, and therefore, the relationship between ΔT and aromatic content must be determined for each sample of xylene in question.

Development of Method

The variables of acid strength, period of stirring, water content, size of stirrer, initial temperature, and change of ΔT with time have been studied.

The procedure was to add 50 cc. of a mixture of benzene and cyclohexane (containing 5.2 per cent of benzene) to 100 cc. of nitrating acid contained in a small-necked pint thermos bottle

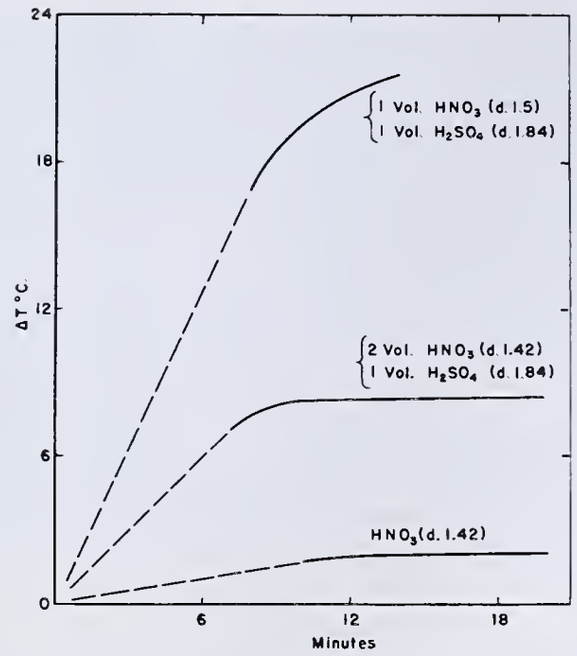

FIGURE 1. VARIATION IN ΔT WITH COMPOSITION OF ACID

Broken lines, temperature during stirring period
Solid lines, temperature after stirring period

whose metal collar was coated with paraffin to protect it against the acid. Both solutions, acid and hydrocarbon, were initially at room temperature, as was the thermos bottle. After stirring for different lengths of time, the stirring was stopped, and the temperature of the acid-hydrocarbon mixture was measured with a 50° thermometer graduated in 0.1° C.

EFFECT OF STRENGTH OF NITRATING ACID. Nitrating acid containing 1 volume of nitric acid (density 1.5) and 1 volume of sulfuric acid (density 1.84) was unsatisfactory, for ΔT continued to increase long after the stirring had been stopped (Figure 1), owing to slow de-emulsification of the acid-hydrocarbon mixture. Only 40 cc. of hydrocarbon were used with this acid because 50 cc. of hydrocarbon raised the temperature to such an extent that the nitric acid rapidly decomposed. The finally accepted nitrating acid contained 2 volumes of nitric acid (density 1.42) and 1 volume of sulfuric acid (density 1.84). This acid gave a definite end point with a sufficiently large ΔT. Nitric acid alone (density 1.42) yielded a ΔT of only 2° C., whereas mixed acid containing one third as much nitric acid produced a ΔT of 8.5° C. (Figure 1).

EFFECT OF STIRRING PERIOD. Figure 2 shows the effect of varying the length of the stirring period. The mixed acid contained 2 volumes of nitric acid (density 1.42) and 1 volume of sulfuric acid (density 1.84). The ΔT was recorded 10 minutes after the end of the stirring period. Evidently the length of the stirring period is critical up to 9 minutes, but after 9 minutes ΔT is essentially independent of small changes in the stirring time ($\Delta T/t$ being 0.03° C. per minute, which corresponds to an analytical uncertainty of less than 0.02 per cent of benzene per minute).

EFFECT OF WATER. A mixture of 5.2 per cent of benzene and 94.8 per cent of cyclohexane was analyzed after drying, and also after saturating with water. The ΔT of the dry sample was 0.1° C. lower than that of the wet sample. Wet cyclohexane gave the same ΔT as dry cyclohexane (0.1° C.). It is recommended that the hydrocarbon under examination be dried by anhydrous calcium chloride before analysis.

EFFECT OF STIRRER. The recommended stirrer is a glass rod, 7 mm. in outside diameter and 24 cm. long, with a flattened end ±13 mm. wide × 19 mm. long (Figure 3); it is operated by a 3000 r. p. m. electric motor. Using a stirrer with a blade twice as large raised ΔT by 0.3° C. with a benzene-cyclohexane mixture containing 5.2 per cent of benzene, and this difference corresponds to an apparent increase in the benzene content of about 0.2 per cent.

EFFECT OF INITIAL TEMPERATURE. It is to be noted that ΔT for the reaction of benzene with nitric acid is greater at 20° C. than at 30° C., which is opposite to the thermodynamic prediction, because $\frac{(d\Delta H)}{(dT)_p} = \Delta C_p$, and ΔC_p is negative. The effect is less with toluene-methylcyclohexane, but is in the

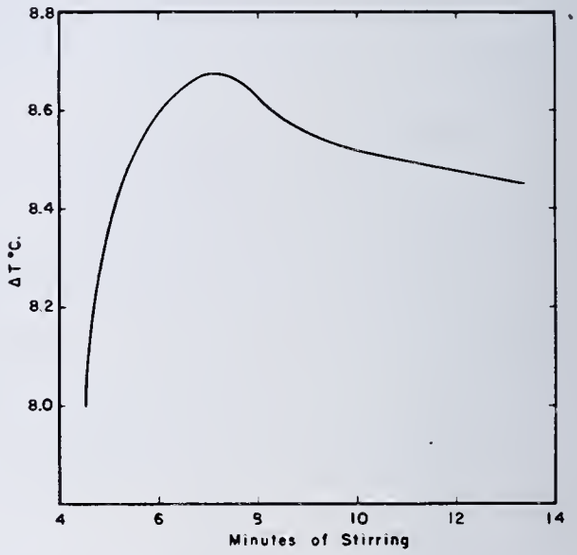

FIGURE 2. VARIATION IN FINAL ΔT WITH TIME OF STIRRING

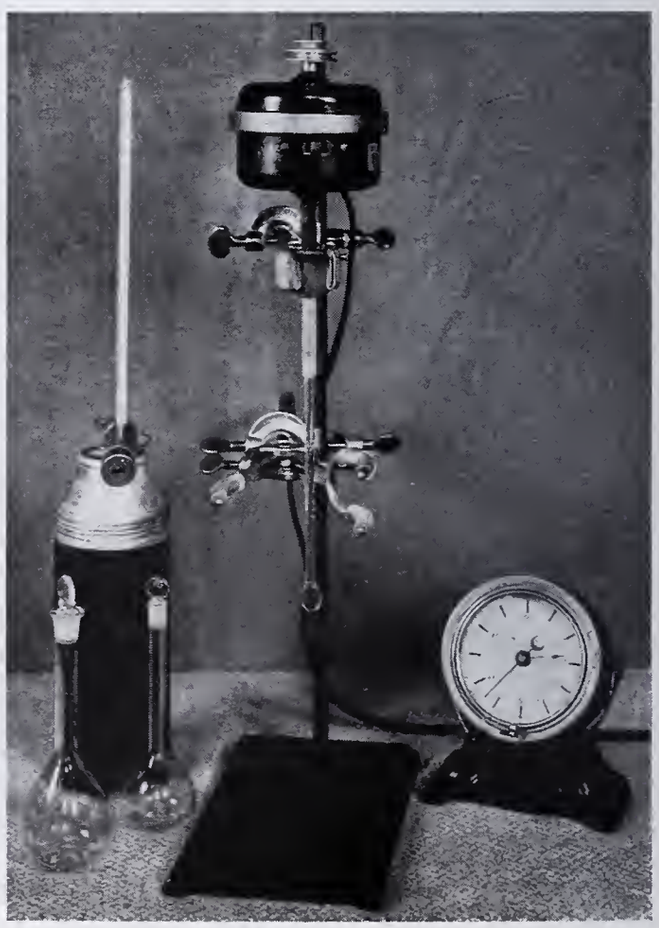

FIGURE 3. EQUIPMENT FOR ACID HEAT TEST

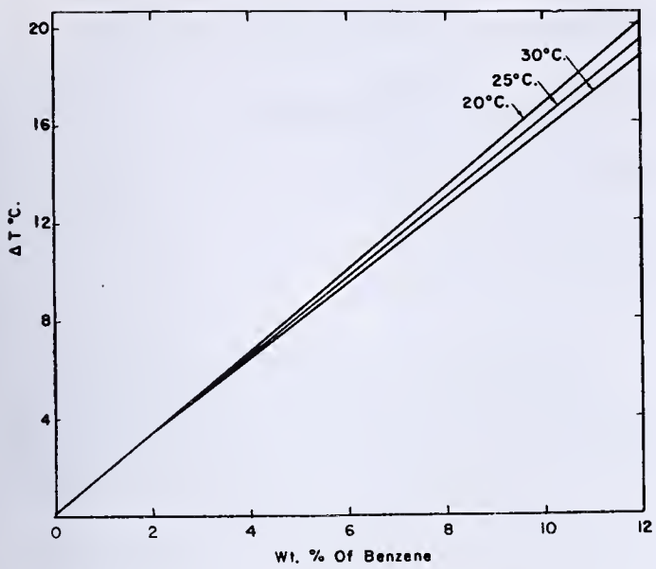

FIGURE 4. ACID HEAT TEST DATA FOR BENZENE-CYCLOHEXANE
Initial temperature, 20°, 25°, and 30° C.

same direction. Presumably this anomalous relationship of ΔT to initial temperature is attributable to the relative speeds of demulsification at the different temperatures.

PURITY OF HYDROCARBONS. The benzene was of reagent quality; the cyclohexane was made from it by catalytic hydrogenation under pressure at about 135° C. The cyclohexane was shaken with nitrating acid, washed with water, and distilled. Its freezing point of 5.9° C. indicated a purity of 99.7 per cent.

TABLE I. ACID HEAT TEST DATA FOR BENZENE-CYCLOHEXANE (FIGURE 4)

Weight Percentage of Benzene	ΔT from Initial Temperatures of 20° C.	25° C.	30° C.
0.00	0.10	0.10	0.10
0.56	1.10	1.10	1.09
1.08	2.07	2.04	2.00
1.56	2.87	2.79	2.70
3.29	5.59	5.40	5.22
6.38	10.82	10.44	10.07
10.15	17.13	16.55	16.00
12.01	20.13	19.44	18.75

TABLE II. ACID HEAT TEST DATA FOR TOLUENE-METHYLCYCLOHEXANE (FIGURE 5)

Weight Percentage of of Toluene	ΔT from Initial Temperatures of 20° C.	25° C.	30° C.
0.00	0.32	0.32	0.32
1.34	3.96	3.95	3.95
3.50	9.71	9.64	9.58
5.49	15.00	14.89	14.80

Determination of Benzene in Cyclohexane

One hundred cubic centimeters of nitrating acid (1 volume of sulfuric acid, d. 1.84, plus 2 volumes of nitric acid, d. 1.42), measured in a 100-cc. volumetric flask, are poured into a thermos bottle, the flask being allowed to drain 15 seconds. The thermometer is placed in the thermos, the bulb resting on the bottom of the latter, and the temperature is read after 2 to 3 minutes (with a magnifying lens). Fifty cubic centimeters of hydrocarbon sample (measured in a 50-cc. volumetric flask and at the same temperature as the acid) are added to the acid in the thermos. An interval timer is started and the flask is allowed to drain 15 seconds.

The stirrer is placed in the thermos, with the bottom of the stirring blade about 1.25 cm. (0.5 inch) from the bottom of the thermos, and when the timer reaches 30 seconds the motor is started. The stirrer is run 9.5 minutes and then removed from the mixture. The thermometer is placed in the thermos (bulb resting on bottom of thermos) and the temperature is read 10 minutes after stopping the stirrer—i. e., 20 minutes after the addition of the hydrocarbon. The mixture is not shaken nor stirred during the last 10 minutes.

For precise work, not only should the acid and hydrocarbon be thermostated to the same temperature, but the thermos bottle should also be at that temperature (although its heat capacity is small).

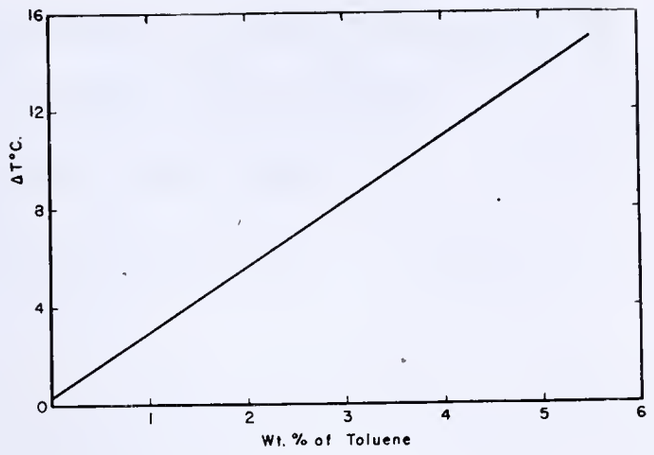

FIGURE 5. ACID HEAT TEST DATA FOR TOLUENE-HEXAHYDROTOLUENE
Initial temperature, 20°, 25°, and 30° C.

The benzene content is read from the family of curves corresponding to the data in Table I, and it is to be noted that ΔT is essentially independent of the initial temperature (20° to 30° C.) up to 1.5 per cent of benzene.

Table II presents acid heat data for toluene-methylcyclohexane mixtures.

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CONTRIBUTION from the Koppers Multiple Fellowship on Tar Synthetics, Mellon Institute, Pittsburgh, Penna.

Cleaning Porcelain Crucibles

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THE method described here can save considerable time and confusion by cleaning "burner grime" and marking inks such as ferric chloride from porcelain crucibles. It does not injure the glaze in any manner, but leaves the crucible with a perfectly clean surface.

Place the crucible in a dish of fused potassium bisulfate for about 5 minutes. Remove, allow to cool, and wash with hot water.

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Sulfanilamide, Sulfapyridine, Sulfathiazole, Sulfaguanidine, and Sulfadiazine

Assay, Differentiation, and Identification

J. A. CALAMARI, ROBERT HUBATA, AND P. B. ROTH
Laboratory, New York Medical Depot, Brooklyn, N. Y.

SULFANILAMIDE, sulfapyridine, sulfathiazole, sulfaguanidine, and sulfadiazine have been studied by the authors, and methods for assay, differentiation, and identification have been established. Sulfanilamide has been adopted as a primary standard for the standardization of the nitrite solution used in the assay of the sulfa drugs, because of the ease with which it may be purified and its stability in air. Rapid differentiation of the sulfa drugs from each other is accomplished by means of the solubility of the original compounds and the solubility and color of some of the easily prepared derivatives. The identity of the sulfa drugs is established by determining the melting points of both the original drugs and the 2-amino hydrolysis products in the case of sulfapyridine, sulfathiazole, and sulfadiazine. The melting points of sulfanilamide and sulfaguanidine and the presence of ammonia in their hydrolysis products identify them.

The authors have selected the diazotization reaction for the assay of the sulfa drugs in preference to an element analysis such as that depending on the estimation of sulfur (*1*), because the former is based on the presence of a characteristic group—the aryl amino group—and because of the greater rapidity of the assay. Using the easily purified sulfanilamide as a primary standard for the standardization of the sodium nitrite volumetric solution, the possibility of error is less than in assay methods employing volumetric solutions standardized by more indirect methods such as the sulfanilamide assay of the U. S. Pharmacopoeia (*4*). Furthermore, the assay method is applicable to all five sulfa drugs either as pure compounds or in tablets, and since the standardization reaction and procedure are identical with those of the assay method, the possibility of an end-point error is minimized.

The information necessary for the differentiation and identification of the sulfa drugs is given in Table I. The methods employed in establishing these characteristics are described below, in following paragraphs numbered to correspond with those in the table.

Table I shows that sulfanilamide can be differentiated from the other sulfa drugs by its solubility in water; sulfaguanidine by its insolubility in sodium hydroxide solution; sulfathiazole by the insolubility of its diazonium chloride at 0° to 4° C.; and sulfadiazine by the insoluble compound formed with stannous chloride.

Differentiation and Identification

1. Follow the U. S. P. XI melting point method (*6*), but preheating the bath to the temperature indicated in Table I, continuing heating at the rate of 0.5° per minute, and stirring vigorously with a propeller type of motor-driven stirrer. Sulfaguanidine is rendered anhydrous by drying at 110° C. prior to the melting point determination.

2. To 0.1 gram of the drug add 10 cc. of an approximately 5 per cent solution of hydrochloric acid, A. C. S., at 25° C. Stir to effect solution.

3. To 0.1 gram of the drug add a 5 per cent solution of sodium hydroxide, reagent grade, at 25° C. and stir.

4. To 0.1 gram of the drug add 20 cc. of distilled water at 25° C. and stir vigorously. The numerical values offered have been obtained from the literature (*3*).

5. Reflux 1 to 2 grams of the drug with 50 cc. of hydrochloric acid, A. C. S., for 1 to 2 hours. Cool and make strongly alkaline with a 25 per cent solution of sodium hydroxide. Cool and extract with ether. Collect the ether extractions, filter, and evaporate. Determine the melting point by the U. S. P. XI (*6*) method, employing vigorous stirring as in paragraph 1.

6. Place 0.50 gram of the drug in a test tube and dissolve in 7 cc. of hydrochloric acid, A. C. S., and 35 cc. of distilled water. Cool in an ice water bath. Add 20 cc. of 0.1 *M* sodium nitrite solution slowly with constant stirring and cooling. Cool the contents of the tube to 0° to 4° C.

7. Dissolve 0.1 gram of the drug in 5 cc. of a 5 per cent solution of hydrochloric acid, A. C. S., and add 1 cc. of stannous chloride–hydrochloric acid reagent. Cool to 20° C. and allow to stand for 10 minutes.

8. Add 20 cc. of acetone, A. C. S., to 0.1 gram of the drug and agitate.

9. Add 20 cc. of ether, U. S. P., to 0.1 gram of the drug and agitate.

REAGENTS. Sodium nitrite solution. Dissolve 7.0 grams of sodium nitrite, A. C. S., to make 1 liter of 0.1 *N* or 0.1 *M* solution. Standardize as directed below.

Stannous chloride–hydrochloric acid reagent. Dissolve 50 grams of stannous chloride, reagent grade, in 50 cc. of hydrochloric acid, A. C. S.

Sulfanilamide (for primary standard). Recrystallize once from acetone and twice from water.

Starch iodide paper, U. S. P. XI (*7*), and starch iodide paste (*2*).

STANDARDIZATION. Dissolve 0.52 gram of pure sulfanilamide (see reagents) in 7 cc. of hydrochloric acid, A. C. S., and 35 cc. of water. Add 30 grams of ice and titrate with 0.1 *M* sodium nitrite volumetric solution, using starch iodide paper or paste as an outside indicator. Each cubic centimeter of 0.1 *M* sodium nitrite is equivalent to 0.01722 gram of sulfanilamide.

TABLE I. IDENTIFICATION OF SULFA DRUGS

	Sulfanilamide	Sulfapyridine	Sulfathiazole	Sulfaguanidine	Sulfadiazine
Melting Point, ° C.	165.5–166.0°	191–192° (decomposed)	201–202° (decomposed)	190.5–191.5°	251–252° (decomposed)
1. Temperature of preheated bath, ° C.	150°	170°	180°	170°	225°
2. Solubility in 5% HCl	Soluble	Soluble	Soluble	Soluble	Soluble
3. Solubility in 5% NaOH	Soluble	Soluble	Soluble	Insoluble	Soluble
4. Solubility in water, mg. per 100 cc. (*3*)	Soluble. Very soluble in hot water. 800 at 25° C.	Insoluble, 49.5 at 37° C.	Insoluble, 94 at 37° C.	Insoluble, 190 at 37° C.	Insoluble, 12.3 at 37° C.
5. Basic hydrolysis product, melting point	NH_3	56°	90°	NH_3	126°
6. Diazonium chloride, color of solution and solubility	Colorless Soluble	Yellow Soluble	Yellow Insoluble at 0–4°	Colorless Soluble	Pale yellow Soluble
7. $SnCl_2$-HCl	No ppt.	No ppt.	No ppt.	No ppt.	White ppt.
8. Solubility in acetone	Soluble	Soluble	Soluble	Soluble	Soluble
9. Solubility in ether	Insoluble	Insoluble	Insoluble	Insoluble	Insoluble

Assay

The assay procedure is identical with that employed in the standardization.

If the approximate quantity of the sulfa drug is not known, run an exploratory titration as in standarization, using the outside indicator after each 1-cc. addition of sodium nitrite. On the final titration, weigh a sample calculated to yield an approximate 30-cc. titration, dissolve, cool the sample as before, and add the sodium nitrite to within about 1 or 2 cc. of the value obtained in the exploratory titration, then in intervals of 0.1 cc., and as the end point is approached in 0.05 and finally 0.025 cc., testing with the outside indicator after each addition. Stir vigorously during and after each addition of sodium nitrite. Tablets of the sulfa drugs contain 5 or 7.7 grains of the drugs and therefore smaller quantities than 30 cc. of sodium nitrite will be consumed if the tablets are assayed individually. The accuracy is proportional to the quantity of sodium nitrite solution consumed.

If the quantity of sample indicated below is used, a titration of about 30 cc. will be obtained:

	Quantity of Sample	Each Cc. of 0.1 *N* $NaNO_2$ Equivalent to:
	Gram	*Gram*
Sulfanilamide	0.52	0.01722
Sulfapyridine	0.75	0.02492
Sulfathiazole	0.75	0.02553
Sulfaguanidine, anhydrous	0.64	0.02142
Sulfadiazine	0.75	0.02501

Discussion

Sodium nitrite solutions standardized against purified sulfanilamide and standardized against sodium oxalate by the U. S. P. sodium nitrite assay method (*5*) have agreed within 0.1 per cent. Sulfathiazole, sulfaguanidine, and sulfadiazine purified by repeated crystallization from acetone have assayed 100.0 ± 0.1 per cent and recrystallized sulfapyridine 99.9 ± 0.1 per cent, using the diazotization method. Tablets have been assayed directly by the method described and the sulfa drugs have been separated from the tablet excipients by extraction and recrystallization for determination of the other characteristics given in Table I.

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Rapid Detection of Gold by the Electrographic Method

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GOLD in alloys and in plating may be detected rapidly by spot tests (*3–7*) and by electrographic methods (*1*), the latter being the more rapid. The authors have discovered a rapid test for gold in alloys and plating which may be completed in about one second and is apparently specific for gold.

When gold is made the anode in a neutral or slightly acid solution of nitrate ion, using an e. m. f. of 6 to 9 volts and a current of about 0.5 to 1.0 ampere, it enters solution as Au^{+++} and reacts with water to form auric hydroxide which deposits on the anode. Hydrogen peroxide added to the solution reduces the auric hydroxide to purple aurous hydroxide and colloidal gold.

APPARATUS AND TEST SOLUTIONS. The equipment and procedure are identical with those described for the electrographic test (*2*). The test solution is prepared by dissolving 30 grams of sodium nitrate, reagent grade, in sufficient hydrogen peroxide, U. S. P., to make 100 ml., and filtering.

METHOD. Ashless filter paper is folded to form a wad three to four layers thick and then dipped into the sodium nitrate–hydrogen peroxide solution. The moistened filter paper is placed on the test metal anode and a graphite cathode about 7.5 cm. (3 inches) long and 0.6 cm. (0.25 inch) in diameter is firmly applied to the free side of the moistened section of the filter paper for about one second. An e. m. f. of about 6 to 9 volts is employed and may be obtained by using 4 to 6 No. 6 dry cell batteries connected in series. The current is about 0.5 to 1 ampere. An e. m. f. of 4.5 to 6 volts, a current flow of 0.1 to 0.5 ampere, and about a 5-second contact are recommended for plating.

If gold is present, a purple stain appears on the paper adjacent to the test metal anode. The intensity of the color increases with the percentage of gold present in the test metal when current density and time remain constant. The stain also increases in intensity upon standing. Very thin or porous plating yields a faint purple to purple stain; heavy plating, a purple stain.

A strong positive test for gold has been obtained with a series of dental alloys ranging from 25 per cent, to pure gold, and with 14- and 18-karat jewelry golds. Copper and silver in the gold alloys tested did not interfere. Numerous other metals and alloys have been tested by this method and negative results have been obtained in each case: nickel-silver, platinum-ruthenium, platinum-iridium, solders, brasses, white metals, 15 per cent silicon steel, bronzes, copper-nickel alloys, carbon steels, platinum, palladium, nickel, copper, manganese, molybdenum, tantalum, tungsten, mercury, cadmium, aluminum, tin, zinc, vanadium, silver, and lead. Chromium yields a blue spot which fades rapidly. Vanadium yields a red spot and silver a black spot.

It is believed that the test is applicable to alloys containing less than 25 per cent of gold, because the purple stain obtained with the alloys containing 25 to 30 per cent of gold is intense.

Heavy gold plating can be detected readily. Thin or porous gold plate on sterling silver yields a spot, faint purple to purple in color, often interspersed with dark areas, due to silver. The characteristic gold stain, however, has been clearly discernible in all tests performed.

Very thin gold plate on copper or brass cannot be detected by this method.

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Estimation of Ortho-, Pyro-, Meta-, and Polyphosphates in the Presence of One Another

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Procedures for the estimation of ortho-, pyro-, hexameta-, trimeta-, and polyphosphates have been developed. Hexametaphosphate is separated as barium hexametaphosphate in an acid solution, and pyrophosphate is precipitated as manganous pyrophosphate at pH 4.1 in the presence of a small amount of acetone after removal of the hexametaphosphate radical. Orthophosphate is precipitated in the cold filtrate after removal of barium phosphates from a solution just acid to methyl red. Trimetaphosphate is obtained in the filtrate from an alkaline precipitation of all other phosphates as their barium salts. Total phosphorus pentoxide is determined so that polyphosphates may be obtained by difference. A qualitative test to identify the polyphosphate as tetraphosphate is given. Alkalinity determination is made by a standard acid titration to phenolphthalein and bromocresol green indicators to aid in calculating the probable form in which the phosphates may be present.

Data on application of method to analysis of samples of glassy metaphosphate, tetra- and tripolyphosphates, and synthetic mixtures of phosphates, carbonates, and silicates, as well as unknowns of these substances, are given.

USE of alkali ortho-, pyro-, meta-, and polyphosphates has recently become widespread, and a suitable method for their estimation in the presence of one another is of considerable importance. This investigation started with the development of a method for the analysis of mixtures of ortho-, pyro-, and metaphosphates, but was later expanded to include polyphosphates such as tripolyphosphate ($Na_5P_3O_{10}$) and tetraphosphate ($Na_6P_4O_{13}$). This work has not been concerned with the controversial question of the existence of additional polyphosphates, but rather with the development of a procedure which can be used analytically to estimate ortho-, pyro-, and metaphosphates as well as the more common commercial polyphosphates. Several published methods for analysis of mixtures of ortho-, pyro-, and metaphosphates were investigated and found more or less unsatisfactory when applied to commercial products. A brief résumé of the published methods and some of the difficulties encountered in their use are presented below.

In the analysis of a mixture of ortho-, pyro-, and metaphosphates, Aoyama (*1*) and Dworzak and Reich-Rohrwig (*6*) first precipitated the phosphates with a measured excess of silver nitrate in the presence of 50 per cent alcohol and determined the excess silver after filtering off the precipitated silver phosphates. Aoyama (*1*) treated the silver phosphates with hydrogen sulfide, while the others (*6*) used hydrochloric acid to form the phosphoric acids, which after separation by filtration, they titrated with sodium hydroxide to both the methyl orange and phenolphthalein end points. From these two titrations and the silver determination they calculated the amounts of ortho-, pyro-, and metaphosphates.

When tests were made in this laboratory on the analysis of known mixtures of phosphates according to these directions, the author found that, in the precipitation of phosphates with silver nitrate, as much as 10 per cent of the total phosphorus pentoxide of the metaphosphate either singly or in mixtures of all three remained in solution. The metaphosphate used in these tests was the glassy variety which is the general commercial type and was prepared by rapid cooling of a molten mass of metaphosphate. Based on the best information available at this time, metaphosphate prepared in this manner is composed mainly of sodium hexametaphosphate, $(NaPO_3)_6$, with a small amount of sodium trimetaphosphate, $(NaPO_3)_3$. As trimetaphosphate is not capable of repressing calcium and magnesium hardness, it may be regarded as an impurity when present in commercial products. Mellor (*12*) points out that trimetaphosphate is not precipitated by any metallic ion, including silver and barium. The author prepared some trimetaphosphate and confirmed this. In addition, he found that in the presence of alcohol some silver trimetaphosphate was precipitated but that a large part of the trimetaphosphate remained in the filtrate. Even though an additional step for the determination and calculation of the unprecipitated phosphorus pentoxide in an aqueous filtrate to trimetaphosphate might be included in the method, other difficulties, such as the conversion of the silver phosphates to the acids, the separation of the acids by filtration, and the neutralization of the acids, which were recognized by these workers, proved to be rather serious. It was desirable to find refinements in the method or an entirely different approach to the analysis.

Certain refinements in neutralization procedures were presented by Lum, Malowan, and Durgin (*11*) in determining the composition of strong phosphoric acid. They employed modifications of the Britzke and Dragunov (*4*), Travers and Chu (*13*), and Gerber and Miles (*7*) methods. The first method included in addition to a neutralization titration to both methyl orange and phenolphthalein end points, a second titration with bromophenol blue as the indicator to the first distinct blue color, followed by the addition of 2.5 to 3 times the amount of zinc sulfate necessary to precipitate the pyrophosphoric acid as the neutral zinc pyrophosphate. The sulfuric acid liberated was then titrated. The second method consisted of a titration by sodium hydroxide with bromocresol green, continued titration after the addition of silver nitrate solution and methyl red as the indicator, and titration of a second aliquot by sodium hydroxide with thymol blue as the indicator in the presence of sodium nitrate to prevent hydrolysis. The titrations were made to pH color standards of pH 4.2, 4.4, and 4.6 to bromocresol green and to pH 8.4, 8.8, 9.0, and 9.2 to thymol blue to aid in determining the exact end points. Although Gerber and Miles' titration of the acids allowed greater refinement in that step of the procedure, the combined procedures were still not generally applicable.

The present investigation has for its purpose the development of procedures intended primarily for the determination of mixtures of phosphates in commercial products such as cleaners and detergents. The volumetric procedures of the new method appear to be reliable and sufficiently accurate to meet the usual requirements in the analysis of such products. They overcome many of the weak points of the previous

methods and also include polyphosphates in the analytical scheme. For greater accuracy longer gravimetric procedures should be used. The present paper describes the new volumetric method, its development, and application to commercial products.

Reagents and Solutions

Disodium Orthophosphate. C. P. disodium orthophosphate was thrice recrystallized from distilled water and dried at 110° C. overnight. The sample, analyzed by magnesia mixture, indicated the presence of 100 per cent disodium hydrogen orthophosphate. Pyro- and metaphosphates (*9*, *10*) were absent.

Tetrasodium Pyrophosphate. C. P. tetrasodium pyrophosphate was thrice recrystallized from distilled water and was dried to constant weight at 250° C. The sample, analyzed by magnesia mixture after conversion to orthophosphate in nitric acid, indicated the presence of 100 per cent tetrasodium pyrophosphate. Ortho- and metaphosphates (*2*, *10*) were absent.

Sodium Hexametaphosphate. C. P. monosodium dihydrogen orthophosphate was heated to about 900° C. for 3 hours in a platinum dish and then the molten mass was cooled rapidly. Different batches of the resulting glassy product contained from 90 to 95 per cent sodium hexametaphosphate and, since ortho-, pyro-, and polyphosphate were absent, the balance of the phosphorus pentoxide must be present as trimetaphosphate. A solution of sodium hexametaphosphate with only 0.10 per cent of trimetaphosphate was prepared by separating the hexametaphosphate from the trimetaphosphate by precipitating the former with silver nitrate in a nitric acid solution acid to methyl orange. The soluble trimetaphosphate was washed out of the silver hexametaphosphate precipitate by decantation until the filtrate showed only a trace of phosphate. The silver hexametaphosphate was then suspended in water and stirred while sodium iodide solution was added slowly. The filtrate from the removal of silver iodide contained the hexametaphosphate.

Sodium Trimetaphosphate. C. P. monosodium dihydrogen orthophosphate was heated for 1 hour at 300° C. The temperature was increased to 610° C. and held there for 3 hours and then slowly decreased to 390° C. where it was held for 14 hours. The phosphorus pentoxide determination indicated that the sample must be 100 per cent $(NaPO_3)_x$. The sample yielded no precipitate to silver nitrate or barium chloride at any pH, thereby indicating the absence of hexametaphosphate, pyrophosphate, and orthophosphate (*12*). Furthermore, tripoly- and tetraphosphate precipitate with either silver nitrate or barium chloride in an alkaline solution.

Sodium Tripolyphosphate. Quantities equivalent to 1 $NaH_2PO_4.H_2O$ and 2 $Na_2HPO_4.12\ H_2O$ were heated in a platinum dish in an oven at 110° C. The salts first dissolved in the water of hydration, and after evaporation to dryness the mixture was transferred to a muffle furnace at 250° C. for 2 hours before increasing the temperature to approximately 800° C. The mixture was allowed to remain at this temperature until the entire mass became molten, then the temperature was lowered to about 650° C. and maintained at this temperature for 18 hours. At the end of this time the temperature was lowered gradually from 650° C. to 250° C. over a period of 8 hours and then was transferred to a desiccator to cool. The white crystalline solid mass disintegrated into a fine white powder upon cooling to room temperature. Analysis of the material by the procedure given below indicated the following composition: $(NaPO_3)_3$ 4.4%, Na_2HPO_4 1.7%, $Na_4P_2O_7$ 16.0%, $Na_5P_3O_{10}$ 77.9%.

A sample of a commercial sodium tripolyphosphate was also obtained, which analysis indicated to have the following composition: $(NaPO_3)_3$ 0.4%, Na_2HPO_4 3.0%, $Na_4P_2O_7$ 10.6%, $Na_5P_3O_{10}$ 86.0%.

Sodium Tetraphosphate. A commercial sodium tetraphosphate made by a large producer was the source of the sample used in this work. Analysis by the current procedure indicated a composition of $(NaPO_3)_3$ 3.8%, NaH_2PO_4 3.8%, and $Na_6P_4O_{13}$ 92.4%.

Standard Sodium Hydroxide, 1 *N* and 0.1 *N*. Prepare a carbonate-free solution of sodium hydroxide and standardize against standard hydrochloric acid with phenolphthalein indicator. Use in determining the phosphomolybdate volumetrically.

Standard Hydrochloric Acid, 1 *N* and 0.1 *N*. Prepare solutions from C. P. hydrochloric acid (sp. gr. 1.18) and standardize against pure sodium carbonate. Use in titrating the excess sodium hydroxide in the phosphomolybdate and alkalinity determinations.

Ammonium Molybdate Solution. Dissolve 100 grams of pure molybdic acid in a mixture of 400 ml. of cold distilled water and 80 ml. of C. P. ammonium hydroxide (sp. gr. 0.90). When solution is complete, pour slowly with constant stirring into a mixture of 400 ml. of C. P. nitric acid (sp. gr. 1.42) and 600 ml. of distilled water. Do not reverse the order of procedure, as nitric acid poured into ammonium molybdate will cause the precipitation of a difficultly soluble molybdic oxide and render the solution practically worthless. Add 0.05 gram of sodium ammonium phosphate dissolved in a little water and agitate. After 24 hours decant the clear solution through a filter paper into a reagent bottle. Sixty milliliters of the reagent are sufficient for 0.1 gram of phosphorus pentoxide.

Barium Chloride Solution. Dissolve 25 grams of C. P. barium chloride dihydrate in 1 liter of distilled water. Use to precipitate the hexametaphosphate radical.

Potassium Nitrate Solution. Dissolve 10 grams of C. P. potassium nitrate in 1 liter of water. Use as a wash solution for the phosphomolybdate precipitate.

Manganous Chloride Solution. Dissolve 100 grams of C. P. manganous chloride tetrahydrate in 1 liter of distilled water. Use to precipitate the pyrophosphate radical.

Phenolphthalein Indicator, 0.5 per cent solution. Dissolve 0.5 gram of C. P. phenolphthalein in 60 ml. of 95 per cent ethyl alcohol, and dilute to 100 ml. with previously boiled and cooled distilled water. Neutralize with 0.1 *N* sodium hydroxide.

Methyl Orange Indicator, 0.1 per cent solution. Dissolve 0.1 gram of methyl orange in 100 ml. of distilled water.

Bromocresol Green Indicator, 0.4 per cent solution. Dissolve 0.4 gram of bromocresol green in 5.75 ml. of 0.1 *N* sodium hydroxide and a few milliliters of alcohol. Dilute with water to 100 ml.

Analytical Procedure

Preparation of Solution for Analysis. Run a rough preliminary test by the method given below for the determination of the approximate total phosphorus pentoxide. Then prepare a solution of the sample in a standard volumetric flask such that a convenient size of aliquot will contain about 0.05 gram of total phosphorus pentoxide.

Conversion of Phosphates to the Ortho Form. This conversion of the phosphates, isolated by the various analytical steps, makes possible application of the volumetric molybdate method for determination of their phosphorus pentoxide content. It is accomplished by boiling gently for 15 minutes the acidified phosphate solution containing a 25-ml. excess of C. P. nitric acid per 100 ml. of solution. Before adding the ammonium molybdate, cool the solution to room temperature, and neutralize the excess acidity with ammonium hydroxide, so that the solution is just acid to litmus paper.

Total Phosphorus Pentoxide. Transfer a suitable aliquot of the prepared solution to a beaker and dilute to 75 to 100 ml. Convert all phosphates to the ortho form as directed above and precipitate the phosphorus pentoxide with 50 ml. of ammonium molybdate. Wash the precipitate with a minimum volume of 1 per cent potassium nitrate wash solution cooled to 10–12° C., until 5 ml. of the wash solution turn alkaline to phenolphthalein indicator with not over 3 drops of 0.1 *N* sodium hydroxide. One hundred milliliters of wash solution should be sufficient. Determine the phosphorus pentoxide content of the phosphomolybdate volumetrically, using 1 *N* or 0.1 *N* reagents depending on the amount of precipitate.

Hexametaphosphate. This procedure should be carried out soon after the solution has been prepared, so that the metaphosphate ion, which undergoes hydration particularly in strongly acid or alkaline solutions, will not have time for conversion.

Transfer an aliquot of the prepared solution of the size used for total phosphorus pentoxide to a beaker and dilute to 50 ml. with distilled water. Add 1 drop of methyl orange indicator, make the solution just acid to the indicator with 1 *N* hydrochloric acid, then add 0.5 ml. of the acid in excess. If the solution is already acid, make slightly alkaline to the indicator, then make final acid adjustment as indicated above. Add slowly with constant stirring 15 ml. of barium chloride solution and let the precipitate settle before filtering. If the solution clouds up without the precipitate separating within a minute, stir vigorously for 15-second intervals until a precipitate commences to separate when stirring is stopped. Filter and wash precipitate by decantation with a small amount of cold barium chloride solution (1 gram per liter), then transfer the precipitate to the filter paper and wash four or five times with small portions of the cold barium chloride wash solution. Save the filtrate for the determination of trimetaphosphate. Dissolve the precipitate in 1 to 1 nitric acid and convert the metaphosphate to orthophosphate as above and determine the phosphorus pentoxide as for total phosphorus pentoxide.

Table I. Conversion of Pyro-, Meta-, and Polyphosphates to Orthophosphate

Phosphate	P_2O_5 by Molybdate After 15 minutes' boiling	P_2O_5 by Molybdate After 30 minutes' boiling	P_2O_5 by Mg Mixture after 2 Hours' Boiling
	Gram	*Gram*	*Gram*
Hexameta 95% } Trimeta 5% }	0.0472	0.0473	0.0472
Pyro	0.0482	0.0481	0.0483
Hexameta 60% } Trimeta 2% } Pyro 33% } Ortho 5% }	0.0798	0.0798	0.0799
Tetra	0.0624	0.0624	....
Tripoly	0.0576	0.0576	....

Trimetaphosphate. To the filtrate from the separation of hexametaphosphate add 1 *N* sodium hydroxide with stirring until the mixture remains definitely alkaline to phenolphthalein indicator. Filter off the precipitated barium phosphates and determine the phosphorus pentoxide of the trimetaphosphate on the resulting filtrate as for total phosphorus pentoxide.

Orthophosphate. Transfer a suitable aliquot of the prepared solution to a beaker, dilute to 100 ml., make just acid to methyl red indicator with 1 *N* hydrochloric acid, then add 25 ml. of barium chloride solution, and filter off any barium phosphate precipitate. To the filtrate cooled to 20–25° C. add 8 to 10 grams of ammonium nitrate before precipitating the orthophosphate radical with ammonium molybdate and determining it volumetrically as above. Allow only 15 minutes for precipitation. Phosphates other than orthophosphate are not precipitated under these conditions.

Pyrophosphate. Transfer a suitable aliquot of the freshly prepared solution to a beaker and make just acid to methyl orange indicator with 1 *N* hydrochloric acid before adding barium chloride 10 per cent in excess of the stoichiometric amount needed for the hexametaphosphate present. In absence of hexametaphosphate add no barium chloride. Stir vigorously to hasten separation and settling out of the barium metaphosphate. Filter off the precipitate, dilute filtrate to approximately 125 ml. with distilled water, and add 5 ml. of manganous chloride solution. Adjust the pH of the solution with the aid of a glass electrode or other suitable pH-meter by adding 0.1 *N* sodium hydroxide drop by drop with stirring until the pH remains 4.1. Then add with stirring 7 to 8 ml. of acetone and allow to stand 12 to 16 hours at 20° to 30° C. to permit the manganous pyrophosphate to precipitate. Filter off the precipitate and wash with a 1 gram per liter manganous chloride solution. Reserve filtrate for polyphosphate qualitative test. Dissolve precipitate in 1 to 1 nitric acid, convert to orthophosphate, and determine the phosphate as for total phosphorus pentoxide.

Polyphosphate. Qualitative test. This test must be performed on the filtrate immediately after removal of manganous pyrophosphate which has been previously precipitated from a freshly prepared solution.

Add 25 ml. of barium chloride solution to the filtrate from the pyrophosphate determination. The formation of a cloudiness or precipitate within 15 minutes, a behavior found to be characteristic of tetraphosphate, is considered indicative of the presence of this compound. Tripolyphosphate gives no visible change under these conditions.

The phosphorus pentoxide of the polyphosphates is taken as the difference between the total phosphorus pentoxide and the sum of the phosphorus pentoxide values of ortho-, pyro-, and the metaphosphates, and is calculated to tripoly- or tetraphosphate as dictated by the qualitative test. The calculation of polyphosphate is subject to an accumulation of additive errors; however, results from numerous experiments indicate that they are satisfactory for the purpose for which the method is intended.

For greater accuracy, the total phosphate may be determined gravimetrically by precipitating with magnesia mixture, igniting the precipitate, and weighing as magnesium pyrophosphate. The barium hexametaphosphate and manganous pyrophosphate precipitates may be fused with sodium carbonate, the fusion extracted with cold water, and the phosphorus pentoxide in the filtrate determined gravimetrically by the magnesium pyrophosphate method. Before adding magnesia mixture, the filtrates must be acidified with nitric acid and boiled to eliminate carbon dioxide and convert the meta- and pyrophosphate radicals to orthophosphate.

Experimental Development

Conversion of Pyro-, Meta-, and Polyphosphate to Orthophosphate. Since ammonium molybdate does not precipitate the phosphorus pentoxide of pyrophosphate, the metaphosphates, and polyphosphates, it is necessary to convert them to the ortho form before determining total phosphorus pentoxide. Results of numerous experiments indicate that gentle boiling for 15 minutes in the presence of nitric acid is sufficient for complete conversion.

Separation of Metaphosphate. In hydration studies of metaphosphoric acid, Holt and Myers (*8*) precipitated the hexametaphosphate ion with barium chloride in the presence of ortho- and pyrophosphoric acids. In ascertaining the composition of the barium metaphosphate, they decomposed it with nitric acid, determined the barium as sulfate, and found the precipitate to have a practically constant composition corresponding to the empirical formula $Ba(PO_3)_2$. In this laboratory the author analyzed the precipitate for the phosphorus pentoxide content according to the above procedure, and confirmed their conclusions.

Table II. Composition of Metaphosphate Used

Hexameta	Trimeta	Total Hexameta + Trimeta Found	Hexameta	Total P_2O_5 by Mg Mixture	P_2O_5 Found (H + T)
Gram	*Gram*	*Gram*	%	*Gram*	%
0.1187	0.0065	0.1246	94.7	0.1253	99.4
0.0704	0.0039	0.0743	94.7	0.0750	99.1
0.0473	0.0028	0.0501	94.4	0.0502	99.8
0.0078	0.0004	0.0082	95.2	0.0082[a]	100.0
0.0025	0.0001	0.0026	96.1	0.0025[a]	104.0

[a] Calculated from size aliquot used.

Kiehl and Wallace (*10*) tested for metaphosphates qualitatively in mixtures of the three phosphates by adding 0.1 *M* barium chloride solution to the phosphates in a 1 *M* nitric acid solution. Tests made in strongly acid solution showed that the precipitation of barium hexametaphosphate was either prevented or incomplete. However, some acidity is necessary to avoid the formation of insoluble barium ortho-, pyro-, and polyphosphates. No barium phosphate precipitate formed when barium chloride was added to solutions of ortho-, pyro-, and polyphosphates when acidified according to directions given in the procedure. This indicated that a solution of this acidity was sufficient to prevent the precipitation of these phosphates. A precipitate did form when a solution of tetraphosphate was just acid to methyl orange, but tripolyphosphate did not produce a precipitate in a solution just acid to either methyl orange or methyl red with barium chloride in the proportions given in the analytical procedure. On the other hand, results of tests carried out on equal volumes of solutions of glassy metaphosphate of widely different concentrations indicated that the hexametaphosphate was precipitated quantitatively. Although the total phosphorus pentoxide content of the sample was not precipitated completely with barium chloride, the amount of unprecipitated phosphorus pentoxide in the filtrate was approximately 5 per cent of the total on the sample under consideration here, even though the concentration of the samples was varied greatly. This lack of complete precipitation in widely varying concentrations points to the presence of another phosphate rather than to errors resulting from solubility of the barium hexametaphosphate.

As the results of the acid and alkaline precipitation with barium chloride were in good agreement with each other, this indicated the absence of ortho-, pyro-, and polyphosphates. The unprecipitated phosphorus pentoxide in the alkaline filtrate is considered to be trimetaphosphate, as its behavior corresponded with that given for it in Mellor (*12*). After development work on the procedure for determining trimetaphosphate had been completed, an article (*14*) applying a similar procedure for it was published. Results of analyses based on the proposed method are given in Table II.

Hexametaphosphate may be determined gravimetrically by drying the barium metaphosphate precipitate and weighing (*8*). The presence of other ions, such as sulfate, which form insoluble barium compounds under the conditions of precipitation, will interfere with such a gravimetric determination. The method described in this paper for the treatment of barium hexametaphosphate avoids such interferences.

Strontium and calcium chlorides were tried in place of barium chloride as a precipitating agent for the separation of hexametaphosphate, but were discarded either because of undesirable physical properties of the precipitate or inability to obtain satisfactory check determinations.

Precipitation of Orthophosphate in the Presence of Pyrophosphate and Silicate. Courtois (*5*) suggested that orthophosphate could be determined with ammonium molybdate in the presence of pyrophosphate but stated that it was necessary to work rapidly. Boratynski (*2*) applied the same test at 25° C. and 15 minutes' standing to the colorimetric determination of small amounts of orthophosphate in the presence of pyro- and metaphosphates. Experiments, carried out in this laboratory on the precipitation of various amounts of orthophosphate alone with ammonium molybdate at 25° and 60° C. and 15 to 30 minutes' standing, indicated that satisfactory results could be obtained at 25° C. and 15 minutes' standing. When ammonium molybdate was added to pyrophosphate solutions at 60° C., considerable phosphomolybdate precipitated because of the accelerated hydration of pyro- to orthophosphate, but at 25° C. no precipitate formed after 15 minutes. Although the silicate radical forms a silicomolybdate, it does not interfere with the phosphate determination since the yellow complex remains in the filtrate. The results of the analysis of ortho- and pyrophosphate and silicate under various conditions indicate that 25° C. and 15 minutes' standing are satisfactory conditions for the determination of orthophosphate. A few precipitations of orthophosphate which were carried out on solutions chilled in an ice bath were found to be incomplete at the end of 15 minutes.

Table III. Orthophosphate Precipitated with Molybdate under Different Conditions

Ortho Added	Pyro Added	$Na_2SiO_3 \cdot 5H_2O$ Added	P_2O_5 Found: 60° and 15 min.	P_2O_5 Found: 25° and 15 min.	P_2O_5 Found: 25° and 30 min.
Gram	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*
0.0586	0.0000	0.0000	0.0585 0.0586	0.0585 0.0585	0.0586 0.0585
0.0586	0.0534	0.0000		0.0586 0.0585	0.0584 0.0586
0.0586	0.0000	0.1250	0.0586 0.0584	0.0584 0.0584	0.0585 0.0586
0.0586	0.0534	0.1250		0.0586 0.0587	0.0585 0.0584
0.0000	0.0000	0.2500	0.0000	0.0000	0.0000

Determination of Pyrophosphate in the Presence of Ortho-, Trimeta-, and Polyphosphates. Were it not for the existence of polyphosphates, pyrophosphate could be obtained satisfactorily by calculating it from the difference between the total phosphorus pentoxide and the sum of the phosphorus pentoxide values of ortho- and the metaphosphates. However, the possibility of the presence of polyphosphates in such mixtures makes it necessary to find a selective precipitant either for pyrophosphate or for the polyphosphates. Zinc salts have been used to separate pyrophosphate from ortho- and metaphosphates at a pH of 3.8 but polyphosphates were found to precipitate also under these conditions, and precipitation at even lower pH values did not furnish a means of separation. Other precipitating agents investigated for this purpose included salts of cadmium, mercurous and mercuric mercury, ferrous iron, nickel, cobaltous and luteo-cobaltic cobalt, and manganous manganese.

Table IV. Determination of Pyrophosphate in Presence of Polyphosphates

Sample	P_2O_5 as $Na_4P_2O_7$ Found	Net $Na_4P_2O_7$ after Deducting Pyro
Gram	*Gram*	*Gram*
0.10 tripoly + 0.00 pyro	0.0161	....
0.09 tripoly + 0.01 pyro	0.0250	0.0105
0.075 tripoly + 0.025 pyro	0.0365	0.0244
0.10 tetra- + 0.00 pyro	0.0004	....
0.09 tetra- + 0.01 pyro	0.0099	0.0096
0.075 tetra- + 0.025 pyro	0.0270	0.0267
0.00 poly + 0.01 pyro	0.0098	0.0098
0.00 poly + 0.025 pyro	0.0242	0.0242

Of these salts manganous chloride was the only one found to precipitate pyrophosphate from a solution just acid to methyl red soon after its addition without doing likewise in the polyphosphate solutions. After several hours' standing at about 25° C. a small amount of precipitate settled out of both the tripoly- and tetraphosphate solutions, but the phosphorus pentoxide in each case was but a small portion of the total of the samples. In solutions containing from 25 to 100 mg. of pyrophosphate, the precipitate formed readily upon addition of manganous chloride, but the formation of a precipitate from solutions of less than 25 mg. of pyrophosphate was slow, requiring several minutes to a few hours for very small amounts. In the presence of polyphosphates the precipitation of pyrophosphate was retarded even longer. Chilling the solution in an ice bath delayed the precipitation still longer. When solutions of polyphosphates acid to methyl red indicator, to which manganous chloride had been added, were heated, not only was the rate of precipitation accelerated but the amount was greatly increased. This action appeared to be dependent on both the temperature and the length of time of heating at any given temperature, and may have been a result of decomposition of the polyphosphates. The most satisfactory conditions of temperature and time for precipitation of pyrophosphate in either the presence or absence of polyphosphate were found to be 20° to 30° C. and 12 to 16 hours.

The volume of manganous chloride solution (100 grams per liter of $MnCl_2 \cdot 4H_2O$) necessary for best precipitation of pyrophosphate alone was determined by varying the amount of manganous chloride solution from 2 to 50 ml. The minimum amount for optimum precipitation was 20 ml., but in the presence of polyphosphates the pyrophosphate results were abnormally high even after correcting for the pyrophosphate known to be present in the polyphosphate samples. In order to reduce this precipitation error of pyrophosphate in the presence of polyphosphates, the volume of manganous chloride solution was progressively reduced and it was shown that a total of 5 ml. was sufficient. At the same time to compensate for the increased solubility of manganous pyrophosphate in solutions not containing polyphosphates, a small amount of acetone was added which appeared to have overcome the solubility problem without affecting the precipitation when polyphosphates are present. As a further aid in establishing best conditions of precipitation, a series of pH control tests from 2.5 to 4.4 was made with the use of a glass electrode pH-meter. In each case manganous chloride was added to the solutions at a lower pH than prevails after final adjustment with 0.1 *N* sodium hydroxide. The results of these experiments indicated that a pH of 4.0 to 4.1 in combination with the other conditions already mentioned were the most satisfactory. Table IV gives data on analysis of pyrophosphate according to the directions given in the procedure.

TABLE V. ANALYSIS OF PHOSPHATE MIXTURES

Sample		Ortho	Pyro	Hexa-meta	Trimeta	Tripoly	Tetra
		%	%	%	%	%	%
1	Present	10.6	78.9	9.6	0.9	....	....
	Found	11.2	78.2	9.9	0.7	....	....
	Difference	+ 0.6	− 0.7	+ 0.3	−0.2	....	....
2	Present	31.9	29.9	....	1.5	....	36.6
	Found	31.1	31.1	....	2.3	....	35.7
	Difference	− 0.8	+ 1.2	....	+0.8	....	− 0.9
3	Present	25.9	27.7	22.8	2.2	21.4	....
	Found	25.5	26.6	25.0	0.7	22.3	....
	Difference	− 0.4	− 1.1	+ 2.2	−1.5	+ 0.9	....
4	Present	26.2	25.0	22.9	3.1	....	22.9
	Found	25.8	25.6	25.5	1.0	....	22.2
	Difference	− 0.4	+ 0.6	+ 2.6	−2.1	....	− 0.7
5	Present	80.5	9.9	....	0.4	....	9.1
	Found	79.7	9.2	....	0.4	....	10.8
	Difference	− 0.8	− 0.7	....	0.0	....	+ 1.7
6	Present	80.1	9.9	9.1	0.8	....	....
	Found	79.9	9.3	9.8	0.9	....	....
	Difference	− 0.2	− 0.6	+ 0.7	+0.1	....	....
7	Present	0.3	91.1	....	0.04	8.6	....
	Found	0.3	91.7	....	0.1	7.9	....
	Difference	0.0	+ 0.6	....	+0.06	− 0.7	....
8	Present	0.5	90.0	....	0.4	....	9.2
	Found	0.8	91.8	....	0.4	....	7.0
	Difference	+ 0.3	+ 1.8	....	0.0	....	− 2.2
9	Present	0.8	2.7	68.6	6.4	21.5	....
	Found	1.8	0.8	68.7	5.6	23.1	....
	Difference	+ 1.0	− 1.9	+ 0.1	−0.8	+ 1.6	....

As manganous ion also precipitates hexametaphosphate radical, although incompletely, the hexametaphosphate radical must be removed before precipitating the pyrophosphate radical with manganous chloride. In the procedure for the analysis of hexametaphosphate, the filtrate free of the hexametaphosphate radical might be used, were it not for the presence of a large excess of barium ions which might precipitate other phosphates when the pH is increased for the pyrophosphate precipitation. The removal of this interference by precipitation of barium as barium sulfate was unsuccessful as a considerable amount of pyrophosphate was found to precipitate with the barium sulfate. Although this difficulty can be reduced greatly in a strong hydrochloric acid solution, an uncertainty still exists as to the extent that pyrophosphate precipitation might occur. It would be desirable then to be able to reduce the excess of barium chloride used in the regular analysis of hexametaphosphate, so as to avoid removal of barium ion and yet effect a satisfactory separation of the hexametaphosphate radical.

In an attempt to accomplish this, stoichiometric quantities of barium chloride were added to hexametaphosphate solutions just acid to methyl orange. Instead of precipitating, the barium hexametaphosphate remained as a cloudy suspension which was almost impossible to filter. However, when barium chloride 10 per cent in excess of the calculated amount was used, the barium hexametaphosphate separated readily upon stirring vigorously and approximately 98 per cent of the hexametaphosphate was removed. These conditions appeared to obviate the difficulties from both the hexametaphosphate and barium, since pyrophosphate determinations, made on the filtrates from the removal of hexametaphosphate by this method, were in good agreement with the actual amounts of pyrophosphate present.

POLYPHOSPHATES. Chemically pure polyphosphates were found not to be available and because of the absence of other independent methods of analysis, the composition of the samples of tripoly- and tetraphosphates has been based on analyses according to the procedures developed in this investigation. This procedure may be open to questioning, especially because of the complex nature of these materials. Therefore, a brief discussion of the nature of the tests is given.

First, it appears that free hexametaphosphate is not present in the samples of polyphosphates used in this work, since no precipitate was obtained when samples were treated with barium chloride according to the directions for determination of hexametaphosphate. Furthermore, upon addition of known amounts of hexametaphosphate to the different polyphosphates, only the amount added was obtained on analysis.

With respect to the phosphorus pentoxide recovered as trimetaphosphate, it might be claimed that this was due to solubility of the barium polyphosphates in the alkaline precipitation. There may be a slight solubility, but the percentages of phosphorus pentoxide calculated to trimetaphosphate from different size samples were in too close an agreement with each other to be considered due essentially to solubility, and according to Bornemann and Huber (*3*) sodium trimetaphosphate, $(NaPO_3)_3$, may be present in polyphosphates depending on the proportions of sodium oxide and phosphorus pentoxide which are fused together and on the manner in which the melts are cooled.

The filtrates from the orthophosphate determinations remained free of phosphomolybdate precipitate too long after filtration to attribute the orthophosphate found to hydration. Both trimeta- and orthophosphate might be considered present as impurities.

Finally, the pyrophosphate determination appears to be open to question. In the case of the tripolyphosphate samples, a manganous phosphate precipitate, corresponding to 16.0 and 10.6 per cent, respectively, as tetrasodium pyrophosphate on samples from two different sources, was obtained consistently regardless of size of sample up to 0.1 gram or with the addition of known amounts of pyrophosphate. However, the tetraphosphate sample gave no or, at best, a very slight amount of precipitate alone, but with pyrophosphate added approximately 15 per cent of the tetraphosphate sample calculated as tetrasodium pyrophosphate was precipitated consistently with the pyrophosphate. This may actually be pyrophosphate not precipitating in absence of added pyrophosphate, or it may be a manganous polyphosphate equivalent of 15 per cent sodium pyrophosphate. Even with this discrepancy, the combined procedures go a long way in enabling one not only to find out whether polyphosphates are present but also to provide a good estimation of the amount. And, as pointed out in the procedures, solutions of tetraphosphate produced a cloudiness or precipitate upon addition of barium chloride to the filtrate from the pyrophosphate determination while tripolyphosphate gave none.

Generally, it was found that the qualitative test for distinguishing between tetraphosphate and tripolyphosphate could be applied satisfactorily even though the solutions had been prepared for several days. However, in one instance a phosphate mixture, containing about 9 per cent tripolyphosphate along with 35 per cent sodium carbonate, gave a qualitative test similar to that for tetraphosphate when carried out on solutions which had been made up a few days before analyzing. But tests on freshly prepared solutions of this phosphate mixture gave no indication of cloudiness or precipitate characteristic of tetraphosphate. Hence, this test must be performed on fresh solutions so as to avoid misinterpretation. In the analysis of several unknowns, the qualitative test was applied correctly to the detection of the presence of tetraphosphate.

At the conclusion of the development of the various procedures, a series of several mixtures of phosphates in widely different proportions was made up and analyzed to check the combined procedures. Data on these results are given in Table V.

Application to Commercial Products

ANALYSIS OF COMMERCIAL METAPHOSPHATES AND PYROPHOSPHATE. The procedures have been applied to the analysis of two different commercial metaphosphates and a pyrophosphate, with the results shown in Table VI.

COMMERCIAL PRODUCTS CONSISTING OF PHOSPHATES AND OTHER MATERIALS. Commercial products may be composed of mixtures of ortho-, pyro-, meta-, and polyphosphates not only in the presence of one another, but also in combination with various other materials, such as sodium hydroxide, sodium carbonate, sodium silicate, abrasives, and soap. Standard procedures of analysis of these materials may be found in reference books. If the mixture contains such soluble substances as sodium hydroxide, sodium carbonate, or sodium

TABLE VI. ANALYSIS OF SAMPLES OF COMMERCIAL PYRO- AND METAPHOSPHATES

Constituent	1 Meta %	2 Meta %	3 Pyro %
$(NaPO_3)_6$	63.8	67.4	...
$(NaPO_3)_3$	7.2	7.0	...
Na_2HPO_4	3.7	3.7	1.1
$Na_4P_2O_7$	...	...	98.9
$Na_2H_2P_2O_7$	5.2	6.6	...
$Na_6P_4O_{13}$	20.1	9.8	...
$NaHCO_3$	...	5.5	...
Total	100.0	100.0	100.0

TABLE VII. ANALYSIS OF PHOSPHATES IN PRESENCE OF CARBONATE AND SILICATE

Phosphate Present					Phosphate Found		
Ortho *Gram*	Pyro *Gram*	Hexa-meta *Gram*	$Na_2SiO_3 \cdot 5H_2O$ *Gram*	Na_2CO_3 *Gram*	Ortho *Gram*	Pyro *Gram*	Hexa-meta *Gram*
0.0586	.....	.....	0.125	...	0.0586	.....	.....
0.0586	.....	.....	0.125	0.5	0.0585	.....	.....
0.0293	0.0267	.....	0.125	0.5	0.0290	0.0270	.....
0.0293	0.00284	0.0250	0.125	0.5	0.0295	0.00280	0.0252
0.04814	0.0267	.....	1.0	0.5	0.04806	0.0270	.....
0.02407	0.00284	0.0250	1.0	0.5	0.02392	0.00280	0.0252
0.02407	0.00142	0.0125	1.0	0.5	0.02422	0.00139	0.0125
0.00481	0.0341	0.0125	1.0	0.5	0.00494	0.0344	0.01235

silicate, the procedures for the phosphates may be applied directly to the solution. If soap or insoluble material such as abrasives is present, it should be removed first. Abrasives may be separated by filtration and washed well to remove completely the soluble phosphates. Mixtures containing soap should be dissolved in the smallest amount of cold distilled water necessary for solution of the soap, then made just acid to methyl orange, and the insoluble fatty acids filtered off and washed well with cold water. The phosphates may then be determined in the filtrate by the indicated procedures.

It would be difficult to evaluate the accuracy of the method by the analysis of commercial products, since there are no standard references on which to base any conclusions. It seems preferable to analyze known mixtures. Hence, samples of various phosphates in combination with sodium silicate and sodium carbonate were prepared and analyzed.

ALKALINITY DETERMINATION. An alkalinity determination is an aid in enabling one to calculate a probable combination of the sodium oxide with the phosphate radicals in solution.

According to Gerber and Miles (7) the pH values at the equivalence points of the phosphates under consideration for titration are:

Phosphate	pH
$Na_4P_2O_7$	9.1
Na_2HPO_4	8.5
NaH_2PO_4	4.6
$Na_2H_2P_2O_7$	4.2

For solutions containing only mixtures of phosphates, the alkalinity is determined by a standard acid titration to a light pink phenolphthalein end point in the presence of sodium nitrate or sodium chloride, which titrates the alkalinity of trisodium orthophosphate to disodium orthophosphate. The titration is then continued to the greenish yellow end point of bromocresol green, which titrates the alkalinity of the disodium ortho- and tetrasodium pyrophosphates to monosodium ortho- and disodium acid pyrophosphates, respectively. From the alkalinity values and the values of phosphate radicals, combinations of the phosphates may be calculated.

If desired, a glass electrode may be used for this titration, but in this case, it will be necessary to determine empirically the actual pH values of the various conversion points in the presence of approximately the same salt concentration as the sample to be analyzed.

If the mixture of phosphates contains sodium hydroxide, sodium carbonate, or sodium silicate, the alkalinity determination is made to methyl orange and the carbonate and silicate determinations must be made also. The sodium oxide must then be adjusted to satisfy the pH of the solution for the different acid radicals present.

ANALYSIS OF UNKNOWN MIXTURES. After each step of the procedure had been checked separately, and the entire method had been applied satisfactorily to the analysis of mixtures of phosphates of known composition, two sets of "unknowns", one of mixtures of phosphates only and the other with sodium carbonate and sodium silicate in addition to phosphates, were prepared by a disinterested chemist and submitted for analysis. Actual titration values and compositions of the phosphate unknowns and the condensed summary of the sodium carbonate–sodium silicate unknowns are given in Table VIII.

TABLE VIII. ANALYSIS OF UNKNOWNS

	Phosphate	NaOH Used *Ml.*	Phosphate Found *Gram*	Phosphate Found %	Present %	Difference %
I.	Total P_2O_5	16.98, 17.00 Av. 16.99 (1 *N*)	....	..	..	...
	$(NaPO_3)_6$	37.8, 37.9 Av. 37.85 (0.1 *N*)	0.0168	17.4	16.7	+0.7
	$(NaPO_3)_3$	2.40, 2.50 Av. 2.45 (0.1 *N*)	0.0011	1.1	1.5	−0.4
	Na_3PO_4	29.80, 30.00 Av. 29.90 (0.1 *N*)	0.0213	22.1	22.1	0.0
	$Na_4P_2O_7$	10.01, 10.03 Av. 10.02 (1 *N*)	0.0579	60.1	59.7	+0.4
	Polyphosphate	(by difference)	None			
II.	Total P_2O_5	18.18, 18.22 Av. 18.20 (1 *N*)	....	..	..	...
	$(NaPO_3)_6$	84.70, 84.80 Av. 84.75 (0.1 *N*)	0.0376	39.7	38.8	+0.9
	$(NaPO_3)_3$	5.7, 5.9 Av. 5.80 (0.1 *N*)	0.0026	2.7	3.6	−0.9
	Na_3PO_4	20.90, 21.1 Av. 21.00 (0.1 *N*)	0.0150	15.8	15.5	+0.3
	$Na_4P_2O_7$	54.7, 54.8 Av. 54.75 (0.1 *N*)	0.0312	32.9	33.4	−0.5
	$Na_5P_3O_{10}$	1.53 (1 *N*) (by difference)	0.0084	8.9	8.7	+0.2
III.	Total P_2O_5	18.37, 18.38 Av. 18.38 (1 *N*)	....	..	..	...
	$(NaPO_3)_6$	None	....	..	..	...
	$(NaPO_3)_3$	1,3, 1.6 Av. 1.45 (0.1 *N*)	0.0006	0.5	1.2	−0.7
	Na_3PO_4	85.8, 85.4 Av. 85.6 (0.1 *N*)	0.0610	53.8	53.4	+0.4
	$Na_4P_2O_7$	39.4, 38.8 Av. 39.1 (0.1 *N*)	0.0226	19.9	14.9	+5.0
	$Na_6P_4O_{13}$	5.76 (1 *N*) (by difference)	0.0294	25.9	30.5	−4.6

Composition	IV Found	IV Present	IV Difference	V Found	V Present	V Difference	VI Found	VI Present	VI Difference
$(NaPO_3)_6$	..	..	...	10.4	10.1	+0.3	20.8	20.8	0.0
$(NaPO_3)_3$	0.6	0.8	−0.2	1.1	0.9	+0.2	2.3	2.0	+0.3
Na_3PO_4	12.6	12.2	+0.4	..	..	...	24.5	24.2	+0.3
$Na_4P_2O_7$	23.6	19.0	+4.6	16.2	16.4	−0.2	23.2	22.3	+0.9
$Na_5P_3O_{10}$	..	..	...	..	..	...	17.5[a]	18.2	−0.7
$Na_6P_4O_{13}$	15.7[a]	19.9	−4.2	..	..	...	..	..	...
Na_2CO_3	47.8	48.1	−0.3	57.3	56.7	+0.6	0.1	0.1	0.0
Na_2SiO_3	..	..	...	14.8	15.6	−0.8	11.1	12.2	−1.1
SiO_2	..	..	...	0.3	0.3	0.0	0.2	0.2	0.0

[a] By difference.

The titration values given in Table VIII are typical of those one might expect with the method, and indicate that good precision can be obtained. They also show that the method can be applied to the analysis of phosphate mixtures containing sodium carbonate and sodium silicate. The method can be applied to mixtures of phosphates in any proportion, but at the discretion of the analyst use of larger aliquots of the prepared solutions may be desirable for small amounts of any constituent.

Summary

A method has been worked out for the analysis of mixtures of phosphates in which hexameta-, trimeta-, pyro-, and orthophosphates are separated and determined. Polyphosphates are obtained from the difference between the total phosphorus pentoxide and the sum of the phosphorus pentoxide values of the other mentioned phosphates. Data on application of the procedures to synthetic phosphate and carbonate and silicate mixtures, commercial phosphates, and "unknowns" are included.

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Pressure-Measuring Device for Moderate Vacua

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THE increasing use of moderate vacua in distillations requires a pressure-measuring device having its maximum utility in the range of 0.05 to 2 mm. of mercury pressure. This range is below that at which the ordinary mercury-filled manometer is accurate and somewhat above that at which the conventional McLeod gage is designed to operate. Efforts to increase sensitivity by the use of lighter liquids of low vapor pressures in closed-end manometers yield unsatisfactory results, since such liquids wet glass and have a most persistent habit of sticking in the closed arm of the manometer. A serious objection to the use of mercury as the working liquid at these pressures is that a substantial amount of distillate vapor is present in the system and pressure variations eventually result in fouling the mercury surface. This is especially serious in McLeod gages where capillary tubes are used.

These difficulties have been overcome in this laboratory by the use of the modified McLeod gage shown. The operating liquid which has been found to give best results is olive oil which has been subjected to prolonged evacuation at 100° C. to remove volatile solvents and moisture. This is introduced through the trap, T, to fill the reservoir, R_1, and the by-pass tube, B, to the level, A-A'. An additional quantity of the oil is added to fill the supplementary reservoir, R_2 to its effective capacity. The effective capacity of R_2 is approximately the capacity of the compression tube, C. The device is attached to the distillation system by a length of pressure tubing forced over the outlet, S, which is bent at right angles to the plane of the figure and away from the operator.

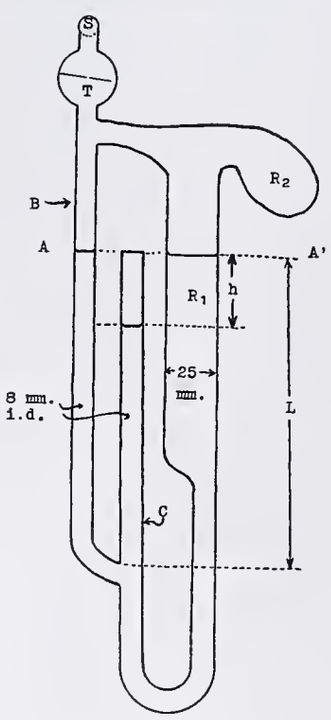

Table I. Calibration of Modified McLeod Gage

(Conversion: h in millimeters to millimeters of mercury pressure)

Units	Tens 0	1	2	3	4	5	6	7	8	9
0	...	0.038	0.163	0.390	0.740	1.24	1.93	2.84	4.05	5.66
1	0.0004	0.047	0.181	0.419	0.784	1.30	2.00	2.94	4.20	5.85
2	0.0015	0.056	0.200	0.450	0.828	1.36	2.09	3.05	4.34	6.06
3	0.0033	0.066	0.220	0.482	0.873	1.43	2.17	3.17	4.49	6.23
4	0.0060	0.077	0.241	0.515	0.921	1.49	2.26	3.28	4.64	6.44
5	0.0094	0.089	0.263	0.548	0.970	1.56	2.35	3.41	4.80	6.65
6	0.0135	0.102	0.286	0.583	1.02	1.63	2.44	3.53	4.96	6.86
7	0.0185	0.116	0.310	0.620	1.07	1.70	2.54	3.66	5.13	7.08
8	0.0244	0.130	0.336	0.659	1.13	1.77	2.64	3.78	5.30	7.30
9	0.0309	0.142	0.363	0.699	1.18	1.85	2.74	3.91	5.48	7.55

This rubber pressure tubing should be of sufficient length to permit rotation of the gage through a 90° arc about S as a center.

During evacuation of the system and between pressure readings this device is supported with tubes B and C nearly horizontal but with sufficient slope to permit drainage into reservoir R_1. Measurements are made by rotating the gage to the vertical with trap T uppermost. The level of the meniscus in B is adjusted to point A by the addition of oil from R_2. The depression, in millimeters, h, of the meniscus in C is a function of the pressure in the system

$$p_0 = \frac{h^2}{(L - h)r}$$

where r is the ratio of the density of mercury to that of the oil and p_0 is expressed in millimeters of mercury. A scale may be attached to bridge the space between B and C. This scale may be graduated in millimeters or to read directly in millimeters of mercury pressure. In actual use in this laboratory no such scale is used. The value of h is determined by the use of a millimeter rule and the value of p_0 is read off a chart which is mounted in a convenient location.

Great precision is not claimed for this apparatus but it is sufficiently accurate for vacuum distillations. While the idea of a rotary gage is not original with the author, the use of a nonvolatile liquid of low density in such a gage does not appear to be mentioned in available literature.

For the benefit of those who do not care to prepare a calibration chart, Table I is given. The gage for which the chart was prepared has L = 188 mm., and for the olive oil used r = 14.6.

Optical Activity of Some Cinchona Alkaloids and Some of Their Salts

In Mixtures of Water and Ethyl Alcohol

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Optical activity can be used as a criterion of the purity of cinchona alkaloids only if exact data are available as to the relationship between this property and such conditions as composition of solvent and degree of neutralization of the cinchona base. Such data are presented for a representative series of the more common quinine derivatives. For each compound studied, data are recorded showing the variation in optical activity in progressively varied mixtures of water and ethyl alcohol of the free base, the sulfate, and the dihydrochloride, as well as the change in optical activity as the free base is progressively neutralized with sulfuric and hydrochloric acids. Studies were made on quinidine, dihydroquinidine, cinchonine, and cinchonidine.

IN A recent paper (*2*), Andrews and Webb reported the results of a systematic investigation of the optical activity of quinine as the free base, the sulfate ($B_2.H_2SO_4$), and the hydrochloride (B.2HCl) in various mixtures of water and ethyl alcohol. At the same time there was reported the effect on optical activity of progressive neutralization of the free base with both sulfuric and hydrochloric acids. The purpose of this work was to establish optimum conditions for the use of optical activity as a criterion of purity of these alkaloids and to fill some of the many gaps in our knowledge of the stereochemistry of these compounds.

It has been found desirable to extend these investigations to other cinchona alkaloids. The present paper records the results of investigations, previously reported for quinine, applied to quinidine, dihydroquinidine, cinchonine, and cinchonidine. This series, while very limited, provides examples of the effect on optical activity of stereochemical isomerism, of the removal of the ethoxy group from quinine, and of hydrogenation of the vinyl group. The procedure used was identical with that described by Andrews and Webb (*2*). In the case of each compound the following data were determined:

1. The specific rotation of the free base, the sulfate, and the dihydrochloride in varying percentages of water and alcohol.
2. The effect on specific rotation of progressive neutralization of the free base with both sulfuric and hydrochloric acids.

The second of the above series was, in each case, carried out at that percentage of ethyl alcohol which had given the maximum rotation when the water-alcohol curve for that particular salt was determined. All figures for $[\alpha]_D$ were determined at 25° C. and refer to the free base, regardless of the salt used. These values are all rounded off to the nearest unit. In the opinion of the writer the reporting of specific rotations in tenths of a unit is usually not justified by the accuracy of the reading, particularly when, as is often the case in the present paper, solubility limitations compel the use of comparatively dilute solutions.

Quinidine

Kahlbaum's quinidine (base) was recrystallized four times by dissolving in 95 per cent alcohol and adding about five

TABLE I. SPECIFIC ROTATION OF CINCHONA ALKALOIDS

(In varying percentages of water and ethyl alcohol)

Quinidine Free Base, Quinidine Sulfate, and Quinidine Dihydrochloride

Free Base: Alcohol (by volume) %	Free Base: $[\alpha]^{25}_D$	Sulfate: Alcohol (by volume) %	Sulfate: $[\alpha]^{25}_D$	Dihydrochloride: Alcohol (by volume) %	Dihydrochloride: $[\alpha]^{25}_D$
20.0	216	0.0	230	0.0	312
28.0	227	7.4	240	10.8	315
36.0	237	19.4	260	18.8	317
44.0	243	27.4	267	26.8	317
52.0	248	35.4	272	34.8	315
60.0	252	43.4	275	42.8	312
68.0	257	51.4	277	50.8	307
76.0	260	67.4	275	58.8	302
84.0	260	75.4	272	66.8	295
92.0	258	83.4	267	74.8	287
100.0	255	91.4	262	82.8	280
		100.0	258	90.8	272
				98.8	265
				100.0	263

Dihydroquinidine Free Base, Dihydroquinidine Sulfate, and Dihydroquinidine Dihydrochloride

0.	190	0.	219	0.	277
20.0	203	17.4	230	9.0	281
40.0	215	27.4	241	17.6	285
50.0	219	35.4	248	26.8	287
60.0	225	43.4	252	34.8	287
70.0	231	51.4	252	42.8	285
80.0	235	59.4	251	50.8	277
90.0	234	67.4	250	58.8	270
100.0	228	75.4	249	66.8	262
		83.4	246	74.8	258
		91.4	244	82.8	250
		99.4	238	90.8	242
				98.8	230

Cinchonine Free Base, Cinchonine Sulfate, and Cinchonine Dihydrochloride

Free Base: Alcohol (by volume) %	Free Base: $[\alpha]^{25}_D$	Sulfate: Alcohol (by volume) %	Sulfate: $[\alpha]^{25}_D$	Dihydrochloride: Alcohol (by volume) %	Dihydrochloride: $[\alpha]^{25}_D$
32.0	150	0.8	195	0.	245
40.0	175	7.8	210	10.6	250
48.0	200	19.4	225	18.6	255
56.0	215	27.4	230	34.6	255
64.0	225	35.4	232	50.6	250
72.0	225	43.4	233	66.6	245
80.0	230	51.4	235	74.6	240
88.0	240	59.4	235	82.6	232
96.0	245	67.4	235	90.6	222
100.0	225	75.4	237	94.6	217
		83.4	235	98.6	210
		87.4	232		
		91.4	227		
		95.4	222		
		99.4	217		

Cinchonidine Free Base, Cinchonidine Sulfate, and Cinchonidine Dihydrochloride

	$-[\alpha]^{25}_D$		$-[\alpha]^{25}_D$		$-[\alpha]^{25}_D$
20.0	44	0.1	140	0.	180
28.0	88	5.9	150	10.6	185
32.0	100	18.7	165	18.6	187
44.0	115	38.7	180	26.6	187
56.0	127	58.7	185	42.6	185
68.0	125	70.7	190	58.6	175
76.0	122	78.7	195	70.6	165
84.0	120	82.7	190	78.6	157
92.0	117	86.7	185	82.6	152
96.0	115	90.7	180	86.6	147
100.0	108	94.7	170	90.6	140
		98.7	155	94.6	130
				98.6	120

volumes of water. The product was dried at 110° C. A constant specific rotation $[\alpha]_D^{25} = +255.0$ in absolute ethyl alcohol was obtained.

This is somewhat higher than the usually accepted figure reported by Rabe (*5*), +243.5, but the ease of contamination of quinidine with dihydroquinidine and resulting lowering of its optical activity have already been commented on by Butler and Cretcher (*3*). On the other hand, the author has never succeeded in obtaining a figure for the anhydrous free base in absolute alcohol as high as that reported by Butler and Cretcher (+262), but they do not specify temperature and the author employed a concentration of 0.300 gram per 100 ml. as compared with 2.0 grams per 100 ml. used by them. The author has found only small concentration effects with the optical activity of these alkaloids and has not investigated the effect of temperature variations. His sample melted at 167.5° C. (corrected). Butler and Gretcher report 162° to 163° C. for U. S. P. quinidine and 170° to 171° C. for their product made by rearrangement of quinine.

The sulfate and dihydrochloride were both made by adding the calculated amount of standard acid to the recrystallized free base, and diluting to volume with absolute ethyl alcohol to make the stock solution. The necessary correction was made for the amount of water introduced with the standard acid.

Neutralization curves were run as described by Andrews and Webb. In all cases, these curves were determined in that concentration of ethyl alcohol which had produced the highest specific rotation when the alcohol-water curve of that particular salt was run. Some deviations from this procedure were dictated by considerations of solubility of the salt. The actual percentage of alcohol used is recorded with each part of Table II.

Table I shows the variation in specific rotation of quinidine free base, quinidine sulfate ($B_2.H_2SO_4$), and quinidine dihydrochloride (B.2HCl). Table II shows the variation in specific rotation of quinidine (as free base) during progressive neutralization with sulfuric and hydrochloric acids at the specified concentrations of ethyl alcohol.

Table II. Effect on Optical Activity of Progressive Neutralization with Sulfuric and Hydrochloric Acids

Sulfuric Acid		Hydrochloric Acid	
Molar ratio of H_2SO_4 to base	$[\alpha]_D^{25}$	Molar ratio of HCl to base	$[\alpha]_D^{25}$
Quinidine Base[a]			
0.	245	0.	216
0.235	260	0.190	220
0.470	276	0.572	235
0.706	291	0.954	257
0.941	300	1.335	280
1.177	306	1.525	294
1.412	310	1.905	316
1.647	312	2.480	338
1.882	313	2.860	339
2.352	315	3.240	346
3.293	316	3.810	345
Dihydroquinidine Base[b]			
0.	219	0.	208
0.177	232	0.415	225
0.355	244	0.830	248
0.532	253	1.245	269
0.710	261	1.660	283
0.888	270	2.075	292
1.065	273	2.490	295
1.242	276	2.905	296
1.420	278	...	...
1.775	280	...	...
Cinchonine Base[c]			
0.	226	0.	150
0.205	230	0.747	230
0.410	235	1.493	251
0.615	240	2.240	260
0.820	243	2.990	265
1.025	247	3.735	267
1.435	252	5.230	268
2.05	255	7.470	267
3.28	255	...	...
Cinchonidine Base[d]			
0.	$-[\alpha]_D^{25}$ 121	0.	$-[\alpha]_D^{25}$ 110
0.160	137	0.584	142
0.320	164	1.168	166
0.480	191	1.752	182
0.640	199	2.336	187
0.800	202	2.920	189
1.120	206	4.380	187
1.600	207	5.84	186
2.400	209	7.30	184
3.200	207	...	...

[a] Sulfuric acid neutralization run in 50% ethyl alcohol by volume; hydrochloric acid in 20% ethyl alcohol by volume.
[b] Sulfuric acid neutralization run in 50% ethyl alcohol by volume; hydrochloric acid in 30% ethyl alcohol by volume.
[c] Sulfuric acid neutralization run in 75% ethyl alcohol by volume; hydrochloric acid in 30% ethyl alcohol by volume.
[d] Sulfuric acid neutralization run in 80% ethyl alcohol by volume; hydrochloric acid, in 40% ethyl alcohol by volume.

Dihydroquinidine

A sample of dihydroquinidine base (kindly furnished by the laboratories of Merck & Co., Inc.) was dried at 110° C. to constant weight and gave in absolute alcohol a value of $[\alpha]_D^{25} = +228.0$ (concentration of base = 1.852 grams per 100 ml. of solution). Since a recrystallized sample produced no measurable difference in optical activity, the original dried sample of the base was used. The value given by Rabe (*5*) is +237.5 at 15° C. and by Henry (*4*) is +230. In neither case is the concentration of alcohol specified. Using the author's sample of the base, the same determinations were made as described above for quinidine. The results for the alcohol-water curves of the free base, the sulfate ($B_2.H_2SO_4$), and the dihydrochloride (B.2HCl) are shown in Table I and the data concerning neutralization of the free base with sulfuric and hydrochloric acids in Table II.

Cinchonine

The fact that commercial samples of cinchonine, as usually obtainable, are highly contaminated with quinine makes necessary special precautions for obtaining a pure product. The sample used as a starting point for purification showed, in absolute ethyl alcohol, a specific rotation of +177 as compared with the figure of +224.4 reported by Rabe and +229 (at 17° C.) by Henry. In substantial agreement with Rabe's figure, the author's highest rotation in absolute ethyl alcohol, reached after successive purification, is +224.0.

The procedure used is based on the fact that the chief contaminant, quinine, is much more soluble in ether than is cinchonine and can therefore be removed by successive extractions. These were repeated until the ether extracts showed a constant optical activity and a constant amount of solid residue on evaporation. As the quinine content of the sample approaches zero, the amount of alkaloid dissolved by the ether and the optical activity shown by the solution gradually approach that produced by the very limited solubility of cinchonine base in ether. This is listed by Allen (*1*) as 0.27 gram of cinchonine per 100 ml. of ether. The specific rotation of +177 for this sample indicates, by interpolation, the presence of about 12 per cent of quinine, assuming no other alkaloids to be present.

A 25-gram sample was shaken mechanically for some hours with 100 ml. of ether. The filtered ether solution was read in a 4-dm. polariscope tube and 25-ml. portions were evaporated to dryness and the residues weighed. Twenty-six such extractions were made before constancy of rotation and of solubility was obtained. During this series the observed rotation changed progressively from −2.90 to +1.20 as the quinine was removed. The last three extractions gave satisfactorily constant values averaging 0.170-gram residue per 100 ml. of ether solution.

Assuming cinchonine to be the only solid phase present, this indicates an ether solubility of this alkaloid at 25° C.

of 0.170 gram per 100 ml. of solution, a figure much lower than that of Allen (*1*). This also gave a specific rotation in ether of $[\alpha]_D^{25} = +176$. The residual cinchonine when dried gave a specific rotation of +224.0. This sample of the base was used for all optical activity determinations as described above under quinidine. The results for the alcohol-water curves of the free base, the sulfate ($B_2.H_2SO_4$), and the dihydrochloride (B.2HCl) are shown in Table I, and data concerning neutralization with sulfuric and hydrochloric acids in Table II. Because of the more limited solubility of cinchonine base in water, the data for the former in Table I could not be extended to alcohol concentrations below 32 per cent. Even at this point only 0.02 gram of base per 100 ml. was possible. The error of the determination is therefore correspondingly large.

Cinchonidine

A commercial sample of the free base was recrystallized from solution in 95 per cent ethyl alcohol by addition of sufficient water to reduce the alcohol percentage to 25 per cent. The resulting precipitate was filtered, washed with 25 per cent ethyl alcohol, and dried. Three such treatments gave a product showing, in absolute ethyl alcohol, a constant optical activity of $[\alpha]_D^{25} = -107.3$. A value of −111.0 was reported by Rabe. Since further recrystallization did not alter the author's figure (−107.3), it was taken as representing the pure base under the conditions specified. Using this sample, the determinations were made as described above for quinidine. The results for the alcohol-water curves of the free base, the sulfate ($B_2.H_2SO_4$), and the dihydrochloride (B.2HCl) are shown in Table I, and data concerning neutralization of the free base with sulfuric and hydrochloric acids in Table II.

It is obvious that the curves of all of these alkaloids follow the same pattern. As in the case of quinine, the water-alcohol curves of optical activity of free base, sulfate, and dihydrochloride all rise to flat maxima at intermediate percentages of water and alcohol, giving lower values of specific rotation in both pure water and pure ethyl alcohol. The position of these maxima varies with the different cinchona derivatives examined, but it may be said that for the free bases, the maxima lie between 60 and 95 per cent alcohol by volume, for the sulfates between 50 and 75 per cent, and for the dihydrochlorides between 20 and 30 per cent.

Using, in each case, the percentage of alcohol giving maximum rotation for that salt, the neutralization curves shown in Table II were run. These follow the course previously reported for quinine: a smooth curve with no breaks corresponding to any stoichiometric ratios. In some cases, particularly with the hydrochlorides, addition of excess acid causes a slight drop in the maximum rotation attained. Some examples of this will be noted in the table.

Acknowledgment

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Oil Absorption of Pigments

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At a certain point in oil absorption of pigments the paste in oil is very easily taken off on the palette knife. This marks the point of saturation and the indication is sharp within 1 drop of oil (0.05 cc.) from an ordinary standard buret.

When pastes, fully or partly saturated with oil according to the above standard, are immersed in a bath of oil stock used for absorption, saturated pastes absorb no more oil, while unsaturated pastes absorb oil almost equal to the calculated deficiency for saturation in one to two days (24 to 48 hours).

THE term "oil absorption" has been vaguely defined and the method of determining its value has not been truly standardized. In view of the circumstances, a rigorous study of oil absorption with strictly accurate and reproducible results has not yet been possible.

Oil absorption has been defined in various ways: as nothing but the filling of a void (*5*) or the filling of interspaces of the pigment particles with oil, the wetting power of the liquid (*3*), or the maximum quantity of oil required with any pigment for an intimate mixture in liquid state with minimum tendency to flow. A point which does not appear to be appreciated and which adds to the difficulty of attaching significance to oil-absorption measurement is the problem of defining experimentally the condition of complete wetting of the pigment. The criterion usually adopted by specifications depends on a rather vague visual judgment of consistency at the end point, which although it may afford a fairly accurate basis for comparing samples of the same pigment, does not necessarily serve well for comparison between different pigments.

Apart from such interpretations, there is in general neither a fixed method nor a sharp finishing point in the determination of oil absorption. The current methods of locating the end point have more or less the common flow of a rule-of-thumb experiment and, as such, the end point determined by these methods is scarcely reproducible with exact precision.

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Table I. Absorption of Oil by Unsaturated Pigment Pastes

Pigment	Quantity Taken	Degree of Saturation	Oil Absorbed 24 hours	36 hours	48 hours	Calculated Deficiency of Oil for Saturation
	Grams	%	*Cc.*	*Cc*	*Cc.*	*Cc.*
Red oxide of iron	10	50	0.8	0.8	0.8	1.0
		75	0.4	0.4	0.4	0.45
White lead	20	50	1.2	1.2	1.2	1.3
		90	0.2	0.2	0.2	0.26
Lithopone	10	50	0.7	0.7	0.7	0.75
		90	0.05	0.09	0.09	0.15

Table II. Percentage of Oil Absorption by Common Pigments

			Raw Linseed Oil	Double-Boiled Linseed Oil	Castor Oil	Tung Oil
	Quantity Taken for Each Experiment[a],	Specific gravity	0.930	0.931	0.953	0.936
		Acid value	2.7	6	7	6.6
		Saponification value	192	198	186	200
		Iodine (Hanus) value	170	145	83	165
Pigments	Grams		Mean Oil Absorption Values			
White lead	20		12.15	12.32	12.12	12.17
White zinc	10		17.89	16.81	17.63	16.85
Lithopone	10		14.18	13.02	13.34	13.1
Whiting	20		19.65	19.29	19.77	19.07
Barytes	20		11.16	10.35	10.48	10.18
Yellow ochre	10		27.9	..	23.83	23.4
Red ochre	10		22.39	18.83	20.1	18.25
Ultramarine blue	10		33.48	33.48	35.26	31.82
Prussian blue	5		56.27	..	53.37	..
Red oxide	10		16.74	14.88	16.20	15.68
Red lead	20		7.44	7.44	7.15	7.02

[a] According to recommendations of I. S. D. specifications.

The Gardner-Coleman method which determines the "oil absorption factor" is accurate within 0.2 cc. (of oil added). The elimination of grinding and incorporation of certain precautions make it easier to duplicate the results within that limit of accuracy. But, unfortunately, the oil-absorption factor does not work out a paste of the conventional consistency approved by painters; and the method, apart from its theoretical importance, has not much to recommend itself to the paint maker in his everyday practice. Obviously, the standard rub-out method and the Gardner-Coleman method give results wide apart, one using much less oil than the other (*2*).

The author of the present communication, after a prolonged search, has observed definite indications locating a sharp end point. The results provide a dependable basis for a comparative study of oil absorption of pigments.

It has been consistently observed by the author that at a certain point, on gradual addition of oil to the pigment with constant rubbing for thorough incorporation of the oil with the pigment, the paste sticks to the palette knife with very little effort. The difference in the coherence of the mass is clearly distinguishable before and after this definite stage is reached. The results are reproducible with very close coincidence.

Following the A. S. T. M. procedure (*1*), using a buret and taking about 20 minutes for each experiment for comparable extent and pressure of grinding, oil absorption of pigments was determined using raw linseed oil of known specifications. The quantity of pigment varied according to its nature from 5 grams in the case of Prussian blue to 20 grams in the case of white lead, following the recommendations of the I. S. D. specifications (*4*). This limits the amount of oil added, in most cases to approximately 40 drops (1 drop = 0.05 cc.). The variation in the results did not exceed 1 drop of oil from a standard buret or 1 per cent in the value of oil absorption.

There is, however, another aspect of the end-point determination by this method. The results have supplied a newer definition of oil absorption of pigments. The smooth pigment pastes which absorbed oil up to the sharp end point located by the author did not absorb more oil when immersed in a bath of the oil stock used for absorption, whereas pastes with lesser oil did actually absorb more oil almost equal to the calculated deficiency of saturation. A graduated cylinder, used as an oil bath, was filled up to a certain point with the paste carefully scraped into, and totally immersed in, oil. The depression in the level of oil in the cylinder was noted after 24, 36, and 48 hours (Table I).

Thus comes a new interpretation of oil absorption, which might now be defined as the minimum quantity of oil required to saturate a pigment by grinding. The end point as located is not only supported on logical and scientific grounds, but also throws a flood of light on the true approach to this obscure problem of a very well-known practice.

Table II gives oil-absorption values for some common pigments with different oils, as determined by the author at the Paint and Varnish Section, Industrial Research Laboratory in Calcutta, Bengal, India. Each result represents a mean of 70 duplications, mostly concurrent. In the few cases where variations were noted, the difference did not exceed 1 per cent. A steel spatula (Sheffield, London) with a 8.75 × 2.5 cm. (3.5 × 1 inch) blade and a marble slab 30.0 cm. (1 foot) square were used for grinding. The temperature ranged from 23.89° to 29.44° C. (75° to 98° F.) and humidity varied between 52 and 67 per cent.

The author's attention has lately been drawn to a recent U. S. Navy Department Specification, 52-Z-7 (int.), September 1, 1941, Bureau of Ships ad interim specification, advocating a method identical with the author's. Incidentally, the experiments by the author were conducted towards the end of 1940. This happy coincidence is in itself a very strong argument in favor of the method for location of the end point.

Conclusion

In view of the fact that pastes produced by various pigments with oil show a considerable difference in characteristics, the end point is better located at the point of saturation as described. The saturated paste is invariably a coherent, stiff, puttylike mass which does not break or separate and which lends itself conveniently for further operation in the preparation of mixed paint.

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Determination of Thiosulfate in Used Doctor Solution

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A method for determining thiosulfate in used doctor solution is based on the observation that carbon dioxide precipitates the lead and simultaneously converts any sulfite present to bisulfite, which subsequently is bound to formaldehyde to render it inactive toward iodine. Under these conditions, thiosulfate is titrated alone.

ONE of the finishing steps in the refining of gasoline and other light petroleum fractions is the "sweetening" operation in which mercaptans, the compounds responsible for the "sour" odor of the product, are converted to less obnoxious disulfides. A process widely used for this purpose is the doctor treatment, in which gasoline is agitated with doctor solution (sodium plumbite in an excess of caustic soda solution) with subsequent addition of free sulfur in carefully controlled amounts. The conversion from mercaptans to disulfides is believed to occur according to the following equations:

$$2RSH + Na_2PbO_2 \longrightarrow (RS)_2\,Pb + 2NaOH \quad (1)$$

$$(RS)_2Pb + S \longrightarrow R_2S_2 + PbS \quad (2)$$

The disulfides remain in the hydrocarbon fraction, which is separated from the used doctor solution containing lead sulfide.

The usual practice is to regenerate the spent doctor by oxidation (air blowing); the reaction proceeding, it is believed, according to the following equation:

$$2PbS + 2O_2 + 6NaOH \longrightarrow 2Na_2PbO_2 + Na_2S_2O_3 + 3H_2O \quad (3)$$

Since sodium thiosulfate, which is the principal by-product of this process, remains in the reactivated doctor solution and reflects its further utility for sweetening operations (*8*), a method for its analytical determination was desired.

The effect of this increasing thiosulfate concentration is to further the tendency to emulsification, decrease the solubility of lead, and increase the specific gravity, so that gravity is no longer an index of the caustic concentration in doctor solution (*6*). A number of methods are available in the literature for determining it in the presence of other reducible sulfur salts, but none of these can be applied directly to the analysis of doctor solution because lead interferes.

Kalmann (*4*) determines thiosulfate plus sulfite by titration with iodine. In the titrated solution hydriodic acid, equivalent to the sulfite,

$$Na_2SO_3 + I_2 + H_2O = Na_2SO_4 + 2HI$$

is titrated with alkali. Thiosulfate is calculated from the two titrations.

Autenrieth and Windaus (*1*) determine thiosulfate by titration with iodine after removing sulfite as strontium salt. A similar method, removing sulfite as barium salt, is described by Scott (*7*).

Giberton (*2*) describes a method based on rapid conversion of silver thiosulfate to silver sulfide, the sulfur of which is determined colorimetrically.

Heinemann and Rahn (*3*) describe a method for determining reducible sulfur in caustic soda, based on reduction of such sulfur compounds to hydrogen sulfide which is determined iodometrically.

Several promising methods were investigated after it was observed that in doctor solution carbon dioxide quantitatively precipitates lead and converts sulfites to bisulfites. When sodium sulfite is added to fresh doctor solution and an excess of carbon dioxide is passed through it and filtered, the filtrate gives no evidence of lead to hydrogen sulfide. When formaldehyde is added to another portion of the filtrate and this is titrated with iodine the latter is immediately in excess, indicating the absence of sulfite.

Most of these procedures, though capable of giving accurate results on knowns, are too long and tedious for routine use. In this class belong such methods as that of Autenrieth and Windaus (*1*) which, involving separation of sulfite as its strontium salt, requires too much time for settling and filtering.

The method of Kurtenacker and Wollak (*5*), which is based on the fact that bisulfite is rendered inactive toward iodine by binding it to formaldehyde, formed the basis of the following method, which has been adopted for determining thiosulfate in used doctor solutions.

Apparatus and Reagents

Apparatus required includes 500-ml. and 250-ml. volumetric flasks, 50-ml. pipet, 50-ml. buret divided in 0.1 ml., 1000-ml., 500-ml., and 250-ml. Erlenmeyer flasks, glass funnels, carbon dioxide reducing valve, and Whatman filter paper No. 4, 11 cm.

The reagents are carbon dioxide, 0.1 *N* iodine, 0.1 *N* sodium thiosulfate, C. P. formaldehyde (35 to 40 per cent), 10 per cent acetic acid, starch indicator, and phenolphthalein indicator.

Procedure

Pipet 50 ml. of sample into a 500-ml. volumetric flask, dilute with water to 500 ml., mix, transfer the entire solution to a 1000-ml. Erlenmeyer flask, add a few drops of phenolphthalein indicator, and pass carbon dioxide through the solution until the pink phenolphthalein color is discharged. The lead is thus precipitated and any sulfite present is converted to bisulfite. As a precaution continue the passage of carbon dioxide for 5 minutes longer.

Filter this solution through Whatman No. 4 filter paper and collect 250 ml. of filtrate in a 250-ml. volumetric flask. For each test put 50 ml. of this filtrate into a 250-ml. Erlenmeyer flask and add 5 ml. of 35 to 40 per cent formaldehyde, followed after 5 minutes by 20 ml. of 10 per cent acetic acid. Titrate the solution with 0.1 *N* iodine immediately after addition of the acetic acid, using starch as indicator.

The 0.1 *N* iodine solution should be checked daily against 0.1 *N* thiosulfate.

Calculations

1 ml. of 0.1 *N* iodine $\simeq$ 0.01582 gram of sodium thiosulfate. 20 $\times$ ml. of 0.1 *N* iodine $\times$ grams of thiosulfate per ml. of standard iodine = grams of sodium thiosulfate per 100-ml. sample.

Discussion

The weight-volume manner of reporting results is in accord with the plant practice of expressing constituents of doctor solution in pounds per barrel.

Frothing usually occurs on passing carbon dioxide through the sample, but subsides after a few minutes, during which a little shaking of the flask will prevent serious difficulty.

The end point fades after a little standing but is sufficiently stable for recognition without difficulty.

After the precipitation of lead by carbon dioxide, the used doctor solution usually is still colored and has a phenolic odor, indicating that not all phenolic compounds have been removed. These compounds, however, do not interfere with the iodine titration. This was demonstrated by comparing

Table I. Thiosulfate Determinations in Used Doctor Solutions

(Known amounts of thiosulfate added)

Sample	Thiosulfate in Original	Thiosulfate Added	Total Thiosulfate Found	Added Thiosulfate Found
	G./100 ml.	*G./100 ml.*	*G./100 ml.*	*G./100 ml.*
1	2.55	3.12	5.60	3.05
		6.23	8.65	6.10
		9.34	11.76	9.21
2	1.74	2.49	4.38	2.64
		4.98	6.80	5.06
		7.48	9.34	7.60
3	1.94	1.25	3.10	1.16
		2.49	4.38	2.44
		3.74	5.45	3.51

results obtained by the proposed method with those obtained by a long gravimetric procedure in which organic matter does not interfere. The latter involved the separation of sulfate and sulfite as barium salts and the oxidation of the thiosulfate to sulfate which was determined gravimetrically. Results obtained by the two methods agreed very well.

The time required for a thiosulfate determination is approximately one hour for the proposed volumetric method as compared to approximately 12 hours' elapsed time for the gravimetric method.

The precision and accuracy of the proposed method, applied in a routine manner, are evident from Table I.

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Apparatus for Continuous Concentration of a Solution under Reduced Pressure

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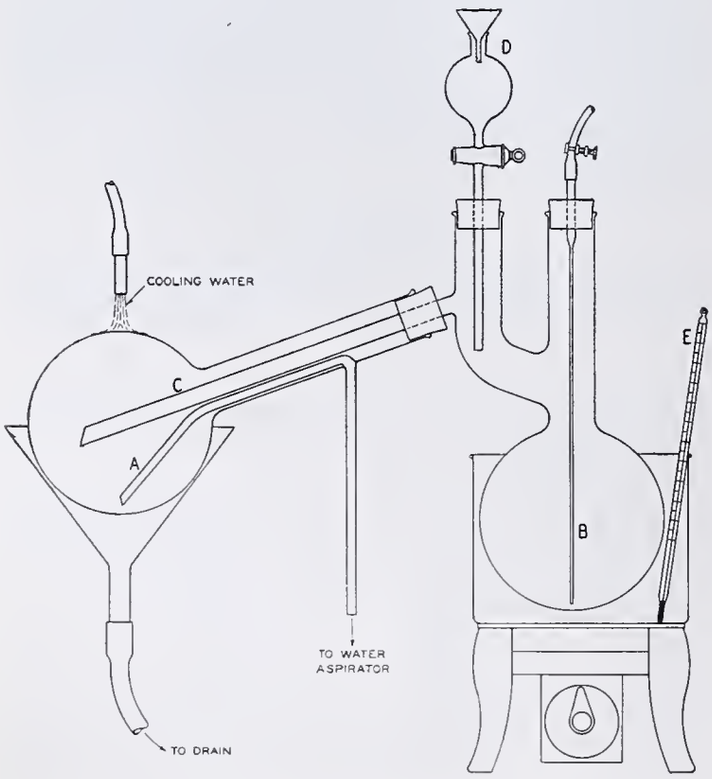
In this laboratory, it is frequently necessary to concentrate large volumes of dilute sugar solutions resulting from the hydrolysis of polysaccharides, the solvent—water or alcohol—being discarded. Because of the danger of decomposition at higher temperatures, distillation at reduced pressure with a maximum bath temperature of 50° C. is used. A water aspirator lowers the pressure enough to obtain rapid distillation. With the usual apparatus (consisting of two side-arm distilling flasks, one being the boiling flask and the other the receiver, cooled with a stream of water), however, the process of concentration must be frequently interrupted in order to empty the receiver and to add more of the dilute solution to the boiling flask.

A modified concentration apparatus, which has been in use in this laboratory for over two years, is described here. It may be operated continuously until the concentration is completed, the receiver being automatically emptied and the dilute solution being added from time to time, or continuously, without breaking the vacuum or stopping the boiling.

A cursory search of the literature shows that a somewhat similar apparatus was reported by Burger (*1*), but it is believed that the apparatus here described necessitates much less manipulation in operation for the purpose outlined above.



The receiving flask was made by sealing a piece of 6-mm. tubing, *A*, into an ordinary 2-liter, side-arm, distilling flask. Another modification of this arrangement consists of a close-fitting rubber tube pushed through the side arm and extending to the bottom of the receiving flask. While not suitable for use with all solvents, this arrangement has been used for several years in this bureau for concentrating sugar solutions. *B* is a fine capillary, to help prevent bumping, with a short rubber tube and screw clamp at its upper end for regulation. Replacing the usual side arm, *C*, of the Claisen distilling flask with a tube of larger diameter (15-mm. outside diameter) hastens the distillation. The dilute solution to be concentrated is added through the funnel, *D*, either intermittently or by a continuous slow drip as the liquid boils out. *E* is a thermometer to indicate the bath temperature. The vapors are condensed in the receiving flask, then drawn off by the aspirator through tube *A* to the drain. The rest of the equipment is obvious from the drawing.

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Agricultural Chemical Research Division Contribution No. 54.

A Dropping Mercury Electrode

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A NUMBER of suggestions have been made for constructing the dropping mercury electrode (*1–6*). The trend is to avoid the use of rubber tubing, since it reacts with the mercury. The rubber connections can be eliminated by a very simple siphon system.

The mercury storage vessel has the form shown in *a*, Figure 1, the capillary itself is *b*, and the glass tubing, *c*, dips into the storage vessel. The height of the mercury can be varied by moving *a* up or down. A mark on the side arm, *d*, makes it easy to reproduce the mercury pressure for every polarogram. The capillary itself is connected to the siphon with an interchangeable ground joint 10/30, held in place by a couple of springs. This makes it easy to clean the capillary separately.

The capillary should be sealed to the glass tubing of the ground joint. To blow out a capillary as used in polarography it is necessary to use the pressure of a gas cylinder. To make such a seal without a cylinder, the capillary tubing is filled with mercury, using an ordinary leveling bulb with mercury connected to the capillary with rubber tubing. The open end of the capillary is closed with one finger and the capillary disconnected from the rubber tubing. One end of the capillary is brought into a very hot flame to close the capillary on this side, the other end being closed with the finger; then the other end is also sealed. The capillary should now be nearly full of mercury. If the glass is heated with a moderately hot flame, the pressure of the mercury will blow it out to a bulb large enough to be sealed to ordinary glass tubing. The sealed end of the capillary is now broken off.

A ground joint 12/18 is sealed to the electrode (Figure 2). The cell to be used with this electrode has a corresponding ground joint, and *g* and *h* are inlet and outlet for the gas. Contact to the mercury pool in the bottom is made through *k*. The capillary tubing between *k* and the cell should have a diameter of approximately 1.5 mm. If an outside anode or reference electrode is to be used, a two- or three-necked cell should be made.

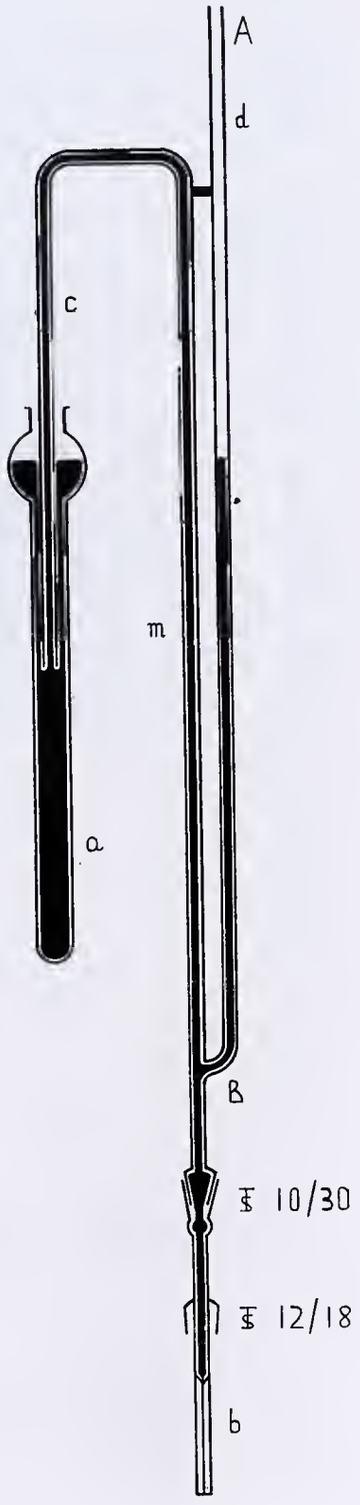

FIGURE 1

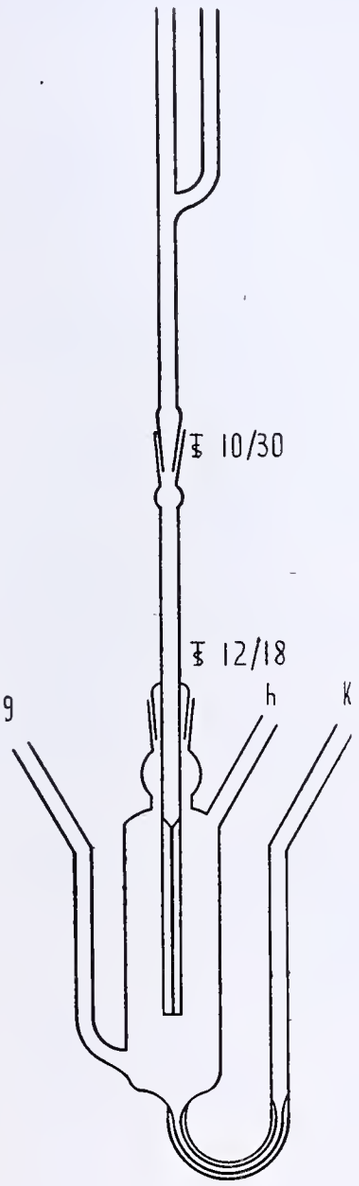

FIGURE 2

Such a capillary should be cleaned before being put into service. Concentrated nitric acid, which is mostly used for this purpose, is not very suitable, as salts form in the reaction of the acid with the mercury left in the capillary and clog it. A solution of iodine in potassium iodide proved to be very effective in cleaning.

To fill the capillary with mercury, *c* is dipped into the storage vessel filled with mercury, and using suction cautiously at *A*, some mercury is brought into *m*. The tapping of *m* causes the mercury to fall and fill out the tubing up to point *B*, where the side arm starts out. Now, using pressure at *A*, all mercury over *B* is driven back into the storage vessel so that the siphon will be free from air bubbles in the end. Then the mercury is brought over by using full suction for a moment. It is necessary to use a safety bottle to save any mercury carried over by the suction.

This construction allows the side arm with the capillary to be pivoted about the storage vessel as center, and swung away from the polarographic cell. When not in use, it is dipped into a test tube, which is provided with the same ground joint as the polarographic cell. This test tube should be filled with pure mercury. The pressure in the test tube, which is closed tightly by the ground joint of the capillary, increases as the mercury flows into the test tube, and this back pressure will soon stop the flow of mercury completely.

An electrode and cell of this design have been in use for more than six months, and they have worked very satisfactorily.

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Nomograph for Computing Compound Compositions of Portland Cements

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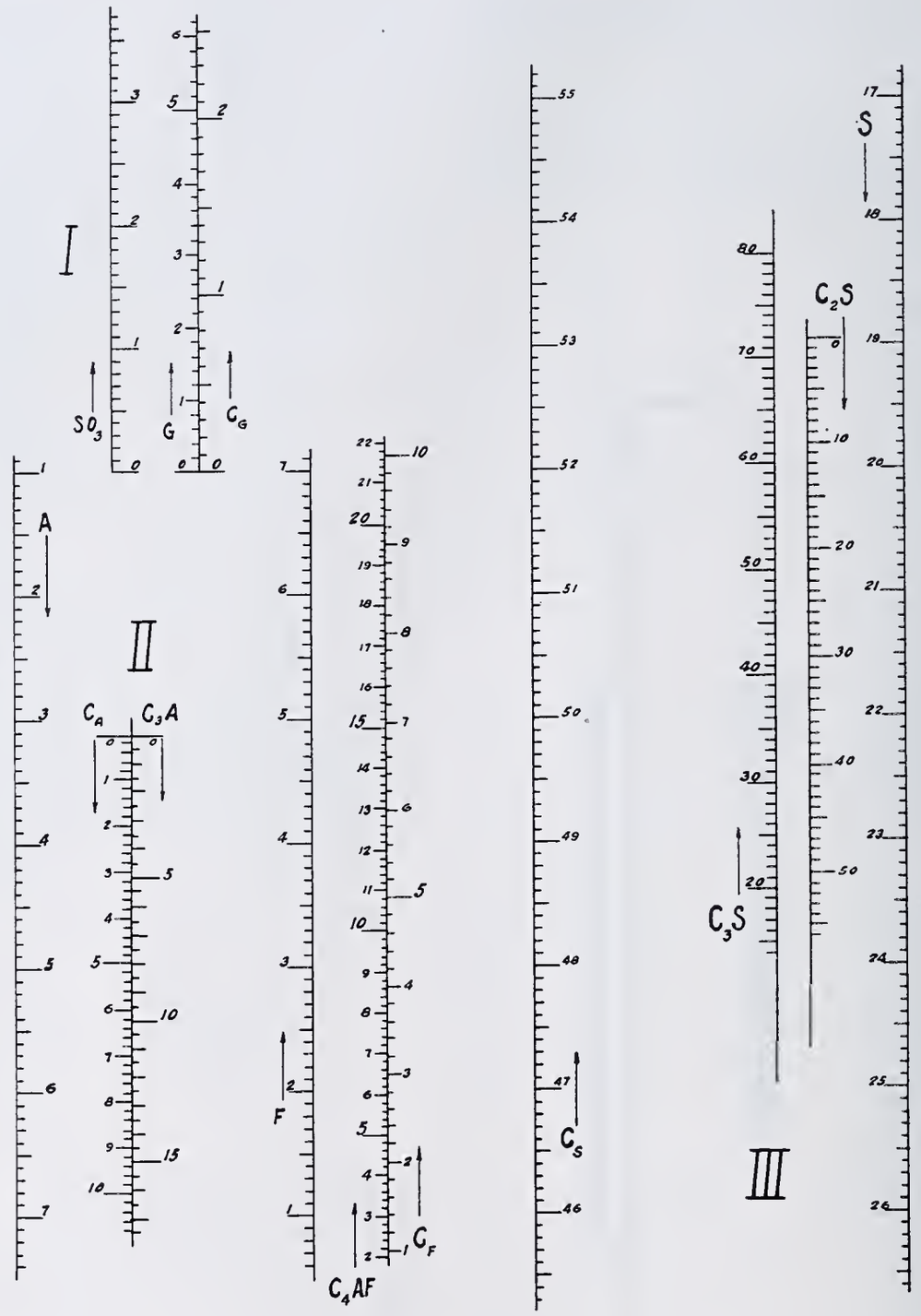

CONSTRUCTION departments of government agencies, federal, state, and municipal, are showing an increasing tendency to specify portland cements on the basis of the computed compound composition (*2*). The latest revision of the specifications for portland cement of the American Society for Testing Materials reflects this trend (*1*). The nomographic chart submitted herewith simplifies considerably the necessary computations from the oxide analyses.

For the values of tetracalcium aluminoferrite, tricalcium aluminate, dicalcium silicate, and tricalcium silicate, we have the following equations:

$$C_4AF = 3.043F$$
$$C_3A = 2.650A - 1.692F$$
$$C_2S = 8.602S - 3.071C_S$$
$$C_3S = 4.071C_S - 7.602S$$

where

C_4AF = tetracalcium aluminoferrite ($4CaO.Al_2O_3.Fe_2O_3$)
C_3A = tricalcium aluminate ($3CaO.Al_2O_3$)
C_2S = dicalcium silicate ($2CaO.SiO_2$)
C_3S = tricalcium silicate ($3CaO.SiO_2$)
A = alumina
F = ferric oxide
S = silica

C_S = net calcium oxide available for silicates
= $C - (C_G + C_F + C_A + C_{free})$
C = total calcium oxide
C_A = calcium oxide in tricalcium aluminate
C_G = calcium oxide in calcium sulfate (gypsum)
C_F = calcium oxide in tetracalcium aluminoferrite

and

C_{free} = free calcium oxide, if the analysis has included this determination

The steps in using the nomographic chart would be:

At I, from the value for sulfuric anhydride, a straightedge parallel to the base line gives the values for calcium sulfate (G) and C_G. At II, a straightedge at the value for F parallel to the base line gives to the right the values for C_4AF and C_F. A straightedge from the value for F to the left for the value for A gives C_3A and C_A, as indicated. From the values of C, C_G, C_F, C_A, and C_{free} (if known), C_S is computed and used at III. A straightedge between the values of C_S and S intersects the lines indicated to give at once C_2S and C_3S.

The accuracy of the calculation by this chart is adequately commensurate with the precision of the oxide analysis, as is readily apparent from two typical analyses and computations shown in Tables I and II. Some of the figures are carried to more decimals than would ordinarily be warranted; the intent is to indicate the fact that the chart is sufficiently accurate for all practical purposes.

Table I. Oxide Analysis

	Cement 1	Cement 2
SiO_2	19.8	22.0
Al_2O_3	5.8	5.35
Fe_2O_3	5.07	4.95
CaO	62.6	61.7
MgO	3.60	3.41
SO_3	1.50	1.37
Ignition loss	0.86	0.80

Table II. Compound Composition, Computed

	Cement 1		Cement 2	
	By logs	By chart	By logs	By chart
$CaSO_4$	2.6	2.6	2.3	2.3
C_4AF	15.5	15.4	15.1	15.1
C_3A	6.8	6.8	5.8	5.8
C_2S	16.1	16.0	35.0	34.9
C_3S	53.9	53.8	37.2	37.1

Literature Cited

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An Injection and Sampling Stopcock

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IN APPARATUS for the automatic, continuous determination or detection of a component of a gaseous mixture, it is imperative that checks with gases containing a known amount of that component be made at suitable intervals to make sure that the sensitivity of the apparatus has not been impaired. This can be done either by displacing the gas being analyzed by a stream of test gas or by injecting a known quantity of test gas into the line. An accepted method for accomplishing the latter is the use of a calibrated by-pass (*1*) which is also a part of the test gas line. By manipulation of three stopcocks the definite volume of test gas contained in the calibrated by-pass may be flushed into the analytical gas line (Figure 1).

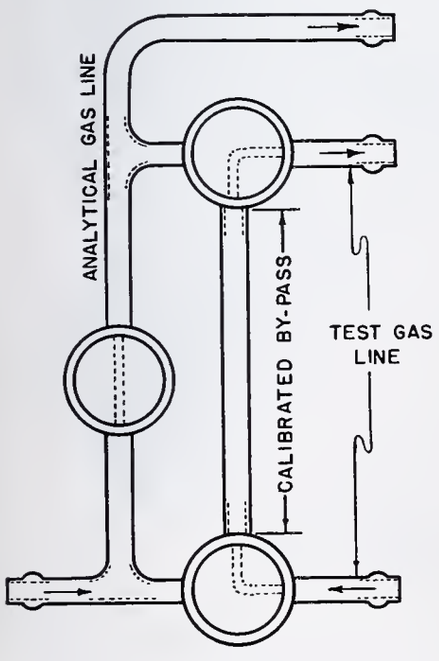

Figure 1. Baldewyns' Injector

A simplification of this injector is shown in Figure 2. In this stopcock, one of the bores is always in the line of flow of gas being analyzed, while the other is in the line of flow of the test gas—that is, there is gas passing through both bores at the same time. To inject a small amount of test gas into the analytical gas line, the stopcock is turned 90 degrees. The quantity of test gas that was in the test gas line stopcock bore just prior to turning is thus transferred to the analytical gas line. By means of calibrated bores, the amount of each injection can be determined. Larger quantities of the test gas may be injected into the analytical gas line by either manual or mechanical rotation of the stopcock plug.

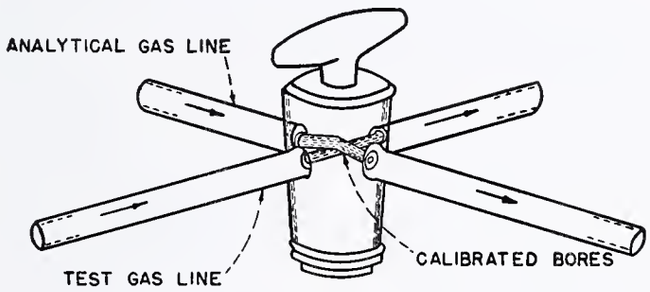

Figure 2. Injection and Sampling Stopcock

The single stopcock injector, when compared with Baldewyns' injector, thus represents a real saving of material and a simplification of manipulations.

By passing an inert carrier gas through the analytical gas line and the gas to be tested through the other line, small samples may be periodically taken for analysis.

In addition to the application mentioned, the device should be useful for mixing and proportioning various fluid combinations.

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Fractionation of Colloidal Systems

Selection of Operating Conditions for the Supercentrifuge

GEORGE FANCHER, S. C. OLIPHANT[1], AND C. R. HOUSSIERE, JR.[2], The University of Texas, Austin, Texas

THE application of the Sharples supercentrifuge to the determination of the size of particles and the distribution of the particles in colloidal and semicolloidal systems has been presented previously (*1–4*). It is possible to operate the machine under such conditions as to separate from a suspension particles varying in size from 1 micron in diameter or larger to particles which approach the size of larger molecules. Compared to other methods of separation, the supercentrifuge method has been found to have the advantages of larger capacity, rapidity of determination, simplicity of operation, and low cost of maintenance and operation. Therefore, it has been adapted to the study of the particle size distribution of oil-well drilling muds and has become a routine operation in the authors' laboratories.

Hauser, Lynn, Reed, and Schachman (*1*, *2*, *3*) developed methods for calculating the size of particles separated by the supercentrifuge under definite conditions of operation, basing the mathematical analysis upon two assumptions—namely, that the flow parallel to the axis of rotation is streamline or viscous, and that Stokes' law holds for dilute suspensions. The effect of the first assumption is to require a rate of feed of the suspension to the centrifuge which is small in comparison to the outward centrifugal force.

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[2] Present address, U. S. N.

It is desirable, therefore, to be able to predict the operating conditions for a particular machine which will result in the separation of particles of definite size or range of size, and Hauser devised a special slide rule to facilitate determination of operating conditions. The expression developed by Hauser and co-workers (for a monodispersed film, *4*) may be used to develop a simpler and less tedious way of accomplishing this desideratum.

Their equation is

$$Y = \frac{18QK_1\eta}{\pi(R_2^2 - R_1^2)D^2\omega^2\,\Delta\rho}\left[\frac{R_2^2}{2}\ln\frac{R_2}{X_0} - \frac{R_1^2}{2}\left(\ln\frac{R_2}{X_0}\right)^2 + \frac{X_0 - R_2^2}{4}\right] \tag{1}$$

in which

$$K_1 = \frac{R_2^2 - R_1^2}{3/4\,R_1^2 + 1/4\,R_2^4 - R_2^2R_1^2 - R_1^4\ln\frac{R_1}{R_2}} \tag{2}$$

Q = rate of feed of suspension
η = viscosity of dispersion fluid
R_1 = distance of overflow weir from axis of rotation
R_2 = inner radius of bowl less thickness of liner
D = equivalent spherical diameter of solid particle
ω = angular velocity of bowl in radians per second
$\Delta\rho$ = density of dispersed phase minus density of dispersing phase at test temperature
X_0 = distance from axis of rotation at which particle begins to settle
Y = vertical distance from bottom of centrifuge bowl

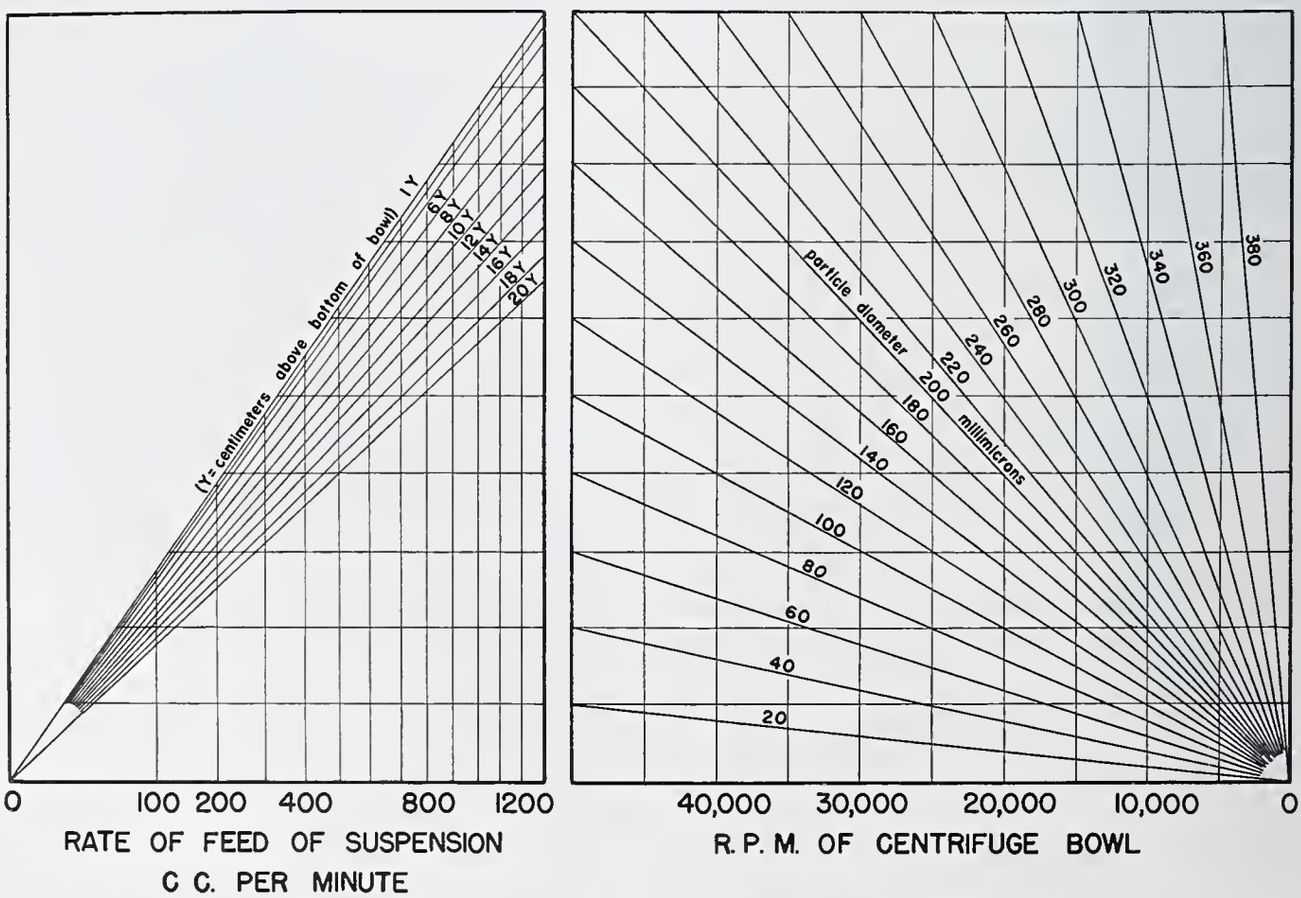

FIGURE 1. ALIGNMENT CHART FOR SELECTION OF R. P. M. AND RATE OF FEED NECESSARY TO SEPARATE A PARTICLE OF DEFINITE SIZE

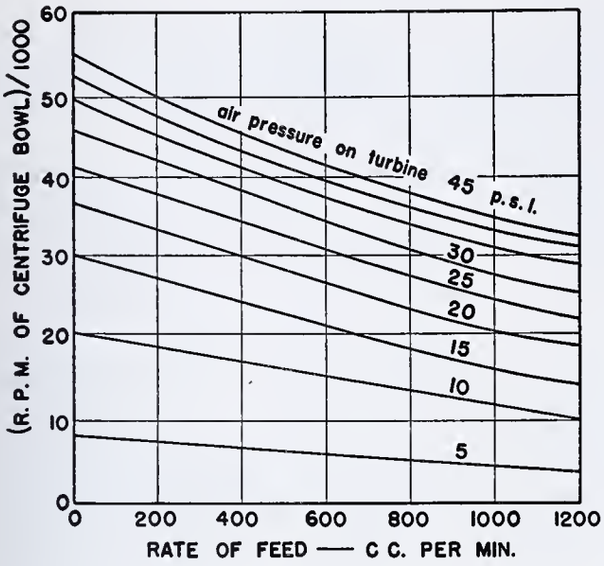

FIGURE 2. AIR PRESSURE REQUIRED TO DRIVE SUPERCENTRIFUGE AT DESIRED R. P. M.

The equation has three variables (Q, ω, D) and is implicit in X_0. This makes the equation soluble only by means of determinants, a family of curves, or some similar method. If, for definite values of Q and in Equation 1, values of X_0 are calculated for any fixed value of D and Y, it is then possible to represent the equation as:

$$Y = \frac{M}{D^2}(C) \tag{3}$$

$$M = \frac{18QK_1\eta}{\pi(R_2^2 - R_1^2)\omega^2\,\Delta\rho} \tag{4}$$

$$C = \left[\frac{R_2^2}{2}\ln\frac{R_2}{X_0} - \frac{R_1^2}{2}\left(\ln\frac{R_2}{X_0}\right)^2 + \frac{X_0^2 - R_2^2}{4}\right] \tag{5}$$

Because Equation 1 is based upon the deposition of a monodispersed film, it follows that values of D at definite values of Y are unique for the particular conditions of operation which prevail, or, in other words, C is only a function of Y and no longer a function of D. The validity of this conclusion was checked by determining independently the range in values of D between the top and bottom of the bowl with the slit-ultramicroscope. Therefore, if dilute enough systems are maintained for fractionation, there is a constant value of C for every value of Y. However, D remains dependent upon Q, ω, and Y. Through readjustment of Equation 3 by bringing Q and ω outside the constant M, we obtain

$$D^2 = \frac{QM'C}{Y\omega^2} \tag{6}$$

$$M' = \left[\frac{18K_1\eta}{\pi(R_2^2 - R_1^2)\Delta\rho}\right] \tag{7}$$

It is then possible for any definite value of Y to establish the relation

$$\frac{D_1^2}{D_2^2} = \frac{Q_1\omega_2^2}{Q_2\omega_1^2} \tag{8}$$

$$D_2 = \left(\frac{Q_2}{Q_1}\right)^{1/2}\left(\frac{\omega_1}{\omega_2}\right)D_1 \tag{9}$$

Using Equation 6 and values of C at various values of Y from independent observation, the diameters of particles were calculated for various r. p. m.'s, ω, and rates of flow, Q. The results of these calculations are graphically illustrated in Figure 1, which provides a rapid and convenient method for determining the diameter of the particles deposited at a definite value of Y for a definite value of Q and of ω. Such a chart can be conveniently used to determine the specific conditions under which the supercentrifuge must be operated to ensure the separation of particles of a definite size.

Further to simplify the operation of the supercentrifuge, the air pressure required to maintain a definite r. p. m. of the centrifuge and the liquid head resulting in definite rates of feed of suspension were determined. Figure 2 shows a plot of the r. p. m. of the centrifuge bowl versus the rate of feed of the suspension at various constant pressures on the air-driven turbine. Figure 3 is a plot of the height of liquid level above the base of the centrifuge versus the air pressure on the turbine for constant rates of feed with three sizes of feed nozzles. The air is supplied to the turbine through two needle valves and a pressure regulator in series. The two valves assure much finer control of pressure than can be obtained with only one valve. The suspension is fed into the bowl through one of three nozzles of different sizes, which can be interchanged while the machine is running. A constant rate of feed is maintained by keeping a definite hydrostatic head on the inlet to the bowl. An automatic overflow siphon, which is adjustable to give any desirable height of liquid level above or below the base of the centrifuge, assures a constant rate of feed. Figures similar to 2 and 3 probably must be determined for each machine because of characteristics inherent in the machine, but they are easily prepared and greatly facilitate routine operation of the machine. The following example is presented to illustrate the use of the chart:

It is desired to obtain from an aqueous suspension containing 1 per cent solids by weight, particles between 125 and 80 millimicrons (mμ) in diameter. Obviously, it is necessary to eliminate from the suspension all the particles larger than 125 mμ by sedimentation or by centrifuging before this can be done. It is assumed that this has already been done.

Using Figure 1, from the intersection of the diagonal line corresponding to a particle diameter of 125 mμ and the vertical line corresponding to 45,000 r. p. m., proceed horizontally to the left; intersect the diagonal line corresponding to $Y = 1$ (1 cm. above the bottom of the bowl); and read below on the abscissa 400 cc. per minute as the rate of feed of the suspension required for the separation. In a like manner, it is seen that a particle of 80 mμ diameter will be deposited at 20 Y (the top of the bowl). It has now been established that, to obtain particles from 125 to 80 mμ in diameter from a suspension containing no particles larger than 125 mμ, the centrifuge is to be operated at 45,000 r. p. m. and the suspension must be fed at a rate of 400 cc. per minute.

Using Figure 2, it is found that 43 pounds per square inch (126 kg. per sq. cm.) air pressure on the turbine are required to drive the bowl at 45,000 r. p. m., when the rate of feed is 400 cc. per minute. Caution should be exercised here in order to prevent the selection of an r. p. m. or flow rate that exceeds the operating limits of the

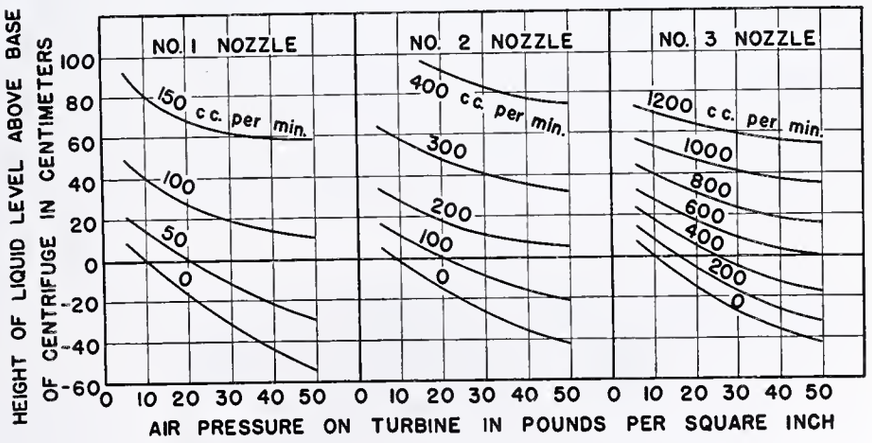

FIGURE 3. CONTROL CHART FOR SELECTION OF FEED NOZZLE AND FLUID HEAD REQUIRED TO MAINTAIN DESIRED RATE OF FEED

Table I. Comparison of Particle Diameter Values Predicted by Calibration Curves and Observed Microscopically

Sample No.	Portion of Liner Represented, Y	Equivalent Spherical Diameter[a] of Particles: Calibration Curves Maximum	Calibration Curves Minimum	Slit-Ultramicroscope (Av. D) Observer 1	Slit-Ultramicroscope (Av. D) Observer 2
	Cm.	*Millimicrons*			
1	0–20	215	150	195	208
2	0–2	150	147	155	158
3	0–20	90	55	...	82

[a] Diameter equivalent to that of a sphere which would settle at a rate equal to that of the actual particle.

centrifuge. From Figure 2 this limit is seen to be 200 cc. per minute at 50,000 r. p. m. and 1000 cc. per minute at 35,000 r. p. m. However, this limit may be altered for the air-driven turbine by adjusting the pressure regulator on the air supply line to a higher driving pressure. If a higher driving pressure is used, care must be exercised never to exceed 50,000 r. p. m. (maximum r. p. m. specified for the supercentrifuge by manufacturer). This might easily be done if a pressure in excess of 45 pounds per square inch (132 kg. per sq. cm.) were applied when the bowl was empty. At the higher rates of feed the basic formula used here for particle size determination may not apply rigidly, owing to the possible development of turbulence within the centrifuge bowl.

From Figure 3, it is seen that to attain a rate of feed of 400 cc. per minute while using 43 pounds per square inch (126 kg. per sq. cm.) it is necessary to use nozzle 3 and keep the liquid feed level at 15 cm. below the base of the centrifuge, or to use nozzle 2 with the liquid level 77 cm. above the base of the centrifuge. Thus, for this example, in order to obtain a separation of particles between 125 and 80 mμ, having previously removed particles larger than 125 mμ, the inlet pressure of the air which drives the centrifuge should be 43 pounds per square inch (126 kg. per sq. cm.), and the suspension should be fed from a level of 15 cm. below the base of the centrifuge through nozzle 3.

These charts may be expected to apply only to suspensions in an aqueous medium and different viscosity and density would require a different chart.

Table I has been prepared to illustrate the reliability and accuracy of the chart used under routine conditions.

Acknowledgment

Major A. E. Sweeney, Jr., U. S. A., aided in preparing the chart shown in Figure 2 and grateful acknowledgment is made to him.

This work was made possible through the Stanolind Oil and Gas Company Fellowship in Petroleum Engineering at The University of Texas. Grateful acknowledgment of financial aid and advice is made to the company.

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Silicon, Manganese, Chromium, Iron, and Copper in a Nickel-Base Alloy

An Aliquot Method for Routine Analysis of an Electrical Heat-Resisting Alloy

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NEWER techniques developed for the analysis of chromium steels (*5*) may be applied to certain nickel-base alloys. This paper outlines an aliquot method adapted to the rapid routine determination of the important elements in electrical heat-resisting alloys.

As with high chromium-nickel steels, the alloy is dissolved in acid and the silica dehydrated in boiling perchloric acid. From the silica-free filtrate aliquots are taken for determination of the elements present. Similarly, the manganese, chromium, and nickel are determined in sequence, in a single aliquot. In a second aliquot phosphorus can be determined, and in another aliquot copper and iron can be separated and determined. This last separation is the point of difference between the alloy steel and the nickel-base alloy, in that the iron determination is not required in the former.

The reagents and apparatus required are the usual equipment found in ferrous and nonferrous laboratories.

Reagents

Copper powder, 325-mesh (*2*).

Arsenite-Nitrite (*3*). Prepare 0.1 *N* arsenite by dissolving 4.95 grams of arsenious oxide in about 20 cc. of 20 per cent sodium hydroxide solution. Using phenolphthalein as indicator, decolorize with dilute sulfuric acid, add 500 cc. of water and 20 to 25 grams of sodium bicarbonate, and decolorize with acid if necessary. Dissolve 7.0 grams of sodium nitrite in 200 cc. of water. Mix arsenite and nitrite, and dilute to 2 liters. This is the stock 0.1 *N* solution. For use, dilute to 0.02 *N*, standardize on a steel of about 1 per cent manganese, to which 20 per cent chromium has been added, and check with Bureau of Standards sample No. 101a or No. 121.

Ferrous Ammonium Sulfate. Dissolve about 46.0 grams (0.117 *N*) of ferrous ammonium sulfate in 500 cc. of water containing about 5 cc. of sulfuric acid. Dilute to 1 liter.

Citric Acid Solution. To 380 grams of ammonium sulfate add 270 cc. of concentrated ammonium hydroxide, 1430 cc. of water, 5 grams of ammonium chloride, and 240 grams of citric acid. Use 35 cc. on a 0.2-gram aliquot.

Standard Silver Nitrate Solution. 2.885 grams of silver nitrate per liter. Use 5 ± 0.1 cc.

Cyanide Solution. Dissolve about 7.5 grams of sodium cyanide and 7 grams of sodium hydroxide in 1000 cc. of water. Dilute to analyst's convenience.

Potassium Iodide. 200 grams per 1000 cc. of water. Use 2 cc.

Silver–Sulfuric Acid–Phosphoric Acid Solution. Mix 500 cc. of water, 1000 cc. of sulfuric acid, 5.77 grams of silver nitrate, and 120 cc. of phosphoric acid. Dilute to 2 liters. Use 5 cc.

Titanium Standard. Dissolve 1 gram of Bureau of Standards No. 121 (titanium 0.394 per cent) in 50 cc. of (1 + 1) hydrochloric acid, cool to 10° C., and dilute to 200 cc. with water. Add 20 cc. of 6 per cent aqueous cupferron solution. Continue with the separation and solution of the titanium, as directed below. Dilute to 200 cc. with 5 per cent sulfuric acid. Preserve as a permanent standard. Add peroxide whenever color fades.

Procedure

Solution of Alloy. The nickel alloy is decomposed by direct attack with hot 70 per cent perchloric acid (*1*). Use 20 cc. for 2.500 grams in a 300-cc. beaker.

If the drillings are contaminated by oil or contain high carbon, or if phosphorus is required, treat a 2.500-gram sample in a 300-cc. tall-form beaker with 25 cc. of mixed acids (300 cc. of hydro-

Table I. Analysis of a Heat-Resisting Nickel-Base Alloy

Carbon %	Silicon %	Manganese %	Chromium %	Copper %	Iron %
0.04	0.16	0.17, 0.18	12.2	0.34, 0.34	8.35
	0.16	0.17	12.25	0.31	8.15
0.06	0.17	0.23, 0.23	13.1, 13.1	0.32, 0.32	8.40, 8.44
	0.18		13.0	0.32	8.40
0.09	0.12	0.13	13.4	0.27	7.24
	0.12	0.15	13.3	0.28	7.25
0.08	0.12	0.12, 0.12	13.5, 13.5	0.12	7.35
	0.12			0.13	7.40
0.04	0.15	0.08, 0.08	13.4, 13.4	0.24, 0.26	7.13
	0.17			0.25	7.24
0.05	0.18	0.16	12.8	0.05, 0.05	6.71
0.09	0.27	0.14	13.3	0.28, 0.27	6.70, 6.71
0.07	0.18	0.14	13.9	0.23	6.75
				0.25	6.71
0.04	0.24	0.11	13.4	0.60, 0.59	7.43, 7.45
0.07	0.30	0.19	16.7	0.14	7.51
			16.8		

chloric acid, 100 cc. of nitric acid, and 400 cc. of water). Cover, and heat at 80° to 90° C. to complete solution. Add 5 cc. of nitric acid (1.4 specific gravity) and 20 cc. of 70 per cent c. p. perchloric acid.

Heat at the boiling point of perchloric acid (204° C.) until the green spot under the cover glass is changed to yellow. Cool, add about 100 cc. of water and 10 cc. of (1 + 1) sulfuric acid, shake to dissolve soluble matter, and heat to boiling. Cool.

Silicon. Filter through a No. 40 Whatman paper into a 250-cc. volumetric flask. Wash 10 times with a hot (1 + 99) sulfuric acid solution, then with hot water. (Change the receiver, and wash with (1 + 4) hydrochloric acid if the precipitate is green.) Burn the paper and weigh as silica. For a 2.500-gram sample, the factor is 0.1864.

Cool the filtrate to room temperature, dilute to the mark, and shake well.

Manganese, Chromium, and Nickel (*5*). Transfer 20 cc. (0.20-gram aliquot) to a 400-cc. low-form beaker. Add 5 cc. of the silver–sulfuric acid–phosphoric acid solution and 75 cc. of hot water. Heat to about 80° C., add 20 cc. of 6 per cent ammonium persulfate solution, and maintain at this temperature until persulfate bubbles cease to form. Cool to 10° to 15° C. Titrate with the arsenite-nitrite reagent (approximately 0.02 *N*).

Dilute to 200 cc. with cold water, and titrate chromium potentiometrically with standard ferrous ammonium sulfate (approximately 0.117 *N*).

For nickel, add 35 cc. of the citric acid solution; add ammonium hydroxide (0.9 specific gravity) till blue; make acid to litmus with (1 + 1) sulfuric acid; dissolve the silver chloride precipitate with ammonium hydroxide, add 2 cc. of potassium iodide solution, and titrate with cyanide solution.

For manganese and chromium standards, a 0.2-gram aliquot of Bureau of Standards No. 101a (0.47 per cent manganese, 18.33 per cent chromium, 8.99 per cent nickel) is used; but a larger portion of a strong nickel sulfate solution, gravimetrically standardized, is required for nickel.

Copper (*6*). Transfer 100 cc. (1.000-gram aliquot) to a tall-form 200-cc. beaker, add 4 cc. of (1 + 1) sulfuric acid, and electrolyze at 4 volts and 0.6 to 0.8 ampere, without agitation. About 90 minutes is sufficient for an electrolyte of 14 per cent chromium, 6 per cent iron, and 0.6 per cent copper. Without any unnecessary agitation, remove the electrolyte. Wash the electrode with water, dry, and weigh for copper.

Iron (*2*). To the electrolyte add about 0.4 to 0.5 gram of powdered copper (325-mesh), sufficient to remain momentarily suspended when stirred. Three 15-second agitations are necessary. Filter through paper (Whatman No. 40, or similar roughened paper) that will not permit fine copper to "creep", and wash with water. To the filtrate add 5 cc. of (1 + 1) sulfuric acid and 5 cc. of 85 per cent phosphoric acid. Add ice, and titrate with 0.1 *N* permanganate, potentiometrically or visually. The first darkening of color corresponds to the potentiometric end point, but for routine visual titration it is best to proceed 0.1 cc. further and subtract the blank.

Titanium (*4*). Transfer 100 cc. (1.000-gram aliquot) to a 400-cc. beaker. Add 20 cc. of (1 + 1) sulfuric acid and about 10 cc. of sulfurous acid (or sulfuric acid plus sodium sulfite) to reduce sexivalent chromium, cool to 10° to 15° C., and dilute to 200 cc. Add 20 cc. of 6 per cent cupferron solution. Let stand in the ice box, stirring occasionally, till the precipitate settles. Filter on No. 40 Whatman paper, and wash with cold (1 + 99) hydrochloric or sulfuric acid. Return the paper to the beaker, add 25 cc. of nitric acid (1.4 specific gravity), mix, then heat to destroy organic matter. Add 15 cc. of 70 per cent perchloric acid, and fume. Cool, dilute, and add 10 cc. of (1 + 1) sulfuric acid and 5 cc. of 3 per cent hydrogen peroxide. Compare with standard titanium solution, or with Bureau of Standards No. 121 (0.4 per cent titanium) which has been prepared as in (*4*).

Phosphorus (*5*). If phosphorus is to be determined instead of titanium, transfer 100 cc. (1.000-gram aliquot) to a 300-cc. beaker. Add 5 cc. or more of ammonium hydroxide (specific gravity 0.9) till a precipitate forms, dissolve with nitric acid (specific gravity 1.4), and add 8 cc. excess. Warm to 40° C., and precipitate with ammonium molybdate as usual.

Results

In Table I, accumulated data are given. Two lines of figures indicate that aliquots were obtained from separately weighed samples.

Copper was determined either on a Sloman analyzer, or on an electrolytic board of stationary electrodes. Duplicates of the iron values were obtained, in some cases, from different standard permanganate solutions, accounting for the odd figures.

Titanium and phosphorus averaged 0.2 and 0.008 per cent, respectively.

Discussion

Dissolution of the nickel-base alloy directly in 70 per cent perchloric acid is much more rapid than by preliminary attack with mixed acids. Only in the case of the high-silicon, high-chromium alloys is the direct attack by perchloric acid likely to fail, since these two elements often prevent complete disintegration of the alloy. Loss of phosphorus is not appreciable for the usual amounts present. Too high a temperature will cause volatilization of nickel from fuming perchloric acid.

The silica residues have been found to retain about 0.02 per cent chromium, but no nickel, iron, or manganese.

Manganese and chromium are determined (*5*) as in steel, but the acidity of the nickel solution must be carefully adjusted, as indicated in the procedure. The nickel result is increased by 0.1 per cent for each 0.3 per cent of copper present. Because of the expected accuracy of the nickel titration, the copper error can ordinarily be disregarded. If, however, copper is present in large amounts (1.0 per cent), another procedure is suggested (*6*).

The unusually long time (90 minutes) required to deposit about 0.003 gram of copper is necessitated by the preliminary reduction of sexivalent chromium and trivalent iron. The platings are firm and adherent, and have the same color as copper depositions from sulfuric acid alone. Phosphoric acid is not required.

Since the reduction of iron is not completed during the electrodeposition of copper (*6*) the method of Percival (*2*) is used; and since beakers are used instead of stoppered flasks, more powdered copper is required (0.4 gram), and the stirring must be vigorous enough to keep the reductant momentarily suspended in the solution. Inadequate agitation is the cause of poor results. After reduction, the copper is filtered off; and the paper selected must not be too smooth, lest some powdered copper creep and be washed into the filtrate. Phosphoric acid does not prevent complete reduction of the iron by copper.

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A Graphic Method of Studying the Separation of Mixtures by Immiscible Solvents

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A graphical procedure is described by which it is possible to predict the ratio of the volumes of the solvents and the number of funnels and separations required to give the best separation of the components of a mixture by the use of immiscible solvents.

THE separation of mixtures by means of immiscible solvents is customarily based on the insolubility of one of the components in one of the solvents. For some mixtures, however, it is not possible to find a pair of solvents which will fulfill this condition. If the distribution coefficients of the components of such a mixture between two solvents differ sufficiently, a separation may be made by passing a solution of the mixture in one solvent through a series of separatory funnels containing the other solvent and repeatedly washing the solution remaining in the funnels with portions of the first solvent. Calculating the proper volume ratio of the solvents, and the number of funnels and washings to give a satisfactorily quantitative separation, is so laborious as to be almost prohibitive. The same results may be obtained rather quickly by a graphical procedure.

The distribution of any solute between two immiscible solvents, where the molecular weight of the solute is the same in both solvents, is expressed by the formula:

$$\frac{x}{1-x} = k\frac{u}{v} \qquad (1)$$

where, considering the amount of solute as unity:

x = fraction of solute dissolved in solvent 1
$1-x$ = fraction of solute dissolved in solvent 2
u = volume of solvent 1
v = volume of solvent 2
k = distribution coefficient

Solving Equation 1 for x,

$$x = \frac{k\frac{u}{v}}{1 + k\frac{u}{v}} \qquad (2)$$

Thus, the fraction present in solvent 1 may be calculated for any ratio of volumes of solvents.

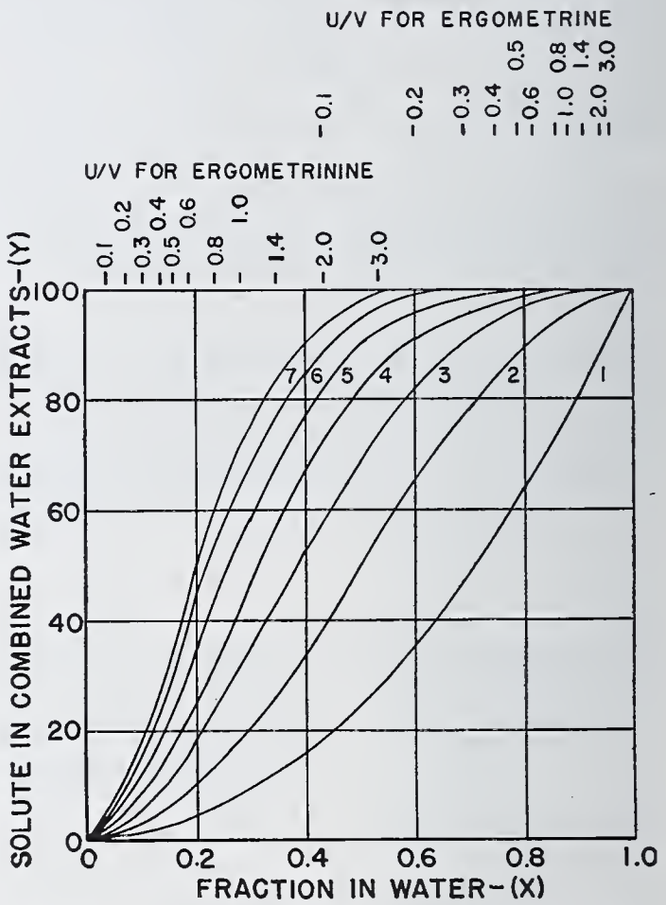

FIGURE 1. SEPARATION OF ERGOMETRINE AND ERGOMETRININE
Using two separatory funnels and one to seven separations

The specific problem used in the development of this method was the separation of the stereoisomeric ergot alkaloids, ergometrine and ergometrinine, wherein it was desired to obtain the ergometrine as free as possible from its stereoisomer. This was accomplished by their distribution between water and ether, and is here used as an example of the general application of the method.

For the purposes of this presentation, the term "separation" indicates the passing of a given volume of one solvent

TABLE I. DISTRIBUTION OF A SOLUTE BETWEEN TWO IMMISCIBLE SOLVENTS

Separation	First Funnel: Total solute	First Funnel: Fraction in water	First Funnel: Fraction in ether	Second Funnel: Total solute	Second Funnel: Fraction in water	Second Funnel: Fraction in ether	rth Funnel: Total solute	rth Funnel: Fraction in water	rth Funnel: Fraction in ether
1st	1	x	$1-x$	x	x^2	$x(1-x)$	x^{r-1}	x^r	$x^{r-1}(1-x)$
2nd	$1-x$	$x(1-x)$	$(1-x)^2$	$2x(1-x)$	$2x^2(1-x)$	$2x(1-x)^2$	$rx^{r-1}(1-x)$	$rx^r(1-x)$	$rx^{r-1}(1-x)^2$
3rd	$(1-x)^2$	$x(1-x)^2$	$(1-x)^3$	$3x(1-x)^2$	$3x^2(1-x)^2$	$3x(1-x)^3$	$ax^{r-1}(1-x)^2$	$ax^r(1-x)^2$	$ax^{r-1}(1-x)^3$
4th	$(1-x)^3$	$x(1-x)^3$	$(1-x)^4$	$4x(1-x)^3$	$4x^2(1-x)^3$	$4x(1-x)^4$	$bx^{r-1}(1-x)^2$	$bx^r(1-x)^3$	$bx^{r-1}(1-x)^4$
5th	$(1-x)^4$	$x(1-x)^4$	$(1-x)^5$	$5x(1-x)^4$	$5x^2(1-x)^4$	$5x(1-x)^5$	$cx^{r-1}(1-x)^4$	$cx^r(1-x)^4$	$cx^{r-1}(1-x)^5$
nth	$(1-x)^{n-1}$	$x(1-x)^{n-1}$	$(1-x)^n$	$nx(1-x)^{n-1}$	$nx^2(1-x)^{n-1}$	$nx(1-x)^n$	$dx^{r-1}(1-x)^{n-1}$	$dx^r(1-x)^{n-1}$	$dx^{r-1}(1-x)^n$

$$a = \frac{r(r+1)}{2!} \qquad c = \frac{r(r+1)(r+2)(r+3)}{4!}$$

$$b = \frac{r(r+1)(r+2)}{3!} \qquad d = \frac{n(n+1)(n+2)\ldots(n+r-2)}{(r-1)!}$$

2!, 3!, etc. = factorial 2, factorial 3, etc.

successively through each of the entire series of funnels containing the other solvent. Let us assume a series of funnels containing equal volumes of ether through which a water solution of the alkaloids is shaken successively. This is followed by successive portions of water, each equal to the volume of water used as the original solvent for the alkaloids. The distribution of the alkaloids in the various layers in the separatory funnels is shown in Table I.

From the data in this table, a series of curves are prepared as illustrated for two funnels (Figure 1) and four funnels (Figure 2). Since we are concerned only with the total amount of solute in the combined water extracts, which is expressed as per cent of the original amount of solute and called "y", the curve for one separation, Figure 1, represents a graph of the values of $y = 100\ x^2$ (see column 6, Table I, first separation), that for two separations represents the values of $y = 100\ [x^2 + 2x^2(1-x)]$ (sum of first and second separations, column 6, Table I), that for three separations represents the values of $y = 100\ [x^2 + 2x^2(1-x) + 3x^2(1-x)^2]$, etc. Only two sets of curves are illustrated, but similar graphs may be prepared for any number of funnels and separations required. Since these values are independent of the nature of the solute and solvents, they can be applied to any combination.

For the application of these curves to any specific problem, a scale, as shown above the curves in Figures 1 and 2, must be constructed for the two substances to be separated. This scale consists of the values of x for various ratios of the solvents as calculated from Equation 2. For example, the distribution coefficients, k, for ergometrine and ergometrinine between water and ether were determined experimentally to be 7.55 and 0.39, respectively. By substituting these values of k independently in Equation 2, together with a volume ratio (u/v = water to ether) of 0.3, the values of x for ergometrine and ergometrinine are calculated to be 0.694 and 0.105, respectively. The values of x thus obtained are marked off on the x scale above the graph, each mark being identified by its appropriate u/v ratio as indicated by the figures above the scale.

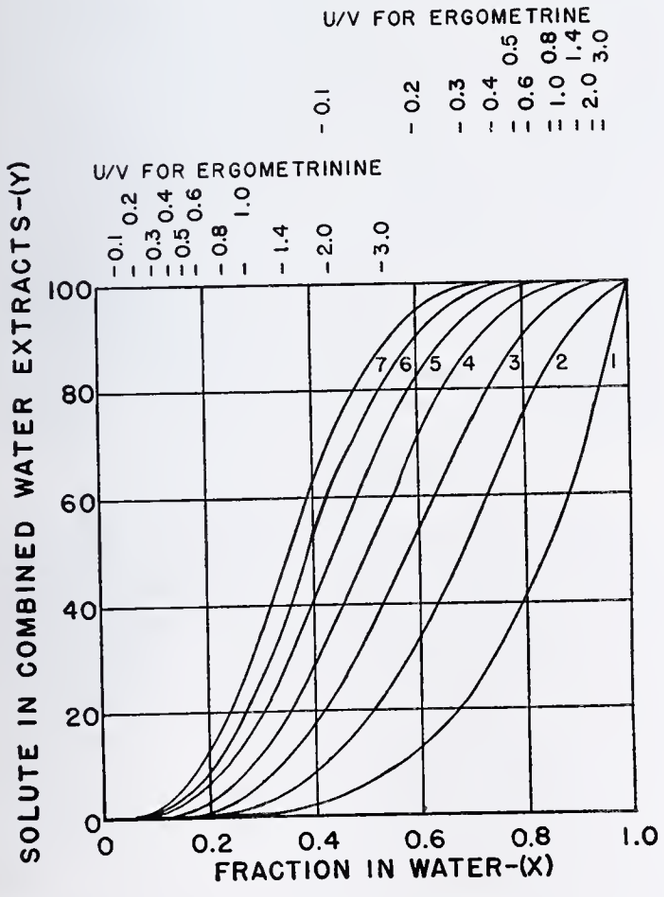

FIGURE 2. SEPARATION OF ERGOMETRINE AND ERGOMETRININE

Using four separatory funnels and one to seven separations

TABLE II. SEPARATION OF ERGOMETRINE AND ERGOMETRININE

u/v	1 Separation		3 Separations		5 Separations		7 Separations	
	A %	B %	A %	B %	A %	B %	A %	B %
				Using Two Funnels				
0.1	18	0	57	1	82	3	93	6
0.4	56	2	94	9	99	19	100	28
1.0	78	8	99	32	100	55	100	72
3.0	93	28	99	74	100	93	100	100
				Using Four Funnels				
0.1	3	0	22	0	48	0	70	0
0.4	33	0	83	0	97	2	100	5
1.0	60	0	97	5	100	17	100	30
3.0	84	9	100	43	100	72	100	89

The intersection of a vertical line projected from these points and each of the curves gives the per cent of the original amount of these alkaloids which has been carried into the combined water extracts for the number of separations indicated. Table II gives a tabulation of some of these values obtained from Figures 1 and 2, where A and B represent the amount of ergometrine and ergometrinine, respectively, in the water extracts from two and four funnels with varying numbers of separations. It is obvious that an optimum separation calls for the highest possible recovery of A and at the same time the lowest possible contamination with B.

An inspection of Table II shows that no separation of these alkaloids approaching quantitative proportions can be obtained with only two funnels. In Table II, however, at a u/v ratio of 0.4, five and seven separations, using four funnels, give practically complete recoveries of ergometrine with only slight amounts of ergometrinine. A more detailed survey of the points in this region on Figure 2 reveals that, at a u/v ratio of 0.3, seven separations give a 99 per cent recovery of ergometrine and 2 per cent of ergometrinine.

It is possible that the use of more funnels would give an even more quantitative separation. The accuracy of the separation to be desired will be dictated by the nature of the problem, and with a set of curves, as illustrated here, it is possible, knowing only the distribution coefficients of the components of a mixture, to determine the optimum ratio of solvents and the least number of funnels and separations required to give the desired results.

In practice, these curves, which are applicable to any situation and need be prepared only once, should be plotted on a fairly large scale to facilitate their use. The single x scale prepared for the specific mixture, if plotted on the same scale on a strip of graph paper, may be applied to all the series of graphs.

To assure sufficient shaking time for the various extractions, it is usually well to determine experimentally the time required for the distribution of the solutes between the solvents to reach equilibrium.

Acknowledgment

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Standard Methods for the Sampling and Analysis of Commercial Soaps and Soap Products, Revised

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THE Committee on Soap and Soap Products of the AMERICAN CHEMICAL SOCIETY has given careful consideration to comments on the methods published in 1937 (*8*), and to cooperative studies carried out by the Soap Analysis Committees of the American Oil Chemists' Society (*18*) and the American Society for Testing Materials (*2*), with the result that the following report was unanimously adopted March 11, 1941.

A. Sampling

The seller shall have the option of being represented at the time of sampling, and when he so requests shall be furnished with a duplicate sample.

I. CAKE SOAPS, FLAKE AND POWDERED SOAP PRODUCTS WHEN PACKED IN CANS OR CARTONS. One cake (can or carton) shall be taken at random from not less than 1 per cent of the vendors' shipping containers, provided such containers contain not less than 50 pounds (22.7 kg.). In the case of smaller containers, a cake (can or carton) shall be taken at random from each lot of containers totaling not more than 5000 pounds (2268 kg.) or fraction thereof. The gross sample shall in all cases consist of not less than three cakes (cans or cartons) taken at random from separate containers. With very large lots where the sample drawn as above will amount to more than 20 pounds (9.1 kg.), the percentage of packages sampled shall be reduced so that the amount drawn shall not exceed 20 pounds (9.1 kg.).

Wrap the individual cakes (cans or cartons) tightly in paraffined paper at once and seal by rubbing the edges with a heated iron. The inspector shall accurately weigh each wrapped cake (can or carton), record its weight and the date of weighing on the wrapper, place the wrapped cakes (cans or cartons) in an air-tight container, which should be nearly filled, sealed, marked, and sent to the laboratory for test. Samples should be kept cool until tested.

II. FLAKE AND POWDERED SOAP PRODUCTS WHEN IN BULK. A grab sample of not less than 0.5 pound (227 grams) shall be taken at random from not less than 1 per cent of the vendors' shipping containers, provided such containers contain not less than 100 pounds (45.4 kg.). In case of smaller containers, a grab sample of not less than 0.5 pound (227 grams) shall be taken at random from each lot of containers totaling not more than 10,000 pounds (4536 kg.) or fraction thereof. The gross sample shall in all cases consist of not less than three grab portions taken at random from separate containers. With very large lots where the sample drawn as above will amount to more than 20 pounds (9.1 kg.), the percentage of packages sampled shall be reduced so that the amount drawn shall not exceed 20 pounds (9.1 kg.). The inspector shall rapidly mix the gross sample and place it in an air-tight container, which shall be filled, sealed, marked, accurately weighed, its weight and date of weighing recorded on the package, and sent to the laboratory for test. Samples shall be kept cool until tested.

III. LIQUID SOAP. A sample of not less than 0.5 pint (236.6 ml.) shall be taken at random from not less than 1 per cent of the vendors' shipping containers, provided such containers contain not less than 10 gallons (37.9 liters) each. In case of smaller containers, a sample of not less than 0.5 pint (236.6 ml.) shall be taken at random from each lot of containers totaling not more than 1000 gallons (3785.4 liters). The gross sample shall in all cases consist of not less than three portions of 0.5 pint (236.6 ml.) each taken at random from separate containers. Before drawing the sample from the container selected, the contents of the container shall be thoroughly agitated. The inspector shall thoroughly mix the gross sample drawn, and place it in clean, dry cans or bottles, which shall be completely filled and securely stoppered with clean corks or caps, then sealed, marked, and sent to the laboratory for test.

IV. PASTE SOAP PRODUCTS. (1) *When packed in cans or cartons of 5 pounds (2.27 kg.) or less.* One can or carton shall be taken at random from not less than 1 per cent of the vendors' shipping containers, provided such containers contain not less than 50 pounds (22.7 kg.). In case of smaller containers, a can or carton shall be taken at random from each lot of containers, totaling not more than 5000 pounds (2268 kg.) or fraction thereof. The gross sample shall in all cases consist of not less than 3 cans or cartons taken at random from separate containers. With very large lots where the sample drawn as above will amount to more than 20 pounds (9.1 kg.), the percentage of packages sampled shall be reduced so that the amount drawn shall not exceed 20 pounds (9.1 kg.). Wrap, seal, mark, and send to the laboratory for test.

(2) *When packed in bulk.* Take a "trier" sample at random of not less than 0.5 pound (227 grams) from not less than 1 per cent of the vendors' shipping containers, provided such containers contain not less than 50 pounds (22.7 kg.). (A trier sample is obtained by inserting a trier into the material. A trier is a half-round steel cylinder 0.5 to 0.75 inch in diameter, 6 to 36 inches in length, pointed on one end, and having a grip handle on the other end. After insertion, the trier is turned 2 or 3 times, and upon removal a core of the material being sampled is obtained.) In case of smaller containers a trier sample shall be taken at random from each lot of containers totaling not more than 5000 pounds (2268 kg.) or fraction thereof. The gross sample shall in all cases consist of not less than three 0.5-pound (227-gram) portions taken at random from separate containers. With very large lots where the sample drawn as above will amount to more than 10 pounds (4.5 kg.), the percentage of packages sampled shall be reduced so that the amount drawn shall not exceed 10 pounds (4.5 kg.). The inspector shall promptly place the gross sample in a clean, dry, air- and water-tight container, which shall be filled, sealed, marked, and sent to the laboratory for test.

B. Preparation of Samples

I. CAKE SOAP. In the case of samples that can be easily disintegrated and mixed, run the entire sample through a suitable chopper. When the sample is large, each cake may be quartered and one quarter of each cake run through the chopper. With samples that cannot be handled as above, select a cake of average weight, quarter it by cutting at right angles in the center, and shave equally from all freshly cut surfaces sufficient soap for analysis. Mix and weigh out all portions for analysis promptly. Preserve the remainder in an air-tight container in a cool place.

II. POWDERED AND CHIP SOAPS. Rapidly disintegrate and mix the sample; if desired, quarter down to about 1 pound (453.6 grams) and weigh out all portions for analysis at once. Unused portions of the sample for analysis shall be preserved in an air-tight container in a cool place.

III. LIQUID SOAP. No preparation of the sample, other than thorough mixing, is necessary unless it is received during very

cold weather, when it should be allowed to stand at least 1 hour after it has warmed up to room temperature (20° to 30° C.) before it is tested, particularly for its lathering qualities.

IV. Paste Soap Products. Mix thoroughly by kneading and quarter down to about 1 pound (453.6 grams). Weigh out all portions for analysis promptly and preserve remainder in an air-tight container in a cool place.

C. Methods of Analysis

When a determination shows nonconformity with the specifications, a duplicate shall be run.

I. Moisture. The oven method given here is generally applicable to all soaps. Experience has shown, however, that certain exceptions to this method must be made if accurate results are desired. These exceptions include: (*a*) For soaps containing appreciable amounts of sodium silicate the distillation method is preferred. (*b*) Soaps of linseed and other oxidizing oils absorb oxygen and if the oven method is used may gain in weight near the end of the test. Therefore, either an inert atmosphere or vacuum oven should be used. The distillation method is also applicable to these types of soaps. (*c*) Soaps containing appreciable amounts of glycerol, such as cold made and semiboiled (including paste soaps), usually give higher results by the oven method. The distillation method is preferred for most accurate results on these types of soaps.

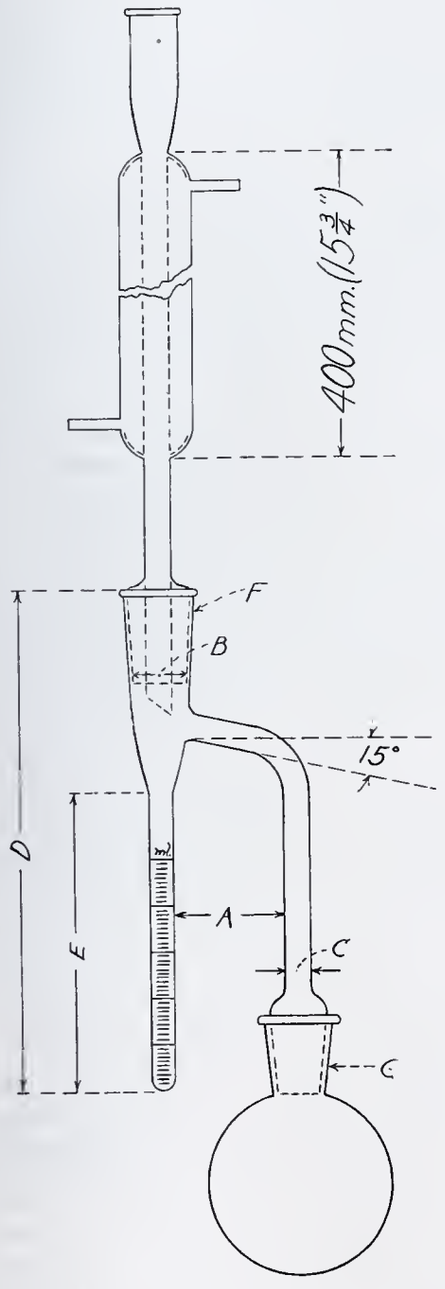

Figure 1. Assembly of Distillation Apparatus

A. 45 to 55 mm.
B. 22 to 24 mm., inside diameter
C. 9 to 11 mm., inside diameter
D. 235 to 240 mm.
E. 146 to 156 mm.
F, G. Interchangeable joints, standard taper 24/40

(1) *Matter volatile at 105° C. (oven method).* Procedure. Weigh 5 grams (±0.01 gram) of the sample in a porcelain or glass dish about 6 to 8 cm. in diameter and about 2 to 4 cm. deep, and dry to constant weight in an air oven at a temperature of 105° ± 2° C. Constant weight is attained when successive heating for 1-hour periods shows a loss (or gain) of not more than 0.1 per cent.

(2) *Distillation method (9).* Apparatus. The apparatus required consists of a glass flask heated by suitable means and provided with a reflux condenser discharging into a trap and connected to the flask. The connections between the trap and the condenser and flask shall be interchangeable ground joints. The trap serves to collect and measure the condensed water and to return the solvent to the flask. A suitable assembly of the apparatus is illustrated in Figure 1.

Flask. A 500-ml. flask of either the short-necked, round-bottom type or the Erlenmeyer type shall be used.

Heat source. The source of heat may be either an oil bath (stearic acid, paraffin, etc.) or an electric heater provided with a sliding rheostat or other means of heat control.

Condenser. A water-cooled glass reflux condenser (Figure 1), having a jacket approximately 400 mm. (15.75 inches) in length with an inner tube 9.5 to 12.7 mm. (0.375 to 0.5 inch) in outside diameter, shall be used. The end of the condenser to be inserted in the trap may be ground off at an angle of 30 degrees from the vertical axis of the condenser. When inserted into the trap, the tip of the condenser shall be about 7 mm. (0.25 inch) above the surface of the liquid in the trap after the distillation conditions have been established. Figure 1 shows a conventional sealed-in type of condenser, but any other condenser fulfilling the detail requirements above may be used.

Trap. A trap made of well-annealed glass constructed in accordance with Figure 1 and graduated as shown to contain 5 ml. at 20° C. shall be used. It shall be subdivided into 0.1-ml. divisions, with each 1-ml. line numbered (5 ml. at top). The error in any indicated capacity may not be greater than 0.05 ml.

Special reagent required. Xylene or toluene. For soaps containing 1 per cent or more of glycerol, toluene shall be used instead of xylene. Saturate the solvent with water by shaking it with a small quantity of water and distill. Use the distillate for the determination.

Procedure. For soaps containing from 5 to 25 per cent of moisture and volatile matter use a 20-gram (±0.04 gram) sample. For soaps containing more than 25 per cent of moisture and volatile matter use a 10-gram (±0.02 gram) sample. Carefully transfer the weighed sample to the 500-ml. flask. Add approximately 10 grams of anhydrous fused sodium acetate to prevent violent frothing, and then follow with 100 ml. of solvent which has been saturated with water. Attach the flask to the trap which is connected to the condenser. Prior to starting the determination, fill the receiver with saturated solvent by pouring it in through the reflux condenser.

So that the refluxing will be under better control, wrap the flask and the tube leading to the receiver with asbestos cloth. Heat the oil bath with a gas burner or other source of heat, or apply heat directly to the flask with an electric heater and distill slowly. The rate at the start should be approximately 100 drops per minute. When the greater part of the water has apparently distilled over, increase the distillation rate to 200 drops per minute until no more water is collected. Purge the reflux condenser during the distillation with 5-ml. portions of solvent to wash down any moisture adhering to the walls of the condenser. The

water in the receiver may be made to separate from the solvent by using a spiral copper wire. Move the wire up and down in the condenser occasionally, thus causing the water to settle at the bottom of the receiver. Reflux for at least 2 hours, and shut off the heat at the end of this period. Adjust the temperature of the distillate to 20° C. Read the volume of water and calculate the percentage of moisture in the soap, as follows:

$$\text{Moisture, per cent} = \frac{V \times 0.998}{S} \times 100$$

where V = volume of water in milliliters at 20° C. and S = weight of sample in grams.

II. Total Matter Insoluble in Alcohol. *Procedure.* Digest a 2- to 10-gram (±0.01 gram) sample with 200 ml. of freshly boiled ethyl alcohol (94 per cent or higher and neutral to phenolphthalein) in a covered vessel on a steam bath until the soap is dissolved. (Formula 3-A or 30 specially denatured alcohol may be used instead of straight ethyl alcohol.) Filter through a counterpoised filter paper neutral to phenolphthalein, or a weighed Gooch crucible with suction, protecting the solution from carbon dioxide and other acid fumes during the operation by covering with a watch glass. Wash the residue on the paper, or in the crucible, with hot neutral alcohol until free from soap and reserve the filtrate and washings. Dry the filter paper or crucible with residue at 100° to 105° C. for 3 hours, cool, and weigh the total matter insoluble in alcohol. (The matter insoluble in alcohol will contain most of the alkaline salts, such as carbonates, borates, silicates, phosphates, and sulfates, as well as starch, and may be used for the approximate determination of these constituents. These salts are not entirely insoluble in alcohol, so for accurate determinations separate portions of the soap should be used. For determination of carbonates see C-XVIII; phosphates, C-XIX; sulfates, C-XX; silicates, C-XVII; borax, C-XVI; starch, C-XXI, C.)

III. Free Alkali or Free Acid. *Procedure.* Heat the reserved filtrate from the determination of total matter insoluble in alcohol (C-II) to incipient boiling, add 0.5 ml. of a 1 per cent solution of phenolphthalein, titrate with standard acid or alkali solution, and calculate to sodium hydroxide (or potassium hydroxide) if alkaline, or to oleic acid if acid.

Note. In the analysis of soaps known to contain little or no alkaline salts, it is unnecessary to filter the hot alcoholic soap solution as described in C-II. However, the filtration should be carried out in all cases where alkaline salts such as silicates, phosphates, borates, and similar salts are present, since these are known to affect the free alkali determination. The presence of carbonates up to 0.5 per cent as Na_2CO_3 does not appreciably affect this determination and filtration may be omitted if the carbonate on a separate sample is not in excess of this amount.

IV. Matter Insoluble in Water. *Procedure.* Proceed as in the determination of matter insoluble in alcohol (starting with a fresh sample of soap and omitting the drying and weighing of matter insoluble in alcohol, C-II). After filtering and thoroughly washing the residue, change the receivers, extract the residue with water at 60° C., and wash the filter thoroughly. (When the matter insoluble in water is all inorganic, boiling water may be used for the extraction and washing.) Reserve the water solution. Dry the filter and residue at 100° to 105° C. for 3 hours, cool, and weigh the matter insoluble in water. The nature of this matter may be determined by further examination.

V. Total Alkalinity of Matter Insoluble in Alcohol (Alkaline Salts). *Procedure.* Titrate the water solution obtained in C-IV with standard acid, using methyl orange as indicator. Calculate the alkalinity to sodium oxide (Na_2O), and, if desired, to any other basis agreed upon by the purchaser and the seller.

VI. Combined Alkali. Total Anhydrous Soap. *Procedure.* Dissolve 5 to 10 grams (±0.01 gram) of the sample, depending upon the anhydrous soap content, in 100 ml. of water in a 250-ml. Erlenmeyer flask. When solution is complete, add dilute sulfuric acid (1 to 1) in slight excess, insert a small funnel in the neck of the flask, and heat the flask at a temperature not exceeding 60° C. until the fatty acids separate as a clear layer. Transfer to a separatory funnel, draw off the acid layer into a second separatory funnel, and shake the acid aqueous liquid with two 20-ml. portions of ethyl ether. Dissolve the fatty acids in the ether used for washing the aqueous liquid and shake with 10-ml. portions of water until they are no longer acid to methyl orange. Unite the water portions used for washing and shake with 20 ml. of ether. Wash this ether until the wash water is neutral to methyl orange. Reserve the acid water for determination of chloride (C-VII).

Unite the ether solutions (if necessary, filter, washing the paper with ether) in a suitable weighed vessel, add 100 ml. of neutral alcohol free from carbon dioxide, add phenolphthalein, and titrate to exact neutrality with standard 0.5 N sodium hydroxide solution. Evaporate off the alcohol, dry to constant weight as in the determination of matter volatile at 105° C., and calculate the percentage of soda soap. This naturally includes any mineral oil and neutral fat, which, if determined separately, must be deducted from the result to obtain the true soap. Calculate the combined sodium oxide (Na_2O) and deduct from the weight of soda soap to give the acid anhydrides. If the original soap was potash soap, proper calculation must be made to reduce to potassium oxide (K_2O), or the titration made directly with standard 0.5 N potassium hydroxide solution. In case the soap shows an excess of free acid, proper corrections must be made in calculating the combined alkali in the original soap. (A blank test should be made on the sodium or potassium hydroxide solution for neutral salts and the proper corrections made if necessary.) In the case of soaps containing a large amount of soluble silicates, and soap products containing a high percentage of finely divided material insoluble in water, the foregoing procedure cannot be applied as given. In such cases the filtrate obtained in the determination of total matter insoluble in alcohol may be used after neutralizing any free acid or alkali. Evaporate off the alcohol on a steam bath, take up in water, and proceed as above.

In the case of soap products containing a high percentage of matter insoluble in alcohol where approximate results will suffice, such as may be the case with cleansers, soap powders, scouring compounds, pastes, etc., and where agreed upon by the purchaser and the seller, the alcoholic solution, obtained after filtering off and washing the matter insoluble in alcohol (C-II), may be evaporated directly in a weighed vessel and dried at 105° C. to constant weight, and the result reported as soap.

VII. Chloride. (1) *Special reagents required.* Silver nitrate solution (0.1 N). Magnesium nitrate solution (20 per cent). Dissolve 200 grams of chloride-free magnesium nitrate, $Mg(NO_3)_2.6H_2O$, in 1 liter of water.

Potassium chromate indicator. Dissolve 5 grams of chloride-free potassium chromate (K_2CrO_4) in water, add silver nitrate solution until a slight red precipitate is produced, filter the solution, and dilute the filtrate to 100 ml.

(2) *Procedure.* Neutralize with chloride-free alkali the acid water obtained in C-VI, using methyl orange indicator. Titrate with standard silver nitrate solution, using potassium chromate as indicator, and calculate the result to sodium chloride or potassium chloride as the character of the soap indicates.

In case the total anhydrous soap is not to be determined, it will be more convenient to use the following method (*6*): Dissolve 5 grams (±0.01 gram) of the sample in 300 ml. of water, boiling if necessary to effect solution. Add an excess of neutral chloride-free magnesium nitrate solution [about 25 ml. of 20 per cent $Mg(NO_3)_2.6H_2O$ solution]. Without cooling or filtering, titrate with standard silver nitrate solution, using potassium chromate as indicator. One milliliter of 0.1 N silver nitrate solution is equivalent to 0.00585 gram of sodium chloride (NaCl).

VIII. Unsaponified Plus Unsaponifiable Matter. (1) *Apparatus.* Extraction cylinder. The extraction cylinder shall be a 250-ml. graduated, glass-stoppered cylinder about 39 mm. (1.5 inch) in diameter and about 35.5 cm. (14 inches) in length.

(2) *Special reagents required.* Ethyl alcohol, 50 per cent, 95 per cent, and 10 per cent. Sodium hydroxide solution 0.1 *N* and 0.04 *N*.

Petroleum ether. The solvent used shall be of the pentane type, containing a minimum amount of isopentane, isohexane, and hexane, and conforming to the following requirements:

Distillation test (Note *a*)	
Initial boiling point	35° to 38° C.
Dry flask end point	52° to 60° C.
Distilling under 54° C., minimum	95 per cent
Distilling under 40° C., maximum	60 per cent
Specific gravity at 15.5°/15.5° C. (60°/60° F.)	0.630 to 0.660
Color	Water white
Doctor test	Sweet
Evaporation residue, 100 ml., maximum	0.0011 gram
Copper-strip corrosion test (Note *b*)	Noncorrosive
Unsaturated compounds (Note *c*)	Trace only permitted
Residue in distilling flask	Neutral to methyl orange
Blotter-strip odor test (Note *d*)	Odorless within 12 minutes
Aromatic compounds (Note *e*)	No nitrobenzene odor
Saponification value	Less than 1.0 mg. of KOH per 100 ml.

Note *a*. The distillation test shall be made in accordance with the standard method of test for distillation of natural gasoline of the American Society for Testing Materials (*1*). As a check on the evaporation residue, 250 ml. of the petroleum ether and 0.25 gram of stearin or other hard fat (previously brought to constant weight by heating) when dried as in the actual determination shall not show an increase in weight exceeding 0.003 gram.

Note *b*. The copper-strip corrosion test shall be made by inserting a small polished copper strip into the petroleum ether in the distilling flask. There should be no appreciable darkening of the copper.

Note *c*. Unsaturated compounds shall be determined by the method for determining olefins (*20*, p. 154).

Note *d*. Odor test. Immerse 1 inch of a strip of white, unglazed blotting paper, approximately 1 × 4 × 0.166 inch in size, in the petroleum ether for 30 seconds, remove the strip, and allow it to dry at room temperature in still air for 12 minutes.

Note *e*. Aromatic compounds. Add 5 drops of petroleum ether to 40 drops of sulfuric acid (sp. gr. 1.84) and 10 drops of nitric acid (sp. gr. 1.42) in a test tube, warm for 10 minutes, allow to cool for 30 minutes, transfer to a shallow dish, and dilute with water.

(3) *Procedure* (*21*). (*a*) Weigh 5 grams (±0.2 gram) of the prepared sample into a 250-ml. Erlenmeyer flask or beaker which contains approximately 0.1 gram of bicarbonate of soda, and dissolve in 100 ml. of redistilled 50 per cent ethyl alcohol. Warm and shake to effect solution, keeping the temperature under 60° C., and filter off any undissolved residue on a Gooch crucible with an asbestos pad or in a funnel, using an asbestos pad deposited on a perforated porcelain disk. Wash three times with hot 50 per cent alcohol and then with 5 ml. of hot 95 per cent alcohol. Wash with a small amount of petroleum ether to remove any traces of unsaponified and unsaponifiable matter. Transfer the entire alcohol-water-and-ether filtrate to the extraction cylinder and make up to the 160-ml. mark with redistilled 50 per cent ethyl alcohol. Add 50 ml. of petroleum ether, shake vigorously for 1 minute (Note 1), and allow to settle until both layers are clear. The volume of the upper layer should be about 40 ml. Draw off the petroleum ether layer as closely as possible, by means of a slender glass siphon, into a separatory funnel of 500-ml. capacity.

(*b*) Repeat the extraction at least six times using 50 ml. of petroleum ether each time (Note 2). Wash the combined ether extracts first with a mixture of 15 ml. of 0.1 *N* sodium hydroxide solution and 15 ml. of 95 per cent alcohol, and then three times with 25-ml. portions of 10 per cent alcohol, shaking vigorously each time. Transfer the petroleum ether extract to a beaker and evaporate the petroleum ether on a steam bath by the aid of a current of air.

(*c*) Test the residue for solubility by treating with 50 ml. of petroleum ether at room temperature. Filter, and wash free from the insoluble residue, if any. Evaporate and dry in the same manner on a steam bath, and finally in an air oven at 100° to 101° C. for 30 minutes. Weigh, and return to the oven, reweighing at 15-minute intervals until constant weight is reached. Take up the residue in 50 ml. of warm ethyl alcohol, neutralized to phenolphthalein, titrate to the same color as the original neutral alcohol with 0.04 *N* sodium hydroxide solution, and calculate to oleic acid. Deduct this figure from the gross weight previously found and report as "unsaponified and unsaponifiable matter".

(*d*) Run a blank test on the petroleum ether by evaporating 250 ml. of the ether with about 0.25 gram of stearin or some other hard fat previously brought to constant weight by heating and drying as in the actual determination. The blank must not exceed a few milligrams [see Note *a* under C-VIII (2)] (Note 3).

Note 1. Thorough and vigorous shaking is necessary in order to secure accurate results. The two phases must be brought into the most intimate contact possible; otherwise low and disagreeing results may be obtained.

Note 2. This method will not remove all the unsaponifiable matter in soaps to which lanolin has been added. More extractions are required when substances of this nature are present.

Note 3. Any blank from the petroleum ether must be deducted from the gross weight of the "unsaponified and unsaponifiable" before calculating the percentage of unsaponified and unsaponifiable matter.

IX. Unsaponifiable Matter. (1) *Apparatus.* Extraction cylinder. See cylinder described for the determination of unsaponified plus unsaponifiable matter [C-VIII (1)].

(2) *Special reagents required.* See the special reagents required for the determination of unsaponified plus unsaponifiable matter [C-VIII (2)]. Potassium hydroxide, 50 per cent.

(3) *Procedure.* Weigh 5 grams (±0.2 gram) of the prepared sample into a 200-ml. Erlenmeyer flask. Add 30 ml. of redistilled 95 per cent ethyl alcohol and 5 ml. of aqueous 50 per cent potassium hydroxide, and boil the mixture for 1 hour under a reflux condenser. Transfer to the extraction cylinder and wash to the 40-ml. mark with redistilled 95 per cent ethyl alcohol. Complete the transfer, first with warm and then with cold water, until the total volume is 80 ml. and finally with a small quantity of petroleum ether. Cool the cylinder and contents to room temperature and add 50 ml. of petroleum ether; then proceed with the extraction as outlined in the procedure for "unsaponified plus unsaponifiable matter" [C-VIII (3); also Notes 1 and 2], except that the alkaline wash may be omitted; weigh the residue and correct for fatty acids in the usual manner. Report the result as "unsaponifiable matter".

X. Unsaponified Matter. From the total unsaponified plus unsaponifiable matter determined in C-VIII (3), deduct the unsaponifiable figure obtained in C-IX (3), and report the difference as "unsaponified matter".

XI. Rosin. McNicoll method (*14*). (1) *Apparatus.* The apparatus required consists of a glass flask connected, preferably by a ground-glass joint, to a reflux condenser.

Esterification flask. A 150-ml. flask of either the round-bottom or Erlenmeyer type shall be used.

Reflux condenser. Any suitable water-cooled, glass reflux condenser may be used.

(2) *Special reagents required.* Potassium hydroxide (0.2 *N*). Accurately standardize a 0.2 *N* solution of potassium hydroxide in neutral redistilled alcohol (owing to volatility of alcohol, this solution should be restandardized frequently).

Naphthalene-β-sulfonic acid solution. Dissolve 40 grams of Eastman grade or equivalent reagent in 1 liter of C. P. absolute methyl alcohol.

Phenolphthalein indicator. Prepare a 0.5 per cent solution in neutral redistilled alcohol.

(3) *Procedure.* Preparation of Fatty and Rosin Acids. For the preparation of the sample for this determination, follow the procedure described in C-XII (2).

Esterification and Titration. Weigh about 2 grams (±0.001 gram) of the fatty acids into the esterification flask. Add 25 ml. of naphthalene-β-sulfonic acid solution. Add a few glass beads to ensure smooth boiling, attach the reflux condenser, and boil for 30 minutes; also, run a blank test using 25 ml. of the reagent. At the end of the boiling period cool the contents of the flask, add 0.5 ml. of phenolphthalein indicator, and titrate immediately with 0.2 N alcoholic potassium hydroxide.

Calculations. Calculate the results as follows (Note 1):

$$R = \frac{(S - B) \times N \times 0.346 \times 100}{W}$$

$$R_1 = R - 1.0$$

$$R_2 = \frac{R_1 \times F}{100}$$

$$R_s = \frac{R_1 \times 1.064 \times A}{100}$$

where

R = percentage of rosin in fatty acids
R_1 = corrected percentage of rosin in fatty acids (Note 2)
R_2 = percentage of rosin on basis of original sample
R_s = percentage of rosin soda soap on basis of original sample
S = milliliters of potassium hydroxide required to titrate sample
B = milliliters of potassium hydroxide required to titrate blank
N = normality of potassium hydroxide
W = weight of sample
F = percentage of total fatty acids in soap
A = percentage of total anhydrous soap

If true fatty acid soap is desired, subtract the rosin soap from the total anhydrous soap.

Note 1. In all cases where the rosin content is found to be less than 5 per cent, the actual presence or absence of rosin should be checked qualitatively by the Liebermann-Storch test, which is as follows:

Transfer 1 to 2 ml. of the sample of fatty acids to a test tube, treat with 5 to 10 ml. of reagent grade acetic anhydride, and warm on a steam bath. After cooling, pour 1 to 2 ml. into a white porcelain dish and allow a drop or two of sulfuric acid (sp. gr. 1.53) to run down the side of the vessel. If rosin is present, a fugitive violet coloration changing to a brownish tinge is immediately produced at the margin of contact of the reagents. The test should be checked with a sample of fatty acids to which a small amount of rosin has been added.

Sulfuric acid of 1.53 specific gravity is prepared by diluting 34.7 ml. of sulfuric acid (sp. gr. 1.84) with 35.7 ml. of distilled water.

Note 2. Cooperative studies have shown that the McNicoll method gives results approximately 1 per cent higher than the amount of rosin present. Consequently, the committee recommends deducting 1 per cent from the percentage of rosin found in the fatty acids.

XII. Preparation of Total Fatty Matter (Fatty and Rosin Acids and Unsaponified Matter). (1) *Special reagent required.* Sulfuric acid (30 per cent by weight of sulfuric acid). Slowly add 650 grams of sulfuric acid (sp. gr. 1.84) to 1400 ml. of distilled water.

(2) *Preparation for rosin and titer tests and iodine and acid numbers.* Dissolve about 50 grams of the sample in 500 ml. of hot water. (If soaps to be tested contain alcohol, the alcohol should be completely removed by evaporation from the soap solution.) Add 100 ml. of 30 per cent sulfuric acid and heat gently until the fatty matter collects in a clear layer.

Siphon off the aqueous acid layer, add 300 ml. of hot water, and boil gently for a few minutes, making sure that all of the fatty acids are melted and clear. High melting point fats are sometimes slow to melt and clear. The fatty acid layer should be carefully inspected while it is quiet, to be sure all has melted. Siphon off the aqueous acid layer.

Wash the acids in this manner three times, or until wash water is neutral to methyl orange. Complete this acidification and washing in a very short period of time, and keep the beaker covered to prevent oxidation of the acids.

After the last washing, remove the last traces of water from the beaker with a pipet, filter the fatty acids through one or two thicknesses of filter paper, and dry at a temperature of 105° C. for 45 to 60 minutes or heat rapidly to 130° C. and allow to cool. Do not hold at 130° C., but if water is present decant the clear fatty acids into another beaker, and again reheat them momentarily to 130° C. These acids may then be used for the titer and rosin determinations.

In preparing the acids for the iodine and acid number determinations, the washed acids should be filtered through one or two thicknesses of filter paper at a temperature not exceeding 15° C. above the titer point of the fatty acids. If the acids are not perfectly clear and dry, refilter.

XIII. Titer Test (Solidification Point of the Fatty Acids (7). (1) *Apparatus.* A 2-liter Griffin low-form beaker.

A wide-mouthed bottle, capacity 450 ml., height 190 mm., inside diameter of neck 38 mm.

Test tubes, length 100 mm., diameter 25 mm., with or without rim. These tubes may have an etched mark extending around the tube at a distance of 57 mm. from the bottom to show the height to which the tube is to be filled.

Laboratory thermometer, 0° to 150° C.

Stirrer, 2 to 3 mm. in outside diameter, one end bent in the form of a loop of 19-mm. diameter. Glass, Nichrome, stainless steel, or Monel wire may be used. The upper end can be formed to accommodate hand stirring or be attached to a mechanical stirrer.

Titer thermometer.

Type, etched stem glass.

Liquid, mercury.

Range and subdivision, −2 to +68° C. in 0.2 degree graduations.

Total length, 385 to 390 mm.

Stem to be constructed of suitable thermometer tubing of either the plain or lens front type.

Diameter, plain front type, 6 to 7 mm.

Thickness of stem, lens front type. The cross section of the stem to be such that it will pass through an 8-mm. ring gage but will not enter a 5-mm. slot gage.

Bulb, Corning normal or equally suitable thermometric glass. Length, 15 to 25 mm. Diameter, 5.5 mm. to not greater than that of stem.

Distance from bottom of bulb to −2° mark, 50 to 60 mm.

Distance to 68° mark from top of thermometer, 20 to 35 mm.

Length of unchanged capillary between the highest graduation and the expansion chamber, 10 mm.

Expansion chamber, to permit heating to at least 85° C. Space above mercury to be evacuated or filled with nitrogen or other suitable gas.

Top finish, glass ring.

Graduations. All lines, figures, and letters to be clear-cut and distinct. Each degree mark to be longer than the remaining lines. Graduations to be numbered at zero and at each multiple of 2 degrees.

Immersion, 45 mm.

Marking. "FAC Titer Test", a serial number, and the manufacturer's name or trade-mark shall be etched on the stem. The words "45-mm. immersion" shall also be etched on the stem, and a line shall be etched around the stem 45 mm. above the bottom of the bulb.

Scale error. The error at any point on the scale shall not exceed 0.2° C.

Standardization. The thermometer shall be standardized at the ice point and at intervals of approximately 20° C., for the condition of 45-mm. immersion, and for an average stem temperature of the emergent mercury column of 25° C.

Case. The thermometer shall be supplied in a suitable case on which shall appear the markings "FAC Titer Test, −2° to 68° C. in 0.2°".

Note. For the purpose of interpreting these specifications, the following definitions shall apply:

The total length is the over-all length of the finished instrument.

The diameter of the plain front round-stem type is that measured with a ring gage or micrometer.

The length of the bulb is the distance from the bottom of the bulb to the beginning of the enamel backing.

The top of the thermometer is the top of the finished instrument.

(2) *Preparation of the fatty acids.* Follow the procedure described in C-XII (2).

(3) *Procedure (solidification of fatty acids).* Fill the water bath so that the level of the water reaches the neck of the wide-mouthed bottle. Adjust the temperature of the water bath. The temperature of the water should be 20° C. for all samples having titers of 35° C. or higher, and 15° to 20° C. below the titer point for all samples with titers below 35° C.

Heat the filtered acids on a hot plate to 130° C. to remove traces of moisture and pour into the test tube. Fill the latter to a height of 57 mm. from the bottom. (The sample should not be held at 130° C. nor should it be reheated to this temperature more than once. If excessive moisture is present, the acids should be decanted after having stood for a few minutes, refiltered and reheated. The acids must be thoroughly dry.) Place the test tube in the assembly as shown in Figure 2. Insert titer thermometer and place in a position equidistant from the sides of the tube, and so that the immersion mark on the thermometer coincides with the upper level of the sample.

Stir with the glass stirring rod in a vertical manner at the rate of 100 complete up and down motions per minute, beginning the stirring at a point 10° C. above the expected titer point. The stirrer should travel through a vertical distance of about 3.8 cm. The stirring may be performed by mechanical means by attaching a small motor with suitable reducing gears to the stirring rod.

Stir at the directed rate and read the thermometer every 15 seconds until the temperature remains constant for 30 seconds or starts to rise. Discontinue stirring immediately and observe the increase in temperature. Report as the titer the highest temperature reached by the thermometer. Duplicate determinations should not differ by more than 0.2° C.

Note. The Committee on Analysis of Commercial Fats and Oils of the American Chemical Society makes the following recommendations:

> The committee has approved vertical stirring. The advantages of this over horizontal stirring are that it gives a sharper end point, is more convenient, and can easily be made mechanical by adapting a suitable motor and coupling arrangement to the stirrer.
>
> The committee wishes to emphasize the fact that no changes have been made in the titer determination which will give results different from those that might be obtained with the horizontal stirring method, providing the latter is correctly performed. The committee believes that the proposed modification will make it easier for different chemists to obtain uniform and consistent results.

XIV. Acid Number of Fatty Acids. (1) *Preparation of fatty acids.* Follow the procedure described in C-XII (2).

(2) *Procedure.* In a 250-ml. Erlenmeyer flask dissolve 2 grams of the fatty acids, accurately weighed, in 20 to 30 ml. of neutral 95 per cent ethyl alcohol. Titrate with standard alkali, using phenolphthalein as indicator. Calculate the acid number (milligrams of potassium hydroxide per gram of fatty acids).

XV. Iodine Number (Wijs Method). (1) *Special reagents required.* Wijs iodine solution. Dissolve 13.0 grams of resublimed iodine in 1 liter of reagent grade glacial acetic acid and pass in washed and dried chlorine gas until the original thiosulfate titration of the solution is not quite doubled. There should be no more than a slight excess of iodine, and no excess of chlorine. When the solution is made from iodine and chlorine, this point can be ascertained by not quite doubling the titration (Note). For preparation of the Wijs solution use glacial acetic acid of 99.0 to 99.5 per cent strength. For glacial acids of somewhat lower strength, freezing and centrifuging or draining as a means of purification is recommended. Preserve the solution in amber, glass-stoppered bottles, sealed with paraffin until ready for use. Mark on the bottles the date on which the solution is prepared; do not use Wijs solution that is more than 30 days old.

Note. For preparation of the solution, McIlhiney (*13*) gives the following details:

> The preparation of the iodine monochloride solution presents no great difficulty, but it must be done with care and accuracy in order to obtain satisfactory results. There must be in the solution no appreciable excess either of iodine or more particularly of chlorine, over that required to form the monochloride. This condition is most satisfactorily attained by dissolving in the whole of the acetic acid to be used the requisite quantity of iodine, using a gentle heat to assist the solution, if it is found necessary; then setting aside a small portion of this solution, while pure and dry chlorine is passed into the remainder until the halogen content of the whole solution is doubled. Ordinarily, it will be found

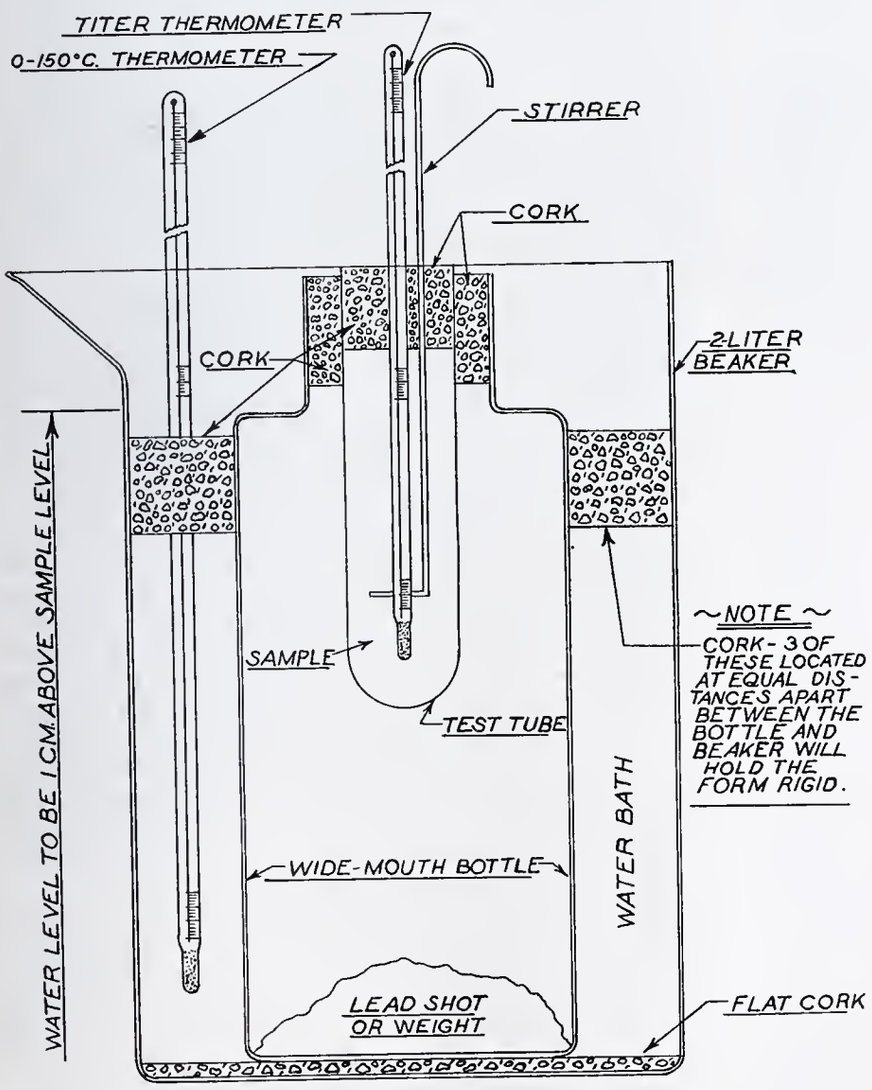

Figure 2. Assembly for Stirring Titers

that on passing the chlorine into the main part of the solution until the characteristic color of free iodine has just been discharged there will be a slight excess of chlorine which is corrected by the addition of the requisite amount of the unchlorinated portion until all free chlorine has been destroyed. A slight excess of iodine does little or no harm, but excess of chlorine must be avoided.

Sodium thiosulfate solution (0.1 *N*). Dissolve 24.8 grams of reagent grade sodium thiosulfate ($Na_2S_2O_3.5H_2O$) in freshly boiled distilled water and dilute to 1 liter at the temperature at which the titrations are to be made. To standardize, place in a glass-stoppered flask 40 ml. of potassium dichromate solution (0.1 *N*) to which have been added 10 ml. of the solution of potassium iodide, add 5 ml. of hydrochloric acid (sp. gr. 1.19), dilute with 100 ml. of water, and allow the 0.1 *N* sodium thiosulfate to flow slowly into the flask until the yellow color of the liquid has almost disappeared. Add a few drops of the starch paste, and while shaking constantly, continue to add the 0.1 *N* sodium thiosulfate solution until the blue color just disappears.

Potassium dichromate solution (0.1 *N*). Dissolve 4.903 grams of reagent grade potassium dichromate ($K_2Cr_2O_7$) in water and dilute to 1 liter at the temperature at which titrations are to be made.

Potassium iodide solution (15 per cent). Dissolve 150 grams of potassium iodide in water and dilute to 1 liter.

Starch paste. Boil 1 gram of starch in 200 ml. of distilled water for 10 minutes, and cool to room temperature.

Note. An improved starch solution may be prepared by autoclaving 2 grams of starch and 6 grams of boric acid dissolved in 200 ml. of water at 15-pound pressure for 15 minutes. This solution has good keeping qualities.

(2) *Procedure.* Weigh accurately from 0.10 to 0.50 gram (depending on the iodine number) of the fatty acids as prepared in C-XII (2) into a clean, dry, 450-ml. (16-ounce) glass-stoppered bottle containing 15 to 20 ml. of carbon tetrachloride. Add 25 ml. of the Wijs solution from a pipet, allowing it to drain for a definite time. The excess of iodine should be from 50 to 60 per cent of the amount added—that is, from 100 to 150 per cent of the amount absorbed. Let the bottle stand in a dark place for 30 minutes at a temperature of 25° ± 2° C., then add 20 ml. of potassium iodide solution (15 per cent) and 100 ml. of distilled water. Titrate the iodine with 0.1 *N* sodium thiosulfate, added gradually while shaking constantly, until the yellow color of the solution has almost disappeared. Add a few drops of starch paste and continue titration until the blue color has entirely disappeared. Toward the end of the reaction, stopper the bottle and shake vigorously, so that any iodine remaining in solution in the tetrachloride may be taken up by the potassium iodide solution. Make two determinations on blanks, employing the same procedure as used for the sample, except that no fat is used in the blanks. Slight variations in temperature appreciably affect the titer of the iodine solution, since acetic acid has a high coefficient of expansion. It is therefore essential that the blanks and determinations on the sample be made at the same time. The number of milliliters of standard thiosulfate solution required by the blank, minus the amount used in the determination, give the thiosulfate equivalent of the iodine absorbed by the amount of sample used in the determination. Calculate the iodine number of the sample tested (centigrams of iodine absorbed by 1 gram of sample) (percentage of iodine absorbed).

XVI. Borax. (1) *Special reagents required.* Sodium hydroxide solution (0.1 *N*); silica, fine powder; precipitated calcium carbonate; and glycerol, reagent grade.

(2) *Procedure* (*16*). Weigh 10 grams (±0.02 gram) of the soap [or 5 grams (±0.01 gram) if more than 5 per cent of borax is present] into a platinum dish and add 2.15 grams of fusion mixture (consisting of 200 grams of sodium carbonate and 15 grams of silica in fine powder). To this mixture add 15 ml. of alcohol, mix with the aid of a glass rod and, after washing the rod with a little alcohol, evaporate the mass to dryness on the water bath. Ignite until the combustible material is destroyed, cover the dish with a piece of platinum foil, and fuse. Completely disintegrate the fusion by boiling with water and transfer the solution to a 250-ml. round-bottomed flask. Acidify with 20 ml. of dilute hydrochloric acid (1 to 1), heat nearly to boiling, and add a moderate excess of dry precipitated calcium carbonate. Connect with a reflux condenser and boil vigorously for 10 minutes. Filter out the precipitate through a folded filter, washing several times with hot water, but keeping the total volume of liquid below 100 ml.

Return the filtrate to the flask, add a pinch of calcium carbonate, and again boil under a reflux condenser. Remove the flame and connect the top of the condenser with a water pump. Apply the suction until the boiling has nearly ceased. Cool to ordinary temperature, add 50 ml. of neutral glycerol, and titrate the solution with 0.1 *N* sodium hydroxide, free from carbonate, using phenolphthalein as indicator. After the end point is reached, add 10 ml. more of glycerol and again titrate. Repeat this process until the addition of glycerol causes no further action on the end point. The number of milliliters of sodium hydroxide required multiplied by 0.00955 will give the equivalent of borax ($Na_2B_4O_7.10H_2O$) present in the solution.

XVII. Silica Present as Alkaline Silicates. *Procedure.* When the material contains no mineral matter that is insoluble in water, ignite a sample of the soap containing not to exceed 0.2 gram of silica in a platinum dish at a low temperature. When charred, extract the soluble salts with water, return the paper and charred residue to the dish, and complete the ignition. Unite the residue in the dish and the water extract, and carefully acidify with hydrochloric acid, finally adding the equivalent of from 5 to 10 ml. of hydrochloric acid (sp. gr. 1.19) in excess. The dish or casserole containing the solution should be covered with a watch glass while adding acid, so as to avoid loss by spray.

When the material contains mineral matter insoluble in water, or a determination of highest accuracy is not necessary, take a portion of the solution after titrating the matter insoluble in alcohol (C-V) containing not more than 0.2 gram of silica and add 5 to 10 ml. of hydrochloric acid (sp. gr. 1.19).

Evaporate the acidified solution (washing off and removing the cover glass if used) to dryness on a steam bath or hot plate at a temperature not exceeding 120° C. Cool, moisten with concentrated hydrochloric acid, and let stand 5 to 10 minutes, breaking up all lumps with a stirring rod. Add about 25 ml. of hot water. Heat a few minutes and filter through a small ashless paper. Wash thoroughly with hot water.

Evaporate the filtrate to dryness and repeat the above treatment, filtering on a second paper. Carefully ignite the two papers and contents in a weighed platinum crucible, first at a low temperature until the paper is consumed, then over the blast lamp. Cool in a desiccator, weigh, and repeat until constant weight is obtained. If extreme accuracy is desired, moisten the weighed contents of the crucible with water, add 10 ml. of hydrofluoric acid and 4 drops of strong sulfuric acid, evaporate to dryness over a low flame, ignite at the temperature of the blast lamp for about 2 minutes, cool in a desiccator, and weigh. The difference between this weight and the previous weight is the weight of the silica (SiO_2) (*10*).

To calculate sodium silicate having the ratio $1Na_2O:3.25SiO_2$, multiply the weight of SiO_2 by 1.308 (*19*).

XVIII. Carbon Dioxide (Carbonates). In most cases the dry matter insoluble in alcohol, as obtained in C-II, will be suitable for this determination. In some cases it might be desired to run the test directly on an original sample of the soap. This should always be done when the highest accuracy is required. Any reliable absorption method for determining carbon dioxide may be used (*4*). A method which has proved satisfactory is described in the following paragraphs.

(1) *Apparatus assembly.* Place a 250-ml. Erlenmeyer flask on a gauze over a burner. Fit the flask with a two-hole rubber stopper, one opening to carry a 25-cm. (10-inch) reflux condenser and the other a thistle tube equipped with a three-way stopcock. Draw out the end of the thistle tube to a small point, and place it in the stopper so that the point is very close to the bottom of the flask. Attach a small funnel to the straightaway end of the stopcock for the introduction of acid into the flask. Attach the other opening of the stopcock (which is to receive air) to a purifying tube containing soda-asbestos (Ascarite) or any other suitable carbon dioxide absorbent. Attach to the top of the reflux condenser a train consisting of the following: (*a*) a drying tube containing a dehydrating agent such as sulfuric acid (sp. gr. 1.84) or magnesium perchlorate, (*b*) a weighed tube containing Ascarite and magnesium perchlorate, and a second weighed tube containing concentrated sulfuric acid. Attach to this train a protective U-tube containing calcium chloride; attach the U-tube to an aspirator.

(2) *Procedure.* Set up the apparatus, leaving out the weighed train, and aspirate with a slow stream of the dry carbon dioxide–free air until the apparatus is free of carbon dioxide. Insert the train and continue to aspirate for 30 minutes. Check the weight of the train to determine whether the air is passing through too fast, or whether the system is free of carbon dioxide. The system must be free from leaks. Weigh a sufficient amount of the sample to yield approximately 0.2 gram of CO_2, transfer to the Erlenmeyer flask, cover with 20 ml. of freshly boiled distilled water, and close the apparatus with the train in place. Add 20 ml. of dilute hydrochloric acid (1 to 1) very slowly through the funnel; do not apply heat to the flask. The rate of adding acid should be carefully controlled, so that the gas does not pass through the train too rapidly. As soon as the acid is added, start aspiration gently. When the absorption begins to slacken, start heating gently and continue until the contents of the flask have boiled 15 to 20 minutes. Stop heating and continue aspirating until the flask has cooled down. Remove the train and weigh. The increase in weight represents carbon dioxide. The amount of this increase multiplied by 2.41 equals sodium carbonate, or by 3.14 equals potassium carbonate.

XIX. Phosphates. If a qualitative test has shown the presence of phosphates and their determination is desired, the matter insoluble in alcohol (C-II) or the ash from the incineration of an original sample may be used. An original sample should always be used when the highest accuracy is desired.

(1) *Special reagents required.* Molybdate solution. Dissolve 100 grams of molybdic acid in dilute ammonium hydroxide [144 ml. of ammonium hydroxide (sp. gr. 0.90) and 271 ml. of water]; pour this solution slowly and with constant stirring into dilute nitric acid [489 ml. of nitric acid (sp. gr. 1.42) and 1148 ml. of water]. Keep the final mixture in a warm place for several days or until a portion heated to 40° C. deposits no yellow precipitate of ammonium phosphomolybdate. Decant the solution from any sediment and preserve in glass-stoppered vessels.

Ammonium nitrate solution. Dissolve 100 grams of phosphate-free ammonium nitrate in distilled water, and dilute to 1 liter.

Magnesia mixture. Dissolve 55 grams of crystallized magnesium chloride ($MgCl_2.6H_2O$) in water, add 140 grams of ammonium chloride and 130.5 ml. of ammonium hydroxide (sp. gr. 0.90), and dilute to 1 liter.

Dilute ammonium hydroxide for washing. Dilute 100 ml. of ammonium hydroxide (sp. gr. 0.90) to 1 liter.

(2) *Procedure* (*3*). Weigh out a 2-gram (±0.01 gram) sample of the alcohol-insoluble or ash, and proceed as described in C-XVII for removal of silica (a platinum dish should not be used), using nitric instead of hydrochloric acid. Boil the covered solution 30 minutes to convert other phosphates that may be present to orthophosphate, wash off and remove cover glass, and evaporate as described. Save the filtrate. Make up to 250 ml., concentrating if necessary. Pipet an aliquot corresponding to 0.50 or 1 gram of the original material into a 250-ml. beaker, add ammonium hydroxide in slight excess, and barely dissolve the precipitate formed with a few drops of nitric acid, stirring vigorously. Add about 15 grams of dry ammonium nitrate or a solution containing that amount. Heat to about 60° C., and add 70 ml. of the molybdate solution for every decigram of phosphoric acid (P_2O_5) present.

Digest at about 65° C. for an hour, and determine if the phosphoric acid has been completely precipitated by adding more molybdate solution to the clear supernatant liquid. Filter, and wash with cold water, or preferably, ammonium nitrate solution. Dissolve the precipitate on the filter with ammonium hydroxide (1 to 1) and hot water, and wash into a beaker, keeping the volume under 100 ml. Neutralize with hydrochloric acid, using litmus paper or bromothymol blue as an indicator, cool, and from a buret add slowly (about 1 drop per second), stirring vigorously, 15 ml. of magnesia mixture for each decigram of phosphoric acid (P_2O_5) present. After 15 minutes add 12 ml. of ammonium hydroxide (sp. gr. 0.90). Let stand till the supernatant liquid is clear (2 hours is usually enough), filter, wash the precipitate with the dilute ammonium hydroxide until the washings are practically free from chlorides; dry, burn first at a low heat (platinum should not be used), and then ignite to constant weight, preferably in an electric furnace, at 1050° to 1100° C.; cool in a desiccator, and weigh as $Mg_2P_2O_7$. Calculate and report the result as percentage of P_2O_5 or alkaline phosphate known to be present.

XX. Sulfates. *Procedure.* For most determinations the matter insoluble in alcohol (obtained in C-II) may suffice. If a determination of the highest accuracy is desired, ignite a 10-gram (±0.1 gram) sample of the soap and use the ash from the ignition. Digest with 100 ml. of water, cover with a watch glass, and neutralize carefully with hydrochloric acid. When neutralized, add 5-ml. excess of hydrochloric acid, filter, and wash the residue thoroughly. (Evaporation to dryness is unnecessary unless gelatinous silica has separated, and should never be performed on a bath heated by gas, *11*). Make up the filtrate to 250 ml. in a beaker, and boil. To the boiling solution add 15 to 20 ml. of 10 per cent barium chloride solution slowly drop by drop from a pipet. Continue boiling until the precipitate is well formed, or digest on a steam bath overnight. Set aside overnight or for a few hours, filter through a prepared Gooch crucible, ignite gently, and weigh as barium sulfate. Calculate to sodium sulfate, or the alkaline sulfate known to be present.

XXI. Glycerol, Sugars, and Starch. *A. Glycerol in the absence of sugars.* (1) Special Solutions Required. Potassium dichromate solution. Dissolve 74.553 grams of potassium dichromate in 500 ml. of water in a 1-liter volumetric flask. Dilute to the mark with water. Sodium thiosulfate solution (0.1 *N*). Potassium iodide solution (10 per cent).

(2) Procedure. (*a*) Weigh a portion of the sample equivalent to not more than 3.0 grams of glycerol and dissolve in 200 ml. of hot water in a 600-ml. beaker. If starch is present, it will be necessary to remove the matter insoluble in water as described in C-II and C-IV. Combine the alcohol and water solutions, evaporate off the alcohol, and proceed. Decompose with 25 ml. of sulfuric acid (1 to 4). If alcohol is present, volatilize it by boiling for 20 to 30 minutes. Cool, remove, and rinse the cake of fatty acids, transfer the acid water and rinsings to a 500-ml. graduated flask, and add about 0.25 gram of silver sulfate to precipitate traces of chlorides and soluble fatty acids. Make up to volume and mix contents thoroughly.

(*b*) Transfer a filtered, accurately measured 50-ml. aliquot of the solution obtained in (*a*) to a 400-ml. beaker, add 75 ml. of accurately measured potassium dichromate solution, followed by 25 ml. of sulfuric acid (sp. gr. 1.84), cover with a watch glass, and oxidize by heating to 90° to 100° C. for 3 hours. Conduct a blank in like manner but using 100 ml. of water, 25 ml. of sulfuric acid (sp. gr. 1.84), and 25 ml. of accurately measured potassium dichromate. Cool and dilute the solutions to 1000 ml. in graduated flasks. The excess of potassium dichromate is determined by taking 50-ml. aliquots of the above, adding 50 ml. of water and 20 ml. of potassium iodide solution (10 per cent), and titrating the liberated iodine with 0.1 *N* thiosulfate, using starch solution as indicator.

(c) Calculate the percentage of glycerol (1 ml. of the potassium dichromate solution is equivalent to 0.0100 gram of glycerol).

B. Glycerol in the presence of sugars. Procedure (*12*). Proceed as in the determination of glycerol in the absence of sugar [C-XXI, A (2)], taking a sample so that the sum of the glycerol and sugar is not more than 3.0 grams. If starch is present, this must first be removed as described in C-XXI, A (2). The solution must be boiled in all cases at least 20 minutes to ensure complete inversion of sucrose as in C-XXI, D (2). Determine the amount of potassium dichromate solution required to oxidize both the sugar and glycerol. Determine also the sugar by the method given in C-XXI, D (2).

Calculate the percentage of glycerol after deducting the amount of potassium dichromate required by the sugar.

1 ml. of potassium dichromate equals 0.0100 gram of glycerol
1 ml. of potassium dichromate equals 0.01142 gram of invert sugar

C. Starch. Procedure (*5*). Separate the matter insoluble in water as described in C-II and C-IV, using a sample of soap that will give not more than 3 grams of starch. Transfer the insoluble matter, without drying, to a 500-ml. flask provided with a reflux condenser, and boil for 2.5 hours with 200 ml. of water and 20 ml. of hydrochloric acid (sp. gr. 1.125). Cool, and nearly neutralize with sodium hydroxide. Complete the volume to 250 ml., filter, and determine the reducing sugars by the gravimetric method as given for the determination of sugar [C-XXI, D (2)].

Calculate the amount of dextrose (*d*-glucose) equivalent to the cuprous oxide obtained. This multiplied by 0.90 equals the amount of starch.

D. Sugars. (1) Apparatus and Reagents Required. The apparatus and reagents used shall be the same as those described in the standard Munson-Walker method (*15*).

(2) Procedure. Dissolve 10 grams (±0.01 gram) of the soap in 200 ml. of hot water in a 600-ml. beaker. Decompose with 25 ml. of sulfuric acid (1 to 4), boil gently for 20 minutes to invert the sucrose completely. Cool, remove, and rinse the cake of fatty acids. Extract the acid liquid with 25 ml. of ether. Neutralize the acid liquid with sodium hydroxide solution, transfer to a 500-ml. graduated flask, make up to volume, and mix thoroughly. Determine invert sugar in 50 ml. of this solution by the Munson-Walker method (*15*). To calculate sugar (sucrose) multiply the amount of invert sugar found by 0.95. [If starch is present, first remove as described in C-XXI, A (2) and then proceed as above.]

XXII. Volatile Hydrocarbons. This method (*17*) requires a source of dry, oil-free steam which is passed through the sample treated with acid, sufficient to liberate the fatty acids from the soap. The steam is next passed through strong caustic solution to scrub out any volatile fatty acids, while the volatile hydrocarbons are condensed with the steam in a suitable arrangement which allows the excess water to flow away, leaving the volatile hydrocarbons in the measuring buret. The method may be applied to samples containing substances immiscible with water and volatile with steam. [For solvents heavier than water the trap as shown in Figure 1 for determining water by the distillation method, C-I (2), should be used.]

(1) *Apparatus.* The apparatus and its arrangement are shown in Figure 3. The following are the important items:

Steam trap, *A*. A 1-liter round-bottomed ring-necked flask equipped with a siphon tube to the drain from the bottom of the flask and provided with a means of regulating the steam flow into the flask.

Evolution or sample flask, *B*. A 1-liter round-bottomed ring-necked flask. In case large samples are desirable the size of this flask may be increased.

Caustic scrubber flask, *D*. A steam-jacketed metal flask is preferred, but a 1-liter Florence flask provided with a steam coil of 0.32 cm. (0.125-inch) copper tubing around the upper half may be used. If the glass flask is used it should be provided with a safety bucket below it and should be renewed frequently, since

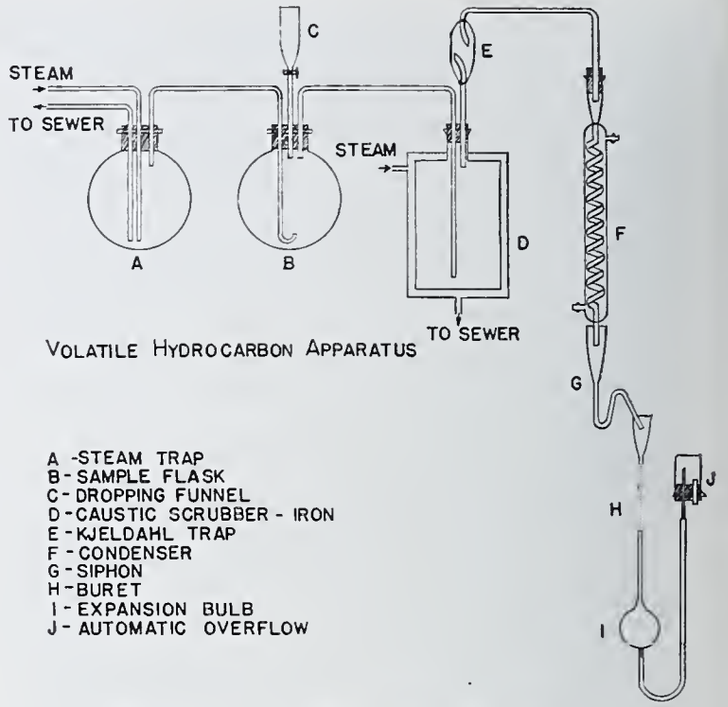

Figure 3. Volatile Hydrogen Apparatus

the strong caustic dissolves the glass rather rapidly. This flask should be connected to the condenser by a Kjeldahl connecting tube, *E*, or similar safety device.

The inlet for the steam into the evolution and scrubber flasks should extend nearly to the bottom of the flasks and be bent at right angles and parallel to the sides of the flask.

Condenser, *F*. A 30.5-cm. (12-inch) or longer spiral condenser of sufficient bore so the condensate will not readily close it.

Measuring buret, *H*. A 10-ml. buret calibrated to 0.1 ml. and carrying a bulb, *I*, of approximately 100-ml. capacity, at the lower end. If desired, an ordinary 10-ml. Mohr-type buret may be used, having attached to it by rubber tubing a bulb of proper capacity which has been blown in the laboratory. An ordinary buret funnel may be placed in the top of the buret in place of the special flared-out top shown in Figure 3.

The stoppers used should be of a good grade of rubber and should have been thoroughly cleaned free from any surface sulfur and should be given a steam-distillation in position for several hours before use on a sample.

Insulating the flasks and tubing to reduce condensation aids distillation and its control.

(2) *Procedure.* Place 150 ml. of sodium hydroxide solution (about 1.47 sp. gr.) and several sticks of solid sodium hydroxide to provide against dilution in the scrubber flask. Rinse out the condenser and buret with acetone. Attach a rubber tubing to the lower end of the buret, fill the buret and tubing with water, and raise the outer end of the tubing so that the water level in the buret is near the top of the scale when the water is flowing to the drain from the automatic overflow, *J*. Be sure the connections are tight and that the tubing contains no air bubbles. Place the condenser in position so that the lower end extends directly into the upper end of the buret just above the water level, or connect to an adapter siphon, *G*, which discharges into the buret. The cooling water should be 15.5° C. or colder. Ice water may be desirable for low-boiling hydrocarbons.

Weigh 100 grams (±0.5 gram) of the soap (cut into cubes of about 1-cm. edges) or 50 grams (±0.3 gram) of soap powder and transfer to the evolution flask. Add about 10 grams of gum arabic (commercial) and 100 ml. of distilled water. Place the flask in position with 100 ml. of sulfuric acid (1 to 3) in a dropping funnel, *C*, carried in the stopper. Connect with the steam line and with the wash flask and the condenser, making sure that the stoppers are tightly fitting and held in place by wiring. Rubber

connections in the lines between the evolution flask and condenser should be avoided.

Add the acid to the sample slowly to avoid excessive frothing. While adding the acid, turn on the steam cautiously, so adjusting the pressure by a bleeder valve that just enough steam flows to prevent any liquid from backing into the steam trap flask.

When all the acid has been added, turn on enough steam to cause brisk distillation, taking care that no liquid is carried over from the evolution and wash flasks and that the condenser water does not become warm.

Continue the distillation until there is no increase in the volume of the upper layer for 45 minutes or no small droplets can be noted in the condensate.

When distillation is completed, shut off and drain the condenser water, and allow the steam to heat up the condenser to drive out the last traces of volatile hydrocarbon. Shut off the steam as soon as vapor begins to issue from the lower end of the condenser. Immediately open the stopcock of the dropping funnel to prevent caustic being drawn into the evolution flask.

Stopper the buret and allow its contents to come to room temperature or bring them to a definite temperature by immersing the buret for 1 to 2 hours in a water bath held at 25° C.

Read the volume of the upper layer to the nearest 0.01 ml. The volume multiplied by the specific gravity equals the weight of the volatile hydrocarbon. The specific gravity should be determined at the temperature at which the volume is read. A small Sprengel tube made of 3-mm. glass tubing is convenient for this purpose.

Calculation. Calculate the percentage of volatile hydrocarbons as follows:

$$V = \frac{M \times \text{sp. gr.}}{S} \times 100$$

where V = per cent of volatile hydrocarbons, M = milliliters of volatile hydrocarbons, and S = weight of sample in grams.

Note. For some samples the volatile hydrocarbon content may be so low that a larger sample than 50 or 100 grams is desirable. The size of the evolution flask may need to be increased if larger samples are used. The amount of water in the evolution flask and acid used should also be correspondingly increased.

XXIII. Combined Sodium and Potassium Oxides. The total combined alkali present in the soap is determined by the method described in C-VI, and calculated as sodium oxide (Na_2O). Determine the combined potassium oxide (K_2O) by the following method, calculate it to the equivalent sodium oxide (Na_2O), and subtract this from the total combined alkali calculated as sodium oxide (Na_2O); the remainder is the combined sodium oxide (Na_2O).

(1) *Special reagents required.* Ammonium chloride solution. Dissolve 100 grams of ammonium chloride in 500 ml. of distilled water, add 5 to 10 grams of pulverized potassium chloroplatinate (K_2PtCl_6), and shake at intervals for 6 to 8 hours. Allow the mixture to settle overnight and filter. (The residue may be used for the preparation of a fresh supply of ammonium chloride solution.)

Platinum solution. Prepare a solution containing the equivalent of 1 gram of metallic platinum (2.1 grams of chloroplatinic acid, H_2PtCl_6) in each 10 ml. of solution. For materials containing less than 15 per cent of potassium oxide (K_2O), a solution containing 0.2 gram of metallic platinum (0.42 gram of H_2PtCl_6) in each 10 ml. of solution is recommended.

(2) *Preparation of sample.* Weigh a 10-gram (±0.01 gram) sample and sinter it in an evaporating dish below a dull red heat. Leach the ash with hot water, filter into a 100-ml. volumetric flask, and wash the paper with three 5- to 10-ml. portions of hot water. Complete the ashing after returning the filter paper and residue to the original dish and sintering as before. Excessive heating should be avoided. Removal of most of the alkali present by thoroughly washing the ash with hot water before completion of the ashing will aid in preventing overheating of the greater portion of the sample. Add a few drops of hydrochloric acid (1 to 1) to the ash and wash the contents of the dish into the volumetric flask. Acidify the solution in the volumetric flask with hydrochloric acid, dilute to 100 ml., mix thoroughly, and pass through a dry filter.

(3) *Procedure.* Acidify an accurately measured 10-ml. aliquot of the solution obtained in Section 2 with a few drops of hydrochloric acid and add 10 ml. of the platinum solution. Evaporate the solution on a water bath to a thick paste which will become solid on cooling to room temperature. Avoid exposure to ammonia fumes while heating the solution. Treat the residue with approximately 6 ml. of alcohol (80 per cent) and add 0.6 ml. of hydrochloric acid (sp. gr. 1.19). Filter on a Gooch crucible and wash the precipitate thoroughly with alcohol (80 per cent) both by decantation and on the filter, continuing the washing until after the filtrate is colorless. Then wash the residue five or six times with 25-ml. portions of the ammonium chloride solution to remove the impurities from the precipitate. Wash again thoroughly with alcohol (80 per cent), dry the precipitate at 100° C. for 30 minutes, and weigh. Calculate the result to potassium oxide (K_2O).

Note. For the conversion of K_2PtCl_6 to K_2O the factor 0.19376 may be used.

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A Dye Extremely Sensitive to Copper

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DURING spectrophotometric measurements of dyestuffs the sensitivity of Benzo Fast Yellow 5GL to the presence of copper was found to be 40 times as great as the carbamate method of test. While not necessarily proposed as an analytical method, it was thought that this sensitivity would be of interest.

Figure 1 compares the curve shapes of a solution of Benzo Fast Yellow 5GL (C. I. 346, diphenylurea-*p,p'*-disazo-bis-salicylic acid) at pH 9.0, temperature 23° C., and concentration of 30 mg. per liter with and without 0.1 mg. per liter of cupric ion added. Both solutions are stable over a 24-hour period.

Analysis of the curve shows that the extinction coefficient of the pure dye and the metallized complex is the same at 397 millimicrons, while the extinction coefficient has increased about 150 per cent at 487 millimicrons (from 2.9×10^3 to 7.4×10^3 sq. cm. per gram). Using magnifying cams, it is possible to get a sensitivity of 0.5 per cent extinction coefficient, which means that a small fraction of this amount of copper can be detected.

In order to determine the sensitivity to a small amount of copper, the data shown in Figure 2 were obtained. Figure 2 shows the curves obtained using a magnifying cam with and without 10^{-6} gram per liter of copper divalent ion present in a Benzo Fast Yellow 5GL solution. Since the linear distance separating the two curves is readily divisible into ten parts, it is evident that 10^{-7} gram of copper per liter can be detected.

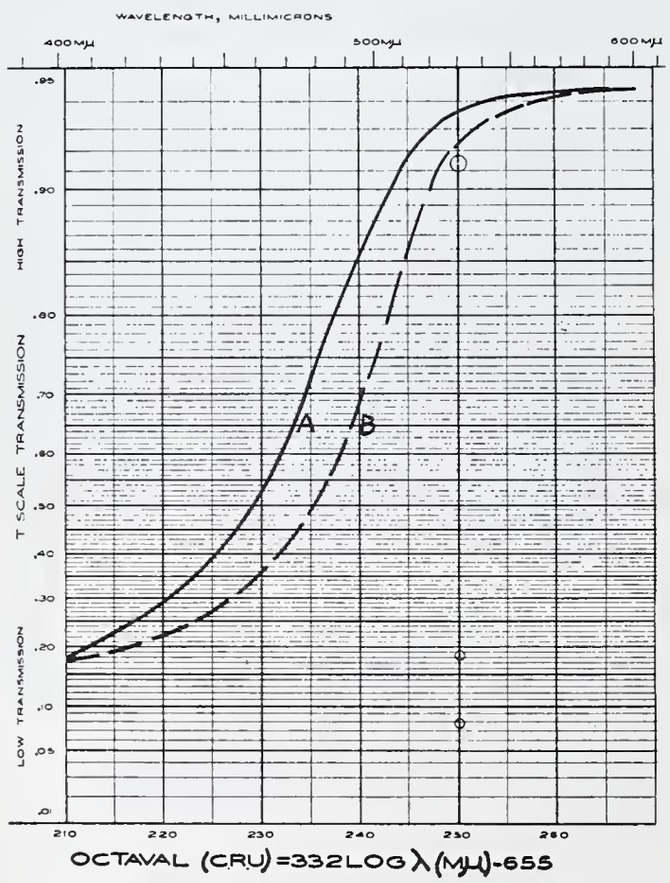

FIGURE 1. EFFECT OF ADDED COPPER ON BENZO FAST YELLOW 5GL

Normal cams
A. 30 mg. per liter of dye in water at pH 9.0, 23° C., measured in 1-cm. cell
B. Same as *A* but with 0.1 mg. per liter of Cu^{++} added

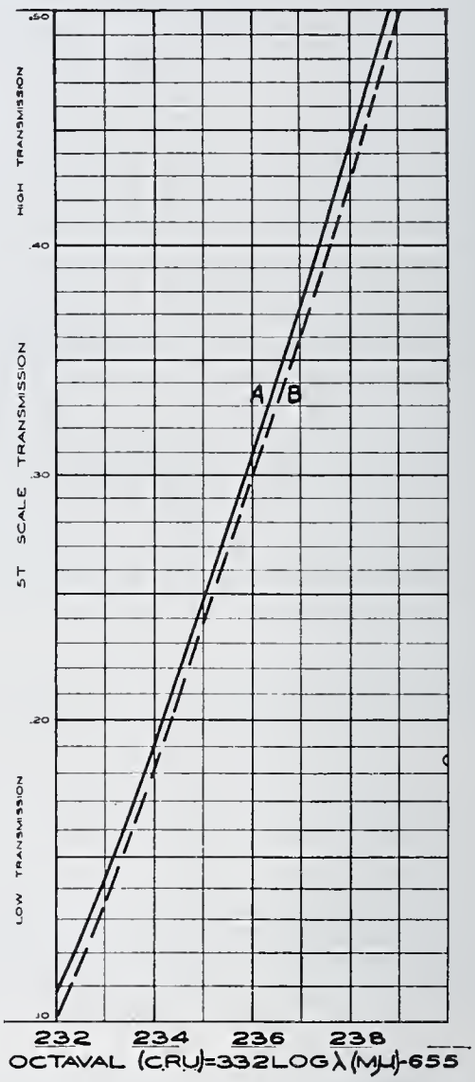

FIGURE 2. EFFECT OF ADDED COPPER ON BENZO FAST YELLOW 5GL

Magnifying cams
A. 30 mg. per liter of dye in water at pH 9.0, 27° C., measured in 5-cm. cell
B. Same as *A* but with 0.001 mg. per liter of Cu^{++} added

The colorimetric reaction with sodium diethyldithiocarbamate and copper as measured on the spectrophotometer gives a test in which 4×10^{-6} gram of copper per liter can be determined in a similar cell. Hence, on the basis of spectrophotometric measurement the Benzo Fast Yellow 5GL is about 40 times as sensitive as the sodium diethyldithiocarbamate.

The carbamate test is ordinarily considered to have a sensitivity based on visual colorimetry with a Lovibond colorimeter of about 10^{-5} gram, which closely approaches the spectrophotometric sensitivity. Visually the Yellow 5GL sensitivity would not so nearly approach the spectrophotometric sensitivity because a change of color, from a yellow to a redder yellow, is not so noticeable as the appearance of a color which is the case in the carbamate method. Hence, for visual work,

the sensitivity of the two methods of test would perhaps be equal.

The Yellow 5GL is also reactive to iron, chromium, and nickel, but to a smaller extent, detecting only about 5×10^{-5} gram per liter of iron.

It has been useful as a measure of the general purity of distilled water as regards presence of metallic ions, but no method has been worked out to make it a specific test for copper. No effort has been made to determine the optimum conditions or to determine whether other closely related dyes are better.

Direct Green B, which is also sensitive to copper, has been proposed (*3*), and has been found to have about the same sensitivity as the carbamate test spectrophotometrically. Hence the Yellow 5GL is also 40 times as sensitive as Direct Green B, based on spectrophotometric measurements.

The use of octaval (logarithm of wave length) for the abscissa of spectrophotometric curves (*2*) is somewhat unusual but is particularly convenient when magnifying cams are used as in Figure 2. Thus the visual range (375 to 750 mu) is divided into 100 logarithmic units and covers the range 200 to 300 c. r. u. This plotting range was used for the data of Figure 1. One fifth of this range, from 230 to 250 c. r. u., was magnified to full width and was the plotting range used for the data of Figure 2. For convenience in range-shifting and magnification changing, as is necessary in problems of this type, the use of a decimal system has been found most convenient.

The $\times$ 5 ordinate magnification need not be so flexible in the case of solutions, since their concentration can be changed to cause them to fall within the plotted range. Hence it is necessary to have the $\times$ 5 log log $1/T$ scale only in the plotting range 10 to 50 per cent transmission. This is the ordinate used in Figure 2. The desirability of the log log $1/T$ scale is generally recognized (*1*).

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Effect of Container on Soluble Silica Content of Water Samples

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IN POWER plant operation, particularly with modern high-pressure boilers, the silica content of the raw water, feed water, boiler water, and steam has become of prime importance.

The silica content of a boiler water sample is of importance in limiting silica in the concentrated boiler water to a tolerable value, so as to prevent scale formation. Particularly at higher boiler pressures, careful control of the silica content of the boiler water and steam may be required for the limitation of siliceous turbine blade deposits. Silica plays an important role in the intercrystalline corrosion of boiler metal, and in evaluating the effect of this constituent under varying conditions it is important that the silica values obtained reflect the true conditions existing in the water sample.

Recently, methods have been developed for the controlled removal of silica from raw and boiler feed waters, and in the formulation of accurate recommendations for such silica removal it is necessary that the water analyses on which these recommendations are based be entirely representative of the silica content of the water. For the proper routine control of silica removal processes, it is obvious that the samples analyzed must be representative.

A recent article by Belyea and Moody covers in detail the sampling of steam and boiler water (*2*). This paper is concerned entirely with the storage of water samples after collection.

For the accurate determination of silica, glass containers should not be employed (*1*). Collins and Riffinburg (*3*) pointed out the large increase in silica that will occur in poor glass bottles over a period of 8 months; they concluded that samples of water in good bottles will not dissolve enough glass in a month to cause any detectable change in the ordinary mineral analysis and that no significant change will be caused in 6 months. They were primarily concerned with the effect of storage on natural or raw water and on distilled water samples, although they also tested dilute sodium carbonate solutions.

Data on resistance to chemical attack for four brands of chemical glassware have recently been presented (*7*).

Table I. Changes in Silica Content

Container	Temperature at Which Sample Added	Silica as SiO_2				
		Original sample	3rd day	5th day	7th day	14th day
	° C.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.
Sample Typical of Raw or Feed Water						
Ordinary soda glass bottle	23	2.0	2.2	2.3	2.4	2.6
	95	2.0	2.4	2.6	2.8	3.0
Pyrex bottle	23	2.0	2.1	2.2	2.2	2.3
	95	2.0	2.2	2.3	2.4	2.4
Hard-rubber bottle	23	2.0	2.0	2.0	2.0	2.0
	95	2.0	2.0	2.0	2.0	2.0
Resin-lined can	23	2.0	2.0	2.0	2.0	2.0
	95	2.0	2.0	2.0	2.0	2.0
Sample Typical of Boiler Water						
Ordinary soda glass bottle	23	32.4	33.4	34.2	35.2	37.7
	95	32.4	33.9	35.1	36.2	38.6
Pyrex bottle	23	32.4	32.8	33.1	33.6	34.9
	95	32.4	33.4	33.8	34.3	35.7
Hard-rubber bottle	23	32.4	32.5	32.4	32.3	32.4
	95	32.4	32.4	32.4	32.4	32.5
Resin-lined can	23	32.4	32.1	32.4	32.6	32.1
	95	32.4	32.4	32.0	32.3	32.4
Sample Typical of Condensate						
Ordinary soda glass bottle	23	0.2	0.3	0.4	0.6	0.9
	95	0.2	0.4	0.5	0.8	1.0
Pyrex bottle	23	0.2	0.3	0.3	0.4	0.5
	95	0.2	0.4	0.4	0.5	0.6
Hard-rubber bottle	23	0.2	0.2	0.2	0.2	0.2
	95	0.2	0.2	0.2	0.2	0.2
Resin-lined can	23	0.2	0.2	0.2	0.2	0.2
	95	0.2	0.2	0.2	0.2	0.2

Table II. Mineral Analysis of Samples Used in Table I

	Raw or Feed Water	Boiler Water	Condensate
Turbidity as SiO_2, p. p. m.	3.6	...	0.0
Color, units	0	200	0
Hardness as $CaCO_3$, p. p. m.	34	0	0
Sulfate as SO_4, p. p. m.	8	224	0.8
Chloride as Cl, p. p. m.	5.5	160	0.0
Total iron as Fe, p. p. m.	0.0	0.0	0.0
Phenolphthalein alkalinity as $CaCO_3$, p. p. m.	0	408	0
Methyl orange alkalinity as $CaCO_3$, p. p. m.	30	628	6
pH value	7.0	11.2	6.2
Silica as SiO_2, p. p. m.	2.0	32.4	0.2

While it has been definitely recognized that silica is picked up from glass bottles, the great majority of water samples are obtained in glass, on the assumption that the increase in silica content will not be of importance. This investigation was conducted partially to determine if such an assumption is justified in the case of samples obtained from the various points in a power plant's cycle.

To avoid the error introduced by glass bottles, tin and tin-lined containers have been suggested (*6*) but have not been found fully satisfactory (*5*, *6*). Schwartz (*5*) has suggested the use of hard-rubber bottles after a short period of aging, describing experience wherein tin-lined containers reacted with alkaline boiler waters to dissolve sufficient tin to interfere in the colorimetric determination of silica.

This study has been primarily directed to any change in the silica content of water samples over a 2-week period, which will cover the time usually elapsing between the collection and analysis of water samples. The samples of water were added to the containers both hot and cold to simulate conditions in practice. The glass containers used to obtain the data illustrated in the tables had, in each case, been "aged" by contact with water. Unless this precaution is taken with new bottles, a large increase in silica may result on first use of a container.

Table I illustrates the change in the silica content when four different types of containers are used with a sample typical of a raw water or feed water, both hot and cold (23° and 95° C.). Maximum increase of 1.0 p. p. m. of silica took place when the sample was added hot to the soda glass bottle. Although this increase was only 1.0 p. p. m. at the end of 14 days, it represents a 50 per cent increase over the original silica content of the water sample.

In similar tests on samples representative of boiler water, in 14 days the samples added hot to the soda glass and Pyrex bottles had increased 6.2 and 3.3 p. p. m. of silica as SiO_2, respectively.

Under similar conditions, using samples typical of condensed steam or condensate, the greatest pickup in silica was shown by the sample added hot to the soda glass bottle. This maximum increase is from 0.2 to 1.0 p. p. m., an increase of only 0.8 p. p. m. of silica as SiO_2, but a silica increase of 400 per cent compared with the original value. Where total solids are determined on a sample of condensed steam, storage of the condensed steam sample in glass may contribute to the sample a solids content several times in excess of the original value.

The silica values shown in Table I are the average of several tests. The original analysis of each of the three samples is shown by Table II. Silica was determined by the photoelectric method developed by Kahler (*4*). The accuracy of this method in the range of silica shown by Table I for raw or feed water and condensate is 0.1 p. p. m. of silica as SiO_2. With boiler water the accuracy is approximately 0.3 to 0.6 p. p. m. of silica as SiO_2. Any pickup of tin from the container will not affect silica determination by this procedure.

Within limits of analytical methods employed, no change was noted in the silica content of samples stored in hard-rubber bottles or in resin-lined cans.

Alkaline boiler water results in a greater increase in the silica content of the sample. The possibility of acidifying the boiler sample to decrease silica picked up from glass could be considered. However, such a procedure would alter the characteristics of the water sample, and it would be necessary to secure an additional sample, not acidified, to obtain a complete analysis. If containers which will not affect the silica content are employed, a complete mineral analysis can be made on the same sample.

In order to check on any change in the silica content of water samples shipped in ordinary tin-lined containers, several tests were made using 5-gallon tin-lined gasoline cans. Table III illustrates the results obtained with a boiler feed water and a boiler water. In each case, corrosion of the can was observed, with increase in the iron content of the sample. Corrosion of the can resulted in the formation of ferric hydroxide, and since this precipitate possesses the property of absorbing silica, a reduction of silica is shown by the tests in Table III. Table IV illustrates the original analysis of the feed water and boiler samples used in these tests. It is evident from these results that the use of tin-lined containers should be avoided, not only because tin interferes with some colorimetric silica determinations but also because removal of the protective tin coating permits corrosion of the iron and subsequent removal of silica from solution.

Table III. Test on Ordinary Tin-Lined Containers

	Original Analysis	3rd Day	5th Day	7th Day
Sample Typical of Feed Water				
Total iron as Fe, p. p. m.	1.0	10.0	20.0	26.0
Silica as SiO_2, p. p. m.	21.6	21.3	18.6	18.2
Turbidity as SiO_2, p. p. m.	9.4	132	260	272
Sample Typical of Boiler Water				
Total iron as Fe, p. p. m.	0.1	5.0	8.0	16.0
Silica as SiO_2, p. p. m.	20.5	20.3	20.0	19.0
Turbidity as SiO_2, p. p. m.	13.8	66	164	200

Table IV. Mineral Analysis of Samples Used in Table III

	Feed Water	Boiler
Turbidity as SiO_2, p. p. m.	9.4	13.8
Color, units	40	30
Hardness as $CaCO_3$, p. p. m.	46	0
Sulfate as SO_4, p. p. m.	56	448
Chloride as Cl, p. p. m.	5.5	276
Total iron as Fe, p. p. m.	1.0	0.1
Phenolphthalein alkalinity as $CaCO_3$, p. p. m.	0	304
Methyl orange alkalinity as $CaCO_3$, p. p. m.	38	436
pH value	6.9	10.7
Silica as SiO_2, p. p. m.	21.6	20.5

In order to obtain data on the effect of long storage periods with resin-lined cans several samples were placed in resin-lined containers, and were withdrawn for analysis after one month and after 13 months (Table V). With the exception of the resin-lined can which was filled with 0.02 *N* sulfuric acid, no change in iron content was noted after one month of storage. The can containing an alkaline boiler water showed an increase in the iron content from 0.2 to 2.0 p. p. m. at the end of 13 months and some drop in silica content. It would appear from these tests that resin-lined cans may safely be employed for storage of samples for a considerable length of time, provided no free mineral acid is present.

Some additional tests have been made on routine water sample shipments to the laboratory. In the case of two water samples, each was submitted in a 1-quart resin-lined can and in a 5-gallon unlined steel drum. In one water supply the resin-lined can showed a silica result of 8.6 p. p. m. as SiO_2 compared with a reduced silica content of the sample in the drum of 3.3 p. p. m. In

Table V. Storage Tests with Resin-Lined Cans

Type of Sample	Silica as SiO_2	Total Iron as Fe	Date of Analysis
	P. p. m.	*P. p. m.*	
Distilled water	0.0	0.0	5/3/40
	0.0	0.0	6/3/40
	0.0	0.1	6/3/41
Distilled water saturated with carbon dioxide	0.0	0.0	5/3/40
	0.0	0.0	6/3/40
	0.0	0.1	6/3/41
Sulfuric acid, 0.02 *N*	..	0.0	5/2/40
	..	1.0	6/3/40
	..	15.0	6/3/41
Alkaline boiler water added to can at 23° C.	29.7	0.2	5/3/40
	29.5	0.2	6/3/40
	28.7	2.0	6/3/41
Philadelphia tap water (Delaware supply) added to can at 95° C.	4.0	0.1	5/3/40
	4.1	0.1	6/3/40
	4.0	0.1	6/3/41

the other case, the resin-lined cans showed a silica result of 5.6 against 2.3 p. p. m. in the sample shipped in the drum. Considerable corrosion of the steel drums was evidenced, together with precipitation of iron. Samples were received in the laboratory 3 days after they had been taken and the analyses were completed on the fourth day after sampling.

In another case, a sample of water was forwarded to the laboratory in two containers, one ordinary soda glass and the other a resin-lined can. The sample secured in glass showed a silica content of 15.5 p. p. m. as SiO_2, whereas the sample in the resin-lined can showed silica as 7.2 p. p. m. Samples were secured January 17, 1941, and analyzed February 6.

The resin-lined can employed was a tin plate container, carrying two interior coats of organic protective coatings. The undercoat is a high baked cured modified alkyd resin varnish. The top coat is a baked vinyl chloride–vinyl acetate copolymer spirit varnish. This container is the standard beer package manufactured by the American Can Company.

Conclusions

In making recommendations for the removal of silica from water, designing feed water conditioning systems, setting silica limits for a boiler water, and determining the solids content of condensed steam samples, it is not safe to assume that the increase in silica from a glass container will be negligible. It is essential to secure water samples in containers that will not impart silica to the sample and to use containers that will not rust and so reduce the silica content of the water sample.

Hard-rubber bottles and resin-lined cans provide excellent containers for sampling and storage of water samples. In the handling of large numbers of water samples for silica determinations, the authors have found resin-lined cans economical and convenient.

Summary

Appreciable increase in the silica content of water samples may result from the use of glass bottles for sampling and for storage. This increase in silica is more marked with alkaline water, such as boiler water, than with raw water, feed water, and condensate. The increase in silica is also greater if the sample is placed in a bottle hot than if it is first cooled to room temperature. Silica pickup from Pyrex containers has been noted, although not to the extent experienced with ordinary soda glass.

The use of steel drums or tin-lined cans results in a corrosive action on the metal, precipitation of iron, and the lowering of the silica content of the water sample. The use of hard-rubber bottles or resin-lined cans prevents any change in the silica content of the water within limits of analytical error.

Acknowledgment

The authors wish to express their appreciation to W. H. & L. D. Betz, in whose laboratories this investigation was conducted. They are also indebted to the American Can Company for the resin-lined containers used in the various tests.

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Presented before the Division of Water, Sewage, and Sanitation Chemistry at the 102nd Meeting of the American Chemical Society, Atlantic City, N. J.

Fluorescein As an Indicator in Bromometric Titrations

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THE use of methyl orange or methyl red in bromometric titrations involves the inconvenience that the indicator may be destroyed by a local or temporary excess of free bromine before the equivalence point is reached.

Fluorescein, a highly sensitive reagent for bromate in the presence of bromide (*1*), permits one to observe the approach and the passing of the end point. The indicator solution may be prepared by dissolving 0.1 gram of fluorescein in 100 ml. of water containing some drops of sodium hydroxide. One drop of indicator is added to each 10 ml. of titrated solution.

In a bromometric titration of arsenious acid (strongly acidified with hydrochloric or sulfuric acid) the greenish yellow of the solution changes to a brownish yellow when the end point is approached. The change to a reddish brown indicates the end point. As the fluorescein reacts only slowly with bromine, it is necessary, near the end point, to wait some 15 seconds after each addition of reagent. Heating the solution to 40° to 50° C. will accelerate the reaction.

A permanganate solution (approximately 0.1 *N*) and a bromate solution (approximately 0.05 *N* plus 20 grams of potassium bromide per liter) were compared iodometrically. Then the arsenite solution was titrated with permanganate, using a little iodide as a catalyst, and with bromate solution. The results were:

1 ml. of $KMnO_4$ = 1.855 ml. of $KBrO_3$ (iodometrically)
= 1.853 ml. of $KBrO_3$ (with fluorescein)

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A Photoelectric Photometer for Rapid Grading of Naval Stores Products

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A photoelectric color grader, especially suited to naval stores plant laboratories, is described. Its design is such that a single meter reading indicates the ratio of the transmissions of a sample for light beams of two colors. By proper choice of variables, any class in a wide range of transparent materials may be graded rapidly and without the necessity of avoiding dirt, haze, or surface imperfections in the samples.

A LARGE number of photoelectric instruments have been described in the literature in which transmission or reflection factors for light of two or more colors are measured (*1*, *9*, *10*, *11*). For certain types of colorimetric work, such as color grading, quantities involving ratios of such factors are required. These ratios must be calculated from the data furnished by instruments of the foregoing type.

In general, these instruments will also measure quantities proportional to the ratios of transmission or reflection factors of two different materials for light of the same color. Another instrument (*12*) measures quantities proportional to the differences between the transmission or reflection factors of materials for light of two different colors.

None of the photoelectric colorimeters investigated by the author has been suitable for the rapid routine color grading which is done in naval stores plants. Heretofore, the method of visual comparison with artificial standards has been used. Recognizing the limitations and inaccuracies inherent in such a procedure, means were sought whereby quantities proportional to the ratios of transmission factors of materials for light of two different colors could be obtained by means of single meter or dial readings from an instrument of the photoelectric type. Furthermore, the optical, mechanical, and electrical characteristics of the design which was finally chosen were so arranged that the readings were proportional to either the x, y, or z trichromatic coefficients (*6*) of the samples being graded, depending on the combination of characteristics chosen for that particular series of measurements.

The justification for this procedure is based on the observation made in this laboratory that the grades of samples having more or less uniform clarity and color characteristics can be based on one of their trichromatic coefficients. The choice of a coefficient for a particular type of sample depends on the position on the I. C. I. color mixture diagram (*6*) of the locus of points representing the complete range of colors of samples of that type. For example, Brice (*2*) has shown that the chromaticity differences for rosin colors are well represented by differences in x trichromatic coefficients. Accordingly, the official U. S. rosin standards have been spaced systematically on a scale of x trichromatic coefficients.

Theory of Instrument

Figure 1 is a schematic diagram showing the essential parts of a typical instrument designed for measuring the color of naval stores products. If a sample be placed in the space between the filter holder and the barrier layer photocell, using a filter having characteristics such that the response of the photocell is proportional to the sum of the X, Y, and Z tristimulus values of the sample, the indicating instrument (galvanometer or microammeter) may be set at some predetermined value by adjusting the iris diaphragm. Then, without changing this adjustment, a filter having characteristics such that the response of the photocell is proportional to the X tristimulus value alone is inserted in place of the first filter. The indicating instrument will then give a reading which will be proportional to the ratio of the transmission of the sample for the X beam to its transmission for the $X + Y + Z$ beam. This ratio, and hence the latter reading, will be proportional to the x trichromatic coefficient of the sample, as may be shown by the following analysis:

Let i_1 = photocell current output with the sample and the $X + Y + Z$ filter in position
i_2 = photocell output with the sample and the X filter in position
E = relative spectral energy of the lamp
s = relative spectral response of the photocell to a source having an equal energy spectrum
T_{X+Y+Z} = transmission of $X + Y + Z$ filter
T_X = transmission of X filter
T = transmission of sample
λ = wave length
k = a constant

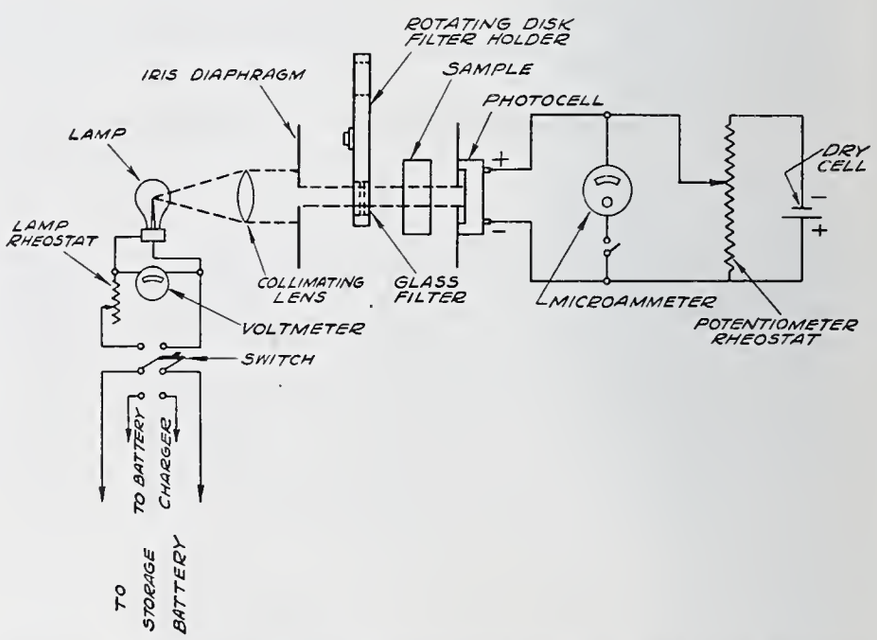

FIGURE 1. SCHEMATIC DIAGRAM OF PHOTOELECTRIC GRADER FOR TRANSPARENT AND TRANSLUCENT SAMPLES

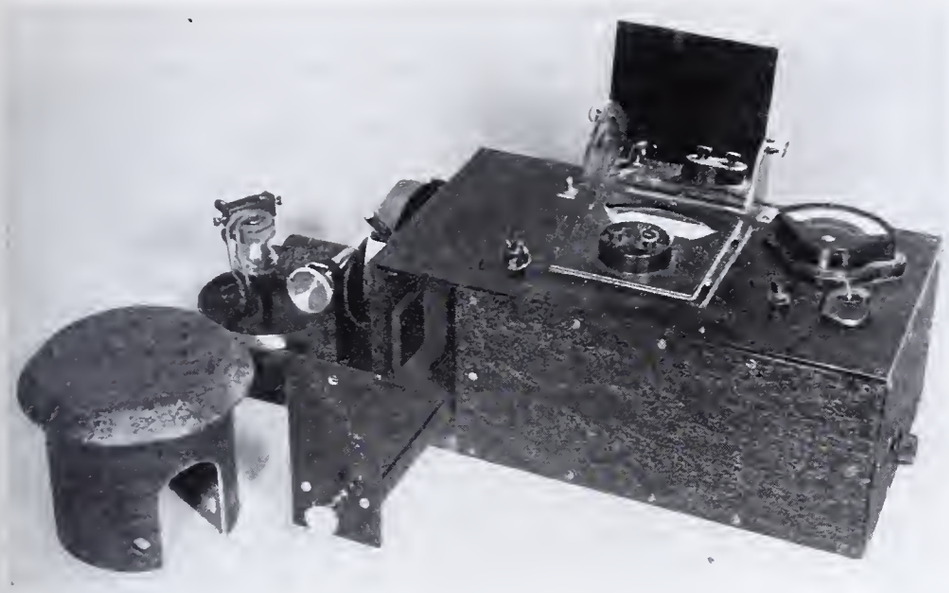

FIGURE 2. PHOTOELECTRIC COLOR GRADER

For low light intensities and low external resistance in the photocell circuit, the photocell current is known to be practically a linear function of illumination (*8*)—that is,

$$i_1 = k \int_0^\infty Es\ T_{X+Y+Z}\ T\ d\lambda \qquad (1)$$

$$i_2 = k \int_0^\infty Es\ T_X\ T\ d\lambda \qquad (2)$$

The integral in Equation 2 is equal to a constant times the X tristimulus value of the sample (*5*), while the integral of Equation 1 is equal to another constant times the sum of the X, Y, and Z tristimulus values of the sample. Dividing Equation 2 by Equation 1:

$$\frac{i_2}{i_1} = \frac{\int_0^\infty Es\ T_X\ T\ d\lambda}{\int_0^\infty Es\ T_{X+Y+Z}\ T\ d\lambda} = \frac{c_1 X}{c_2 (X + Y + Z)}$$

or

$$i_2 = \frac{i_1 c_1}{c_2} \frac{X}{X + Y + Z} = cx \qquad (3)$$

where

$c = \frac{i_1 c_1}{c_2}$ (i_1 is set at a constant value for each measurement)

and

$$x = \frac{X}{X + Y + Z} \quad \text{(see 6)}$$

A similar analysis applies to the measurement of y and z trichromatic coefficients.

By varying the constants of the circuit shown schematically in Figure 1, the scale of values can be expanded beyond a point which would be possible if the microammeter were connected directly to the photocell. In this circuit, a "bucking" potential is applied to the photocell by means of the voltage drop across a portion of a potentiometer circuit through which current from a 1.5-volt dry cell is flowing. In effect, a depressed zero is achieved by electrical means, and the scale may be magnified as much as desired within practicable limits.

Construction of Instrument

Figure 2 is a photograph of one of the instruments which was built in accordance with the foregoing principles. Three of these instruments have been in more or less continuous operation for more than a year, and have proved themselves to be well suited for the grading of naval stores products.

An 8-volt storage battery supplies current for a 50-candle power automobile spotlight lamp. A series rheostat controls the lamp voltage, which is maintained at 6 volts while the instrument is in operation. The lamp is situated at the focal point of a lens, from which a collimated beam emerges. After passing through an iris diaphragm, the aperture of which may be varied by means of a knob and worm and pinion gears, the narrowed beam is filtered. It is then transmitted by the sample and strikes the sensitive surface of a No. 732 Electrocell photocell mounted in a hermetically sealed case behind the sample compartment. The sample compartment is provided with a sliding sample drawer, so constructed that when the sample is properly placed in the drawer and pushed into the light beam, the compartment is tightly closed. A circular filter holder provided with a handle permits rapid changes from one filter to another. Space for eight filters has been allowed in anticipation of possible future requirements. The photocell current is indicated on a Rawson Type 507C microammeter, graduated with 100 divisions, and giving full-scale deflection at 30 microamperes. For the comfort of the operator an adjustable mirror is provided for reading the meter while the operator is in a sitting position.

A three-position selector switch permits the operator (1) to make an initial adjustment of the bucking voltage; (2) to make color measurements using the bucking voltage; and (3) to make measurements without the bucking voltage, or with the photocell connected directly to the meter. Once a day, the operator turns the selector switch to position 1. He then adjusts the rheostat on the right side-panel until the microammeter reads 100 or full scale. If measurements are to be made on pale and medium-colored samples, he then switches to position 2. The switch is turned to position 3 for the measurement of dark-colored samples. With the selector switch in this position, the color scale is a compressed version of the scale obtained with the selector switch in position 2.

In order to avoid the effect of variables such as aging of the lamp, dust on the filters, possible small changes in the spectral characteristic of the photocell, etc., calibrations are prepared each day with the aid of a set of permanent glass standards closely approximating in appearance the color series which is to be measured. Brice (*2*) has described the construction of a typical set of such standards for rosin.

It will sometimes be found that, in order to grade accurately very light or very dark samples, filters other than those giving the trichromatic coefficients must be used—for example, red and blue filters have been used in this laboratory for the grading of pale yellow samples, while deep red and neutral filters have been used for the grading of dark red samples. The range of sample colors must be studied carefully before a choice of filters is made.

By means of such filter combinations, it is possible to grade almost colorless liquids such as pine oil, turpentine, etc., pale liquid and solid resins, and dark resins such as Vinsol and Belro. The color bodies in the latter resins first must be diluted by dissolving the resins in a suitable organic solvent or mixture of solvents.

At present, no specific rules can be laid down for the design of the foregoing special filter combinations, since their selection in this laboratory has been based on cut-and-try procedures. However, further study on this problem is being planned.

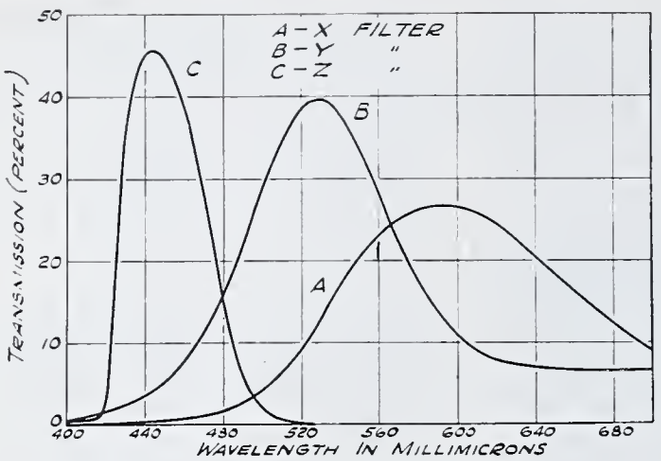

FIGURE 3. TRISTIMULUS FILTERS

A. *X* filter, 4.5-mm. Corning No. 330 + 3.0-mm. Corning No. 399
B. *Y* filter, 2.0-mm. Corning No. 450
C. *Z* filter, 1.0-mm. Corning No. 511 + 1.5-mm. Corning No. 038

Design of Filters

In Figure 3, *A*, *B*, and *C*, respectively, are the spectral transmission curves of the amber, green, and blue filters used in the instrument in conjunction with a lamp operated at a color temperature in the neighborhood of 2800° K. and an Electrocell barrier layer photocell to give first approximations to *X*, *Y*, and *Z* responses referred to Illuminant C (*6*). A Corning Aklo filter is being used in conjunction with the foregoing lamp-photocell combination to give a first approximation to the $X + Y + Z$ response. The spectral transmission curve of this filter is shown in Figure 4. For the purpose of grading, these approximations to the ideal filters have proved satisfactory. However, the need for an instrument which will evaluate the trichromatic coefficients more accurately has led to a consideration of the design of filters which will more closely approximate the ideals. The requirements for the spectral characteristics of source-filter-photocell combinations which will give *X*, *Y*, and *Z* tristimulus values have been listed by the National Bureau of Standards (*5*). Gage (*4*) has described a method by which glass filters may be selected with respect to kind and thickness to fit such requirements.

In order to evaluate x trichromatic coefficients accurately, the blue component of the *X* function must be taken into account in the filter design. Hunter (*7*) overcomes this difficulty in the measurement of *X* tristimulus values by adding a fraction of the reading obtained with his *Z* filter to that obtained with his *X* filter. In order to avoid this complication, a combination of glasses having the proper relative transmission in the blue as well as in the other parts of the spectrum is being sought. Calculations for this filter as well as for new *Y*, *Z*, and $X + Y + Z$ filters are now under way, and will be reported in a separate communication.

Operation of Instrument

The ultimate criterion for the success of an instrument designed to replace visual methods of grading must be its closeness of agreement with visual observations. Accordingly, a number of representative samples of both wood and gum rosins were graded independently by eighteen more or less experienced observers. Comparisons were made visually with the official U. S. rosin standards. The observers were asked to estimate to the nearest one-tenth grade. Although this was recognized as being a difficult task, results reported by the different observers were in surprisingly good agreement. The averages of the grades assigned by the eighteen observers to two samples of gum rosin and seven of wood rosin are given in Table I, together with the photoelectric instrument readings on the same samples. The photoelectric instrument had been calibrated previously by noting the readings obtained for the official U. S. rosin standards. In practice, such a calibration is made once each day, although it has been found that only very small changes take place over periods of several days. Progressive changes do occur, however, as the color temperature of the lamp changes with age.

In the visual grading of gum rosin the factor of brightness as well as that of chromaticity must be considered. However, the photoelectric instrument grades on the basis of chromaticity alone, so that if decreases in brightness due to dirt become large, grading errors will occur if the photoelectric method is used. No difficulty is encountered in the correlation of photoelectric with visual grading of either wood rosin or reasonably clean gum rosin.

The average time for a single photoelectric grading is approximately 0.5 minute. This compares favorably with the time required by the most experienced visual graders. The chief advantage of the photoelectric method is the elimination of discrepancies caused by eye fatigue, inaccurate grading by partially color-blind individuals, errors of judgment, and the like.

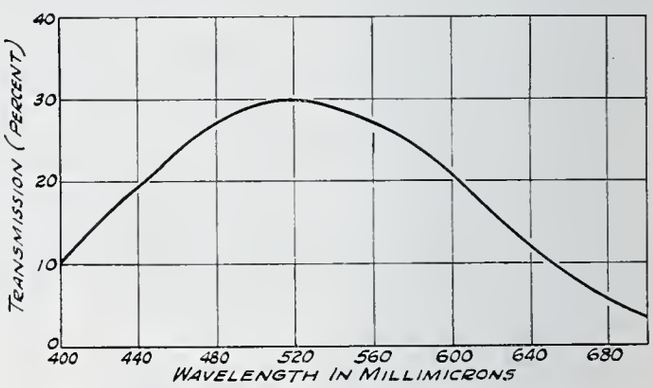

FIGURE 4. $X+Y+Z$ FILTER, 2.0-MM. CORNING NO. 398

Within limits, varying amounts of dirt or haze in gum rosins, smeared cover glasses, cracked samples, etc., introduce little or no error in the grades obtained with the instrument. Cut or poured cubes can be measured equally well. Of course, care must be taken that all samples of a given class are of the same thickness. In the case of rosins and resins, 0.875 inch is standard, while in the case of pale liquid products, 5 cm. is commonly used.

Other Uses of the Instrument

By employing "monochromatic" filters (*3*), the photoelectric grader has been used as a chemical colorimeter for the

TABLE I. COMPARISON OF VISUAL AND INSTRUMENT GRADING OF ROSIN

Sample	Instrument Grades[a]	Visual Grades
Gum rosin	I + 0.7	I + 0.5
Gum rosin	M + 0.6	M + 0.5
Wood rosin	G + 0.5	G + 0.5
Wood rosin	I + 0.0	I + 0.0
Wood rosin	M + 0.6	M + 0.6
Wood rosin	I + 0.9	I + 0.7
Wood rosin	K + 0.8	K + 0.7
Wood rosin	M + 0.1	M + 0.0
Wood rosin	H + 0.3	H + 0.3

[a] I + 0.7 means that the rosin is 0.7 grade lighter than I.

determination of small amounts of both organic and inorganic substances. In this respect, the methods employed do not, in general, differ from those described in the voluminous literature on the subject.

The tristimulus values X, Y, and Z of a sample may be measured by setting the selector switch to a position which connects the photocell directly to the meter; then, with one of the tristimulus filters in position, but with nothing else in the light beam, the iris diaphragm is adjusted until the meter reads 100 (full scale). The sample is then inserted in the beam, and the resultant meter reading is referred to a chart which gives the corresponding tristimulus value. The Y tristimulus value (brightness) is very often specified with the x and y trichromatic coefficients to describe completely the color of a transparent sample.

The instrument has also been used for the determination of haze. For this measurement, the brightness of the sample (Y tristimulus value) is compared to the known maximum brightness of a clear sample having the same chromaticity as the unknown.

Several of these instruments have been constructed in the company's shops. At the present time others are being manufactured for their use by a commercial instrument maker.

Summary

A photoelectric instrument suitable for the rapid routine color grading of transparent liquid or solid naval stores products is described. Essentially, the apparatus consists of a light source, colored glass filters, and a photocell. The design, however, is characterized by the fact that single readings obtained with the instrument are proportional to the ratio of the transmissions of a sample for light beams of two different colors. The electrical circuit is arranged so that the instrument scale may be expanded or contracted. In this manner, both light and dark colored samples may be graded. Within limits, the grades obtained with the instrument are independent of dirt, haze, or imperfections in the sample.

In addition to its use as a grader, the instrument has been used as a chemical colorimeter, to obtain tristimulus values of transparent samples, and to determine haze.

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A Simple Hydrogen Sulfide Generator

BRYANT W. POCOCK, L. SCHOLTEN, AND PAUL J. ERICKSON

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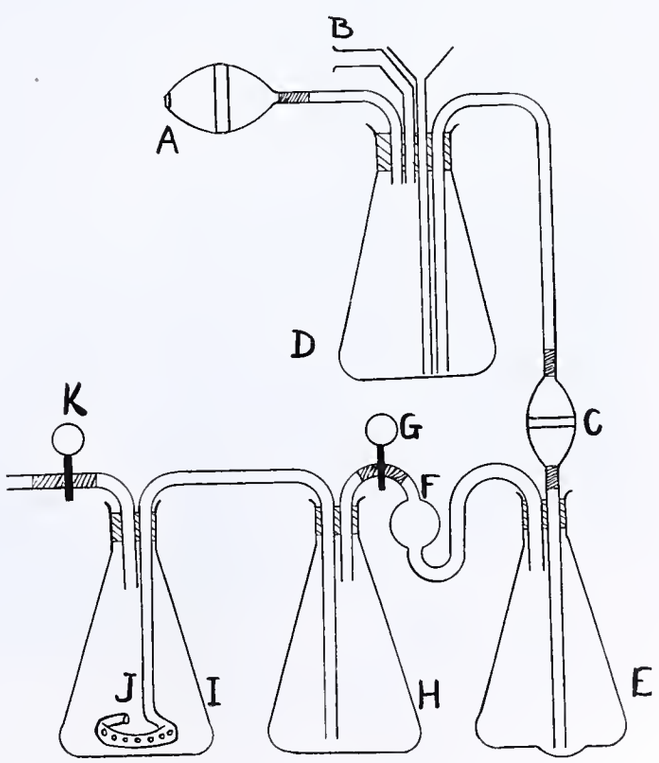
A HYDROGEN sulfide generator is a necessity in any chemical laboratory where the analysis of metals is to play an important role, particularly in these days of increasing demands for metal analysis and of correspondingly growing numbers of new control laboratories, which are springing up all over the United States.

The apparatus described is of a type which any chemist can probably construct from supplies already at hand. The parts are held together by clamps and support stands.

The most novel feature of the device is the delivery tube, J, which consists of a celluloid drinking straw purchased at a dime store and bent to form a circle at one end. The extreme end of the circle is crimped nearly together and the circle is bent to form a plane at right angles to the shaft. Small holes are bored into the top of the circle a few millimeters apart. The gas is delivered through these holes, thus increasing the area of contact between the gas and the liquid. (This is a modification of the Abson method for the recovery of asphalts from road mixes.)

The generating flask, E, is bumped outward somewhat at the bottom, to form a small interior concavity in which the acid will collect, to facilitate complete elimination of the acid by gas pressure. The reservoir, C, is a two-way rubber compression bulb, placed on a level with the bottom of the acid flask, D. Thus C will always hold enough acid to maintain a positive siphoning action.

H and F are traps, K and G are pinch clamps, and I is the precipitation flask. Ferrous sulfide sticks are placed above a layer of glass wool in E, and hydrochloric acid is poured into D through the funnel. To start the action initially, the finger is held at the safety tube, B, and acid is pumped into C and E by the rubber compression bulb, A.



High-Speed Rotational Viscometer of Wide Range

Confirmation of the Reiner Equation of Flow

HENRY GREEN
Interchemical Corporation, New York, N. Y.

IN A recent article by the writer on the tack of printing inks, (*5*) mention was made of the use of a high-speed rotational viscometer for measuring pseudoplastic materials and thixotropic plastics. Such an instrument is described in this paper, with data showing the application of the Reiner equation for determining yield value and plastic viscosity, a term employed here for the reciprocal of mobility.

The great importance of Reiner's work is not generally realized by industrial rheologists. Even Reiner has not published data tending to establish the validity of his own equation. This is regrettable, for it has caused this particular rheological contribution to remain practically unknown to industry since its initial publication in 1929.

In order to comprehend fully the advantages inherent in the rotational viscometer for measuring many types of industrial products, it is necessary to understand the nature of the rheological background immediately preceding Reiner's work.

The first important contribution to modern rheology was that of Bingham (*1*), who worked with a rather difficult medium, clay-water suspensions. Such materials have a tendency to separate, causing seepage and slippage. Bingham used a capillary-tube type of viscometer and produced flow curves by plotting force against rate of flow. So far as he could tell, he seemed to obtain straight-line relationships. The fact that the lower points of his curves fell off toward the origin was attributed to possible seepage and slippage, and it was also assumed that all curves for a given material intersected the force axis at the same point, regardless of the dimensions of the capillary used. With these ideas in mind Bingham felt justified in writing the equation of flow for plastics in capillary tubes as follows:

$$\text{Mobility} = \frac{8L\ V/t}{\pi R^4\ (F - f)} \qquad (1)$$

where R and L are the radius and length of the capillary, V/t is the volume of flow per second, F is the force causing the flow, and f is the intercept on the pressure axis. This is simply the well-known equation of liquid flow with the intercept, f, introduced. Obviously, it can fit only a linear flow curve.

Later, Bingham and Green (*2*) working with more tractable materials, pigments suspended in oil, showed that f was not independent of R and L. The lower points, however, still fell off toward the origin and this condition was still believed to be caused by seepage and slippage. Subsequent careful analysis showed that the pigment-vehicle ratio was exactly the same for the low points on the curve as for the high ones, proving that seepage could not be responsible for the condition at the lower end of the curve. This led to the micro-

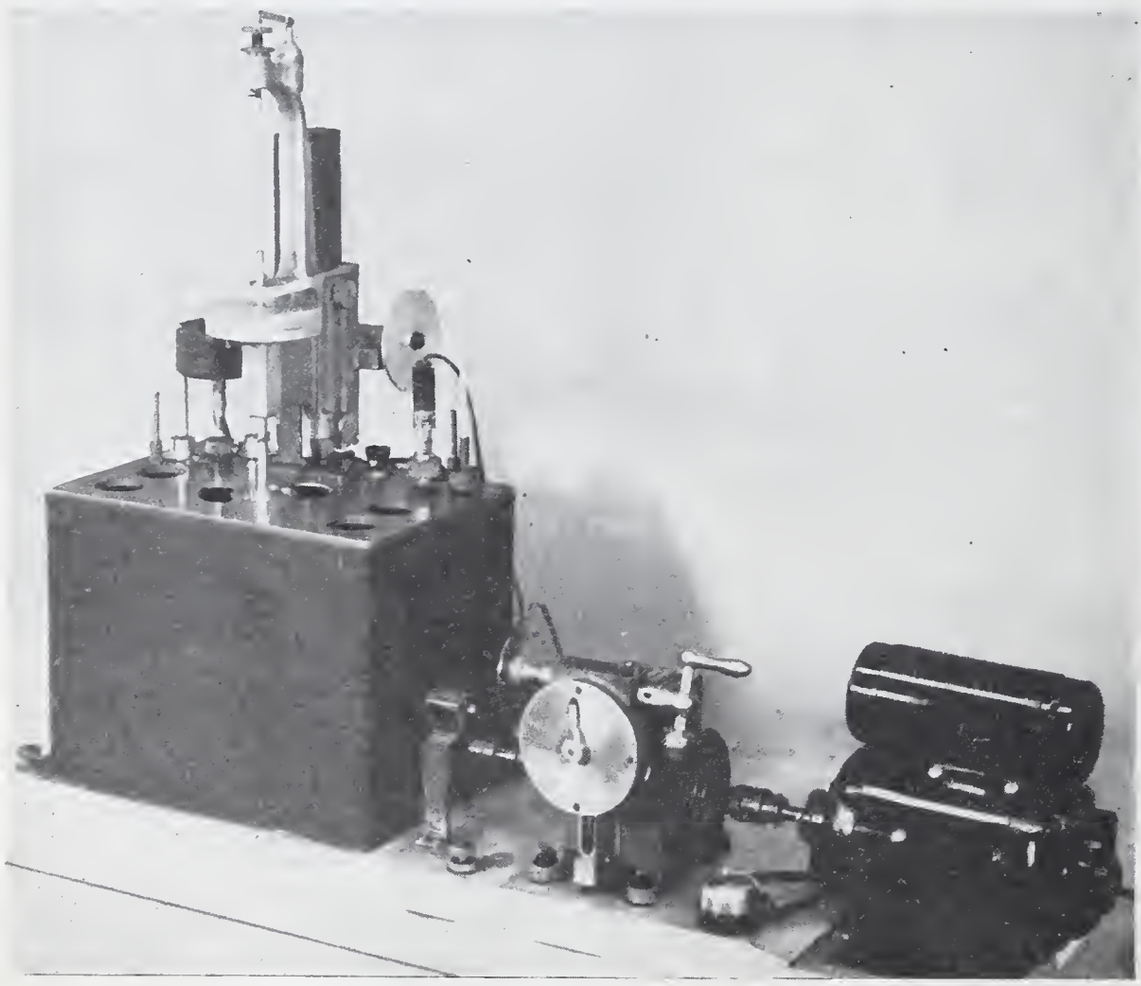


FIGURE 1. ROTATIONAL VISCOMETER

scopical examination of plastic flow in capillary tubes (*7*, *8*), which established the existence of plug flow and slippage.

Plug flow occurs whenever the force of flocculation is strong enough to hold the body of pigment together, a condition that exists at the lower end of the curve where the applied force is insufficient to cause telescopic shearing. As the force is raised, however, shearing commences, starting at the capillary wall and developing inward as the force is increased. This means that the model of flow is changing continuously until a state of complete laminar flow ensues; and it was believed that this state was attained at a finite distance from the origin.

Plug flow was also seen to be a contributory cause of slippage. In the case of pigment-oil suspensions the plug at the wall was always observed to be lubricated with a thin layer of the oil vehicle when examined microscopically. Since such a vehicle is a Newtonian liquid, it will flow under any pressure, no matter how small; hence the slightest applied force induced the lubricated plug to flow—i. e., to slip en masse. Obviously, if a small force causes slippage, larger forces cause more slippage, and the entire plastic flow curve becomes misleading by having imposed upon it a slippage flow curve (Figure 5).

Realizing that plug flow and slippage must modify the Bingham equation, Buckingham (*3*) in 1921 deduced the following equation as a substitute:

$$V/t = \frac{\pi R^4}{8LU}\left(F - \frac{4f}{3} + \frac{f^4}{3F^3}\right) + \frac{\pi R^3 e\phi F}{2L} \qquad (2)$$

where U, used throughout this paper as the reciprocal of mobility, is the plastic viscosity of the material, ϕ is the fluidity of the vehicle, and e is the thickness of the lubricating layers. The last term of Equation 2 gives the volume of flow per second caused entirely by slippage. [In the author's previous papers on tack (*5*, *6*) the symbol μ is used to designate plastic viscosity.]

Buckingham's equation, though resembling the experimentally determined flow curve, failed to coincide with it at its most important point—the place where the initial shearing of the plug occurs, which unfortunately, cannot be found by inspecting the experimental curve. It is necessary to know the location of this point in order to calculate yield value. The attempt to derive the yield value from the equation of the curve proved to be impracticable. Consequently, while Buckingham's equation is of great theoretical importance, it has little useful laboratory value.

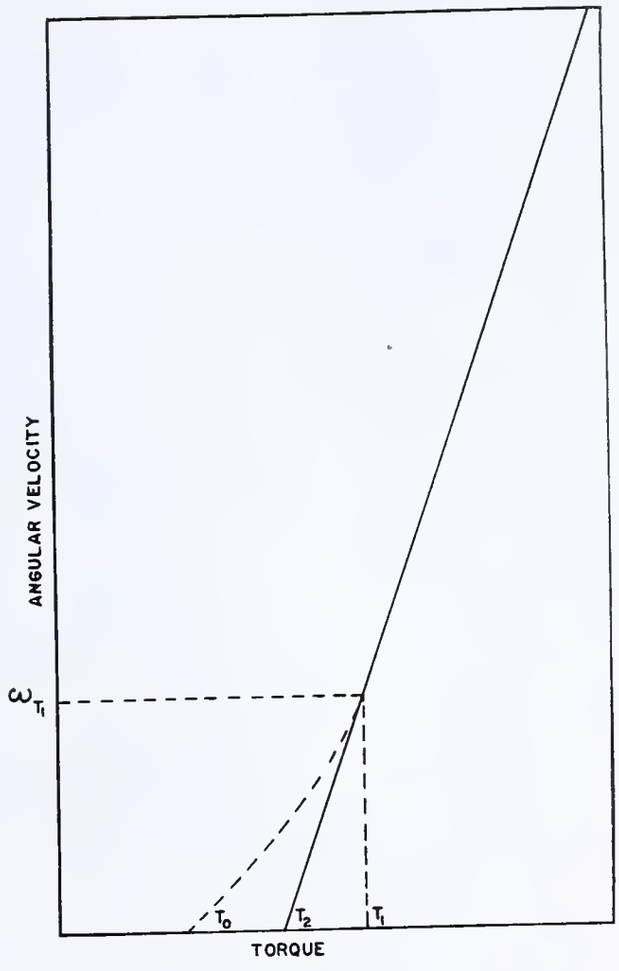


FIGURE 3

Buckingham's equation cannot be dismissed, however. Having based it on the Bingham assumption that yield value and mobility ($1/U$) remain constant during a change in rate of flow, Buckingham was able to show that the plastic flow curve could never be linear for capillary tubes and that no "straight" part of the curve exists except at the lower end, which is comprised entirely of slippage flow. This discovery definitely invalidated Bingham's equation for capillary tubes and left nothing useful in its place. Industrial rheology was in an unenviable position and remained so until Reiner in 1929 showed that the rotational viscometer gives flow curves from which yield value and plastic viscosity can easily be determined.

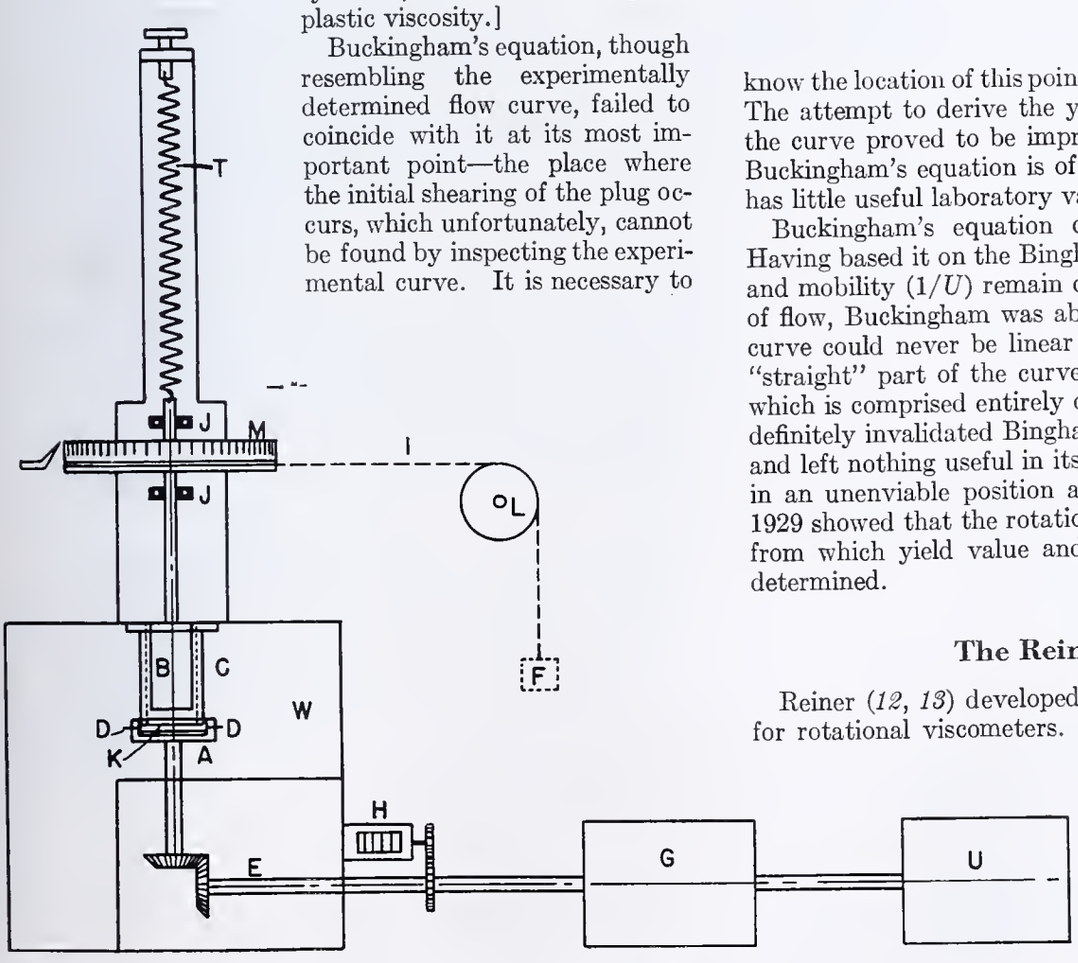


FIGURE 2. DIAGRAM OF VISCOMETER

The Reiner Equation

Reiner (*12*, *13*) developed the theoretical equation of flow for rotational viscometers. He used the Bingham assumption of the constancy of f and U and showed that under these circumstances the plastic flow curve in a rotational viscometer becomes linear at a finite distance from the origin. The curved portion exists only where the flow is not com-

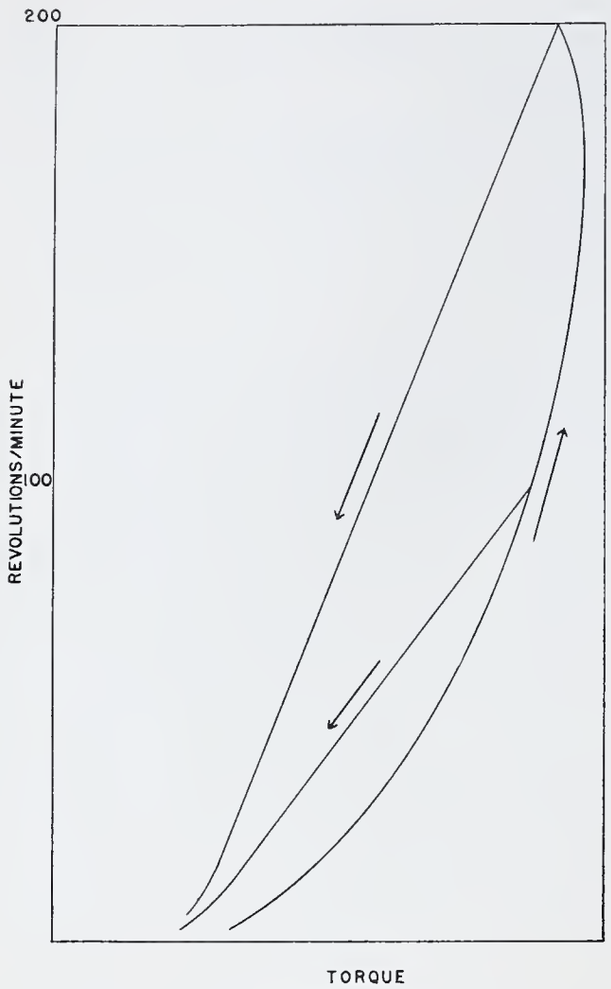


FIGURE 4

pletely laminar—i. e., in the transition period from total plug to complete laminar flow. The Reiner equation is

$$\Omega = (T/4\pi hU)(1/R_b^2 - 1/R_c^2) - (f/U) \ln (R_c/R_b) \qquad (3)$$

where

U = plastic viscosity
f = yield value
Ω = angular velocity
T = torque
R_b = radius of the bob
R_c = radius of the cup
h = height of the bob

(In the original Reiner equation R^4 occurs in place of the correct term, R^2, used throughout this paper.)

Reiner's equation can be rewritten as follows:

$$U = 9.55(T - T_2)\, S/\text{r. p. m.} \qquad (4)$$

and

$$f = T_2 C \qquad (5)$$

where T_2 = intercept on the torque axis
r. p. m. = revolutions per minute

$$S = (1/R_b^2 - 1/R_c^2)/4\pi h \qquad (6)$$

$$C = S/\ln (R_c/R_b) \qquad (7)$$

Equations 4 and 5 are of great practical value because they are applicable to the experimental data and easily solved, but they demand a viscometer so constructed that the necessary data can be obtained.

The Rotational Viscometer

The instrument described in this paper (Figure 1) is for the consistency range covered by such materials as oils, varnishes, paints, and printing inks. The object in designing the viscometer in the manner described here is (1) to obtain consistency curves, and (2) to eliminate certain detrimental features usually inherent in commercial rotational instruments. These faults are likely to be: either no means or very inadequate means for maintaining constant temperature control while the cup is rotating; no way of changing the speed of cup rotation quickly and noting immediately the new r. p. m. (the necessity for this is discussed in connection with thixotropy); insufficient strength in the torsion members (springs, wires, tapes), hence measurements at high speeds cannot be obtained with heavy materials without damaging these members; and no simple means for calibrating the torsion members without resorting to light and heavy standard oils, the viscosities of which are determined by capillary-tube methods. Light oils cannot be used for the heavy members. The serious mistake involved in using heavy oils for calibration will be shown in a subsequent paper by Ruth N. Weltmann.

The Viscometer

The essential parts of the viscometer are shown diagrammatically in Figure 2. They consist of a synchronous motor, *U;* a device, *G,* for quickly changing the speed of the shaft, *E;* a constant temperature bath, *W;* a rotatable cup, *C,* for holding the material being tested; a bob, *B,* suspended in the material; a torsion member *T,* from which *B* is suspended; and a scale, *M,* to record the degrees (arc) through which *B* is turned by the viscous or plastic drag imposed upon it.

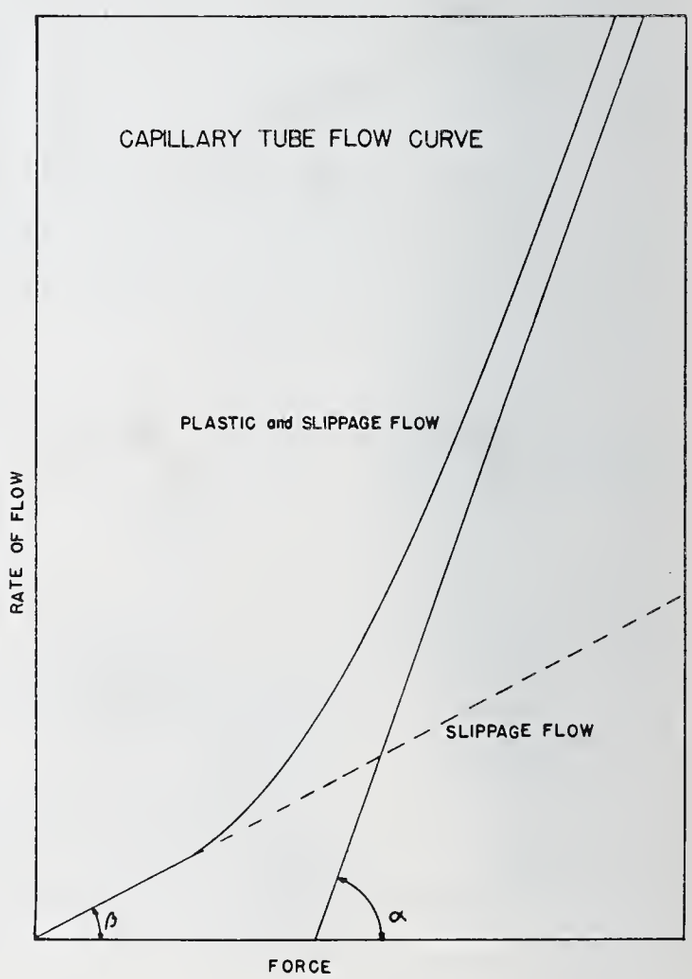


FIGURE 5

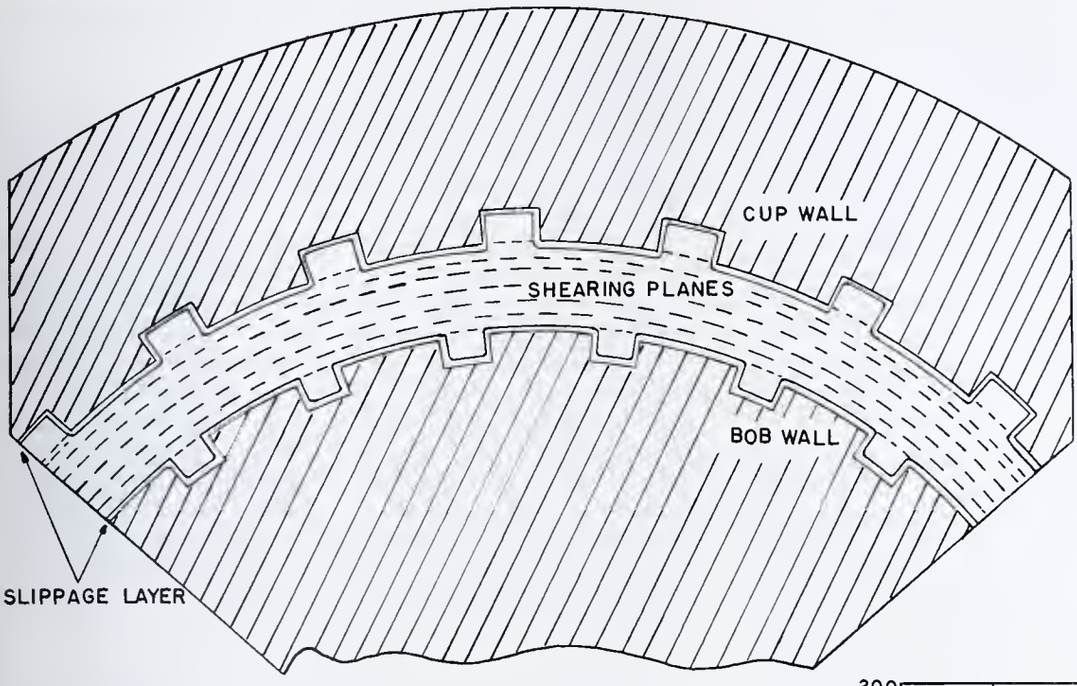


FIGURE 6. PLAN FOR REDUCING SLIPPAGE

For high-speed work on materials as heavy as lithographic inks, the viscometer must be substantially built. The motor, in the instrument shown in Figure 1, is 0.25 horsepower and can produce a maximum cup, C, speed of 400 r. p. m. The transmission, G, is made by the Briggs and Stratton Corporation and modified by changing somewhat the original speed-indicating dial, adding a fine-adjustment dial and a suitable handle. By turning the handle on the transmission, the cup speed can be continuously varied from 10 to 400 r. p. m., without stopping the rotation of the cup. This is essential in the measurement of thixotropic systems.

C has a removable bottom, K, for convenience in cleaning; this is an important time saver in plant control work. The cup is keyed onto the rotating platform, A, by means of two small pins, D, projecting from the bottom of the cup (Figure 2). By twisting the cup slightly in the direction of rotation, it is released and easily removed from the bath.

Reiner has shown that the force existing at any instant in the material between the cup and the bob is at a maximum at the wall of the bob and a minimum at the wall of the cup. Therefore, if the yield value is equal to or greater than the maximum existing force, the material will hold together as a whole—i. e., there will be no shearing within the plastic mass. If the yield value is less than the minimum force, shearing will be complete throughout and no mass or plug flow will occur.

Buckingham showed that in a capillary tube shearing is not complete until the shearing force reaches infinity. Reiner has shown that in a rotational viscometer shearing is complete when the torque at the wall of the cup attains a value of $T_1 = 2\pi R_c^2 hf$, and that shearing at the wall of the bob commences when the torque is equal to $T_0 = 2\pi R_b^2 hf$ (Figure 3). Torques greater than T_1 produce complete laminar flow and consequently give a linear curve. Torques lying between T_0 and T_1 give a mixed regime—i. e., partly plug and partly laminar flow—hence, a nonlinear curve ensues in this region, owing to a continuously changing model of flow.

It is the aspiration of all rheologists to produce a completely linear curve for plastics of the nonpseudoplastic type. It is now evident that this ideal can be attained theoretically when $T_0 = T_1$—in other words, when $R_b = R_c$. From a practical consideration this is impossible, but Reiner has shown that the extent of curvature can be reduced by decreasing the difference between the lengths of R_c and R_b.

The first point for consideration is the allowable magnitude of T_1. Since the useful part of the curve (for which Reiner Equation 3 applies) extends from T_1 upwards, it is evident that T_1 must be kept considerably below the strain limit of the torsion member. If the range of yield values to be covered is known, an approximate idea of R_c can be had from the equation $T_1 = 2\pi R_c^2 hf$. Unfortunately, the capillary-

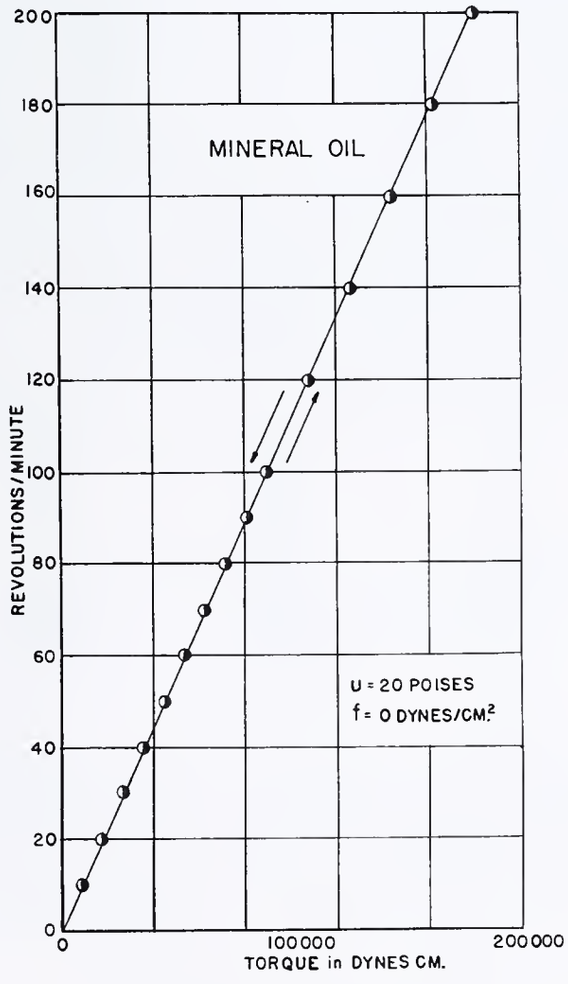


FIGURE 7. CURVE FOR NEWTONIAN LIQUID

Down Curve

Torque, T	$R.\ p.\ m.$	$T/r.\ p.\ m.$
181,500	200	907.5
161,980	180	899.9
143,960	160	899.7
125,980	140	899.9
107,840	120	898.7
89,860	100	898.6
80,880	90	898.7
71,890	80	898.6
62,900	70	898.6
53,920	60	898.7
44,930	50	898.6
35,950	40	898.7
26,960	30	898.7
17,970	20	898.5
8,990	10	899.0

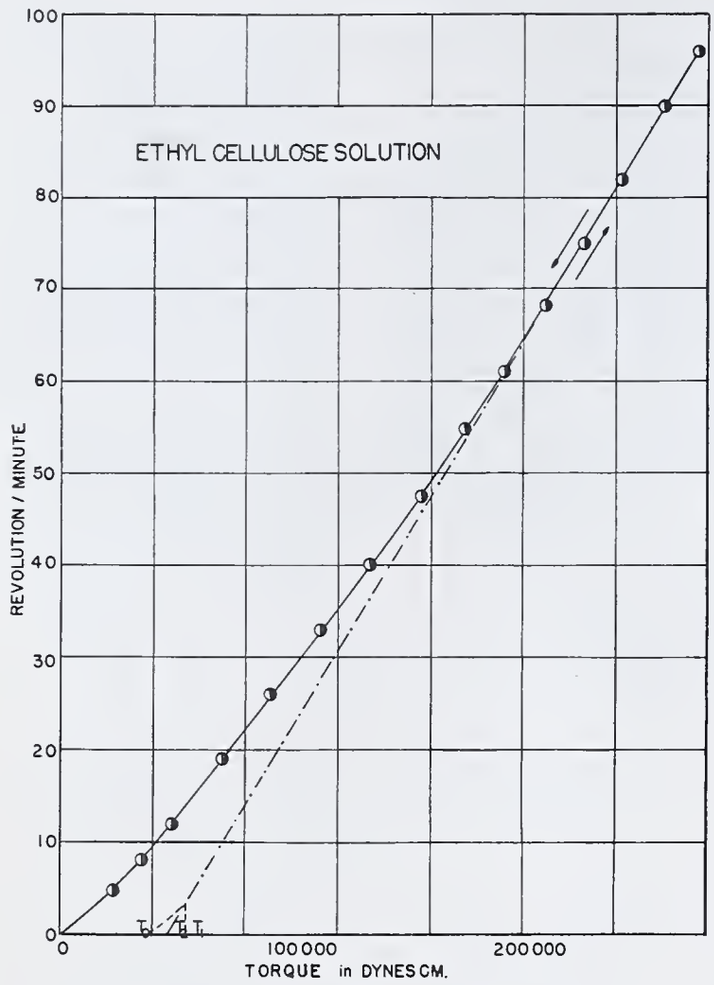


FIGURE 8. PSEUDOPLASTIC CURVE

R. p. m.	Up and Down Curve Torque, Up Curve	Torque, Down Curve
8	35,000	34,000
12	48,000	48,000
19	70,000	69,000
26	90,000	88,000
33	113,000	110,000
40	135,000	132,000
47	155,000	152,000
55	175,000	173,000
61	193,000	191,000
68	211,000	209,000
75	228,000	226,000
82	243,000	242,000
90	261,000	260,000
96	277,000	276,000
102	291,000	291,000

tube viscometer cannot give dependable measurements of yield value. Consequently, there is no practical way of determining yield value prior to the construction of the rotational viscometer; hence the upper safe limit for R_c cannot be calculated, but must be determined by trial and error.

The nonlinear portion of the curve (Figure 3) can be made smaller by decreasing ω_{T_1}. From Reiner's work it can be shown that the angular velocity for torque, T_1, is

$$\omega_{T_1} = \frac{f}{U}\left[\frac{R_c^2}{2R_b^2} - \ln\left(\frac{R_c}{R_b}\right) - 1/2\right] \qquad (8)$$

From this equation it is obvious that if f and U were known and a particular ω_{T_1} desired, satisfactory values for R_c and $R_c - R_b$ could be calculated. Since f and U are not known, except for values obtainable with capillary-tube viscometers, the investigator is again forced to resort to trial and error methods. Though ω_{T_1} is not directly dependent on h (see Equation 8) the ultimate selection of R_c and R_b will be influenced by U, the plastic viscosity. This factor, h, is a good starting point. Any h can be selected that the operator finds convenient, but the rest of the procedure is trial and error, guided by the principle that $R_c - R_b$ must be kept as small as operating convenience and the strain limit of the torsion member will allow.

Briefly, the variables involved in designing a suitable bob and cup are: R_c, $R_c - R_b$, h, the range to be covered by f and U, the strain limits of the various torsion members that are required to cover the desired consistencies, and the available r. p. m.

The bob is a solid cylinder, having the top end level with the material in the cup. The bottom end being immersed, will give an "end effect" which is not taken into consideration in the Reiner equation. The farther the bob is withdrawn from the bottom of the cup, the less will be the viscous or plastic drag upon the flat lower end of the bob. By trial and error it can be ascertained how much this distance must be before the end effect can be considered negligible. In the instrument shown in Figure 1, this distance is 1 cm.; other dimensions are $R_c = 1.5$, $R_b = 1.3$, and $h = 5.1$ cm. Mooney and Ewart (*10*) have devised an ingenious means for taking end effect into consideration. They use a conical end of predetermined angle from which the additional end torque can be calculated.

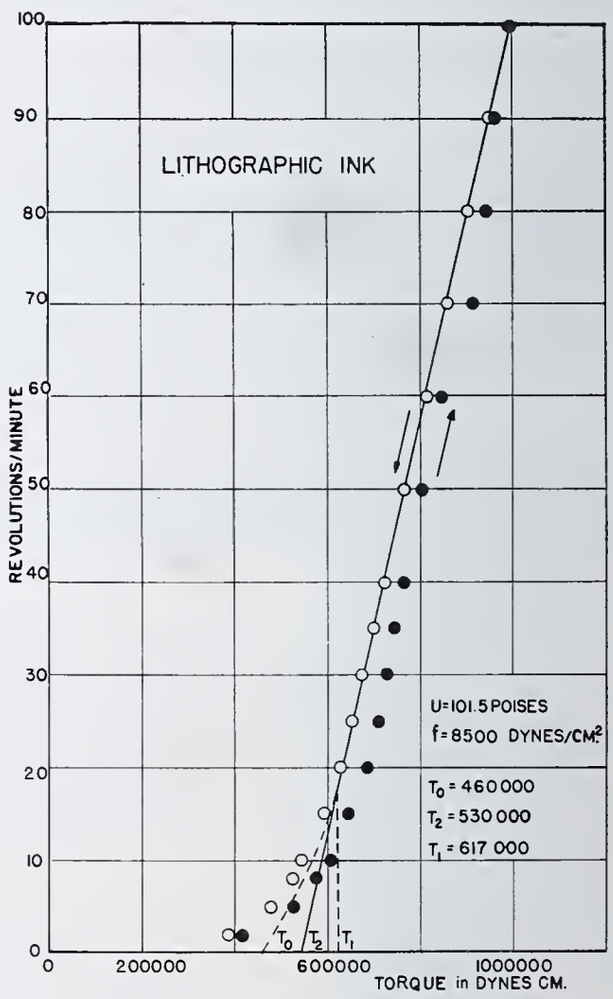


FIGURE 9. TYPICAL CURVE FOR THIXOTROPIC PLASTIC

Down Curve. T_2 = 320,000 Dynes per Cm.

Torque, T	R. p. m.	$(T - T_2)/r.\ p.\ m.$
1115×10^3	100	7.95×10^3
1040	90	8.00
945	80	7.81
875	70	7.93
788	60	7.80
725	50	8.10
640	40	8.00
600	35	8.00
558	30	7.93
518	25	7.92
468	20	7.40
420	15	6.67
355	10	3.50
335	8	1.88

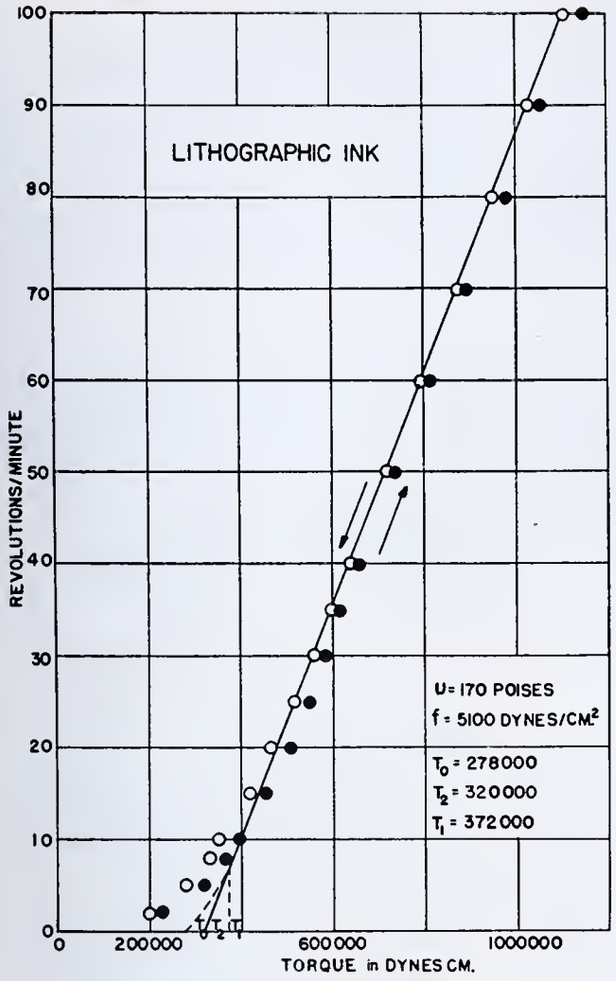


FIGURE 10. TYPICAL CURVE FOR THIXOTROPIC PLASTIC

Down Curve. T_2 = 530,000 Dynes per Cm.

Torque, T	$R.\ p.\ m.$	$(T - T_2)/r.\ p.\ m.$
990 × 10³	100	4.60 × 10³
958	90	4.76
900	80	4.63
860	70	4.71
810	60	4.67
760	50	4.60
720	40	4.75
700	35	4.86
665	30	4.50
650	25	4.80
620	20	4.50
585	15	3.67
538	10	0.80
518	8	..
470	5	..

The bob, B, is at the lower end of a vertical shaft which rotates on two sets of ball bearings, J, one set above and the other set below scale M. This plan gives great stability to the system, so that the bob once accurately centered with the cup remains that way indefinitely. The torsion medium is a helical spring, T. When in operation this spring supports the shaft and bob, thereby eliminating friction except the negligible amount existing in the ball bearings. The ball bearings simply function as a guide, keeping the shaft and bob rigidly centered. The bob, shaft, scale, and torsion spring are all attached to a single frame support which can be raised to bring the bob sufficiently above the top of the cup so that the cup can be removed when desired.

One of the principal faults of commercial rotational viscometers is their lack of adequate temperature control. Usually such instruments are made so that the surrounding bath must rotate with the cup making temperature control difficult. This difficulty has been completely overcome in the instrument described here. Because the cup is rotated by a shaft penetrating the bottom of a substantial bath, the necessity for an inadequately small rotating bath is eliminated, and thermostatic control can be maintained. In addition there is ample room for a stirrer, a cooling coil, and a thermometer, all of which are shown in Figure 1.

Calibration and Use of Viscometer

A series of six springs of different torsion constants is desirable in order satisfactorily to cover the entire range of consistencies found in the printing ink field. These springs can be calibrated very easily on the viscometer. Each spring can withstand a twist of one revolution without being strained beyond the elastic limit. The instrument is provided with a stop for this purpose. Liquids of known viscosity are usually employed for calibrating purposes, but this is not necessary on this instrument and in the case of the heavy springs it is not even permissible.

The procedure for calibration is shown diagrammatically in Figure 2. A string is attached to and wrapped around the scale M and thence over a wheel, L. Various weights, F, are attached to the string and the degrees of rotation of M are noted. When F is plotted against the scale reading, a linear curve (for helical springs and straight wires) intersecting the origin is obtained. The calibration value for the spring is the torque ($F \times g$ times radius of M) divided by the scale reading in degrees of arc. F is the weight shown in Figure 2 and g is the acceleration due to gravity. Since the calibration curve is linear and commences at the origin, the calibration value of the spring is a constant independent of the applied force. The scale on the variable-speed transmission, G, must also be calibrated in terms of r. p. m. This is accomplished by the use of the revolution counter, H, and a stop watch. The curve, r. p. m. *vs.* scale reading, is not linear and must be used whenever interpolated values are required.

Since most pigment vehicle suspensions are thixotropic and consequently subject to changes in consistency while being measured, it was necessary to give considerable thought to the best means of meeting this situation. It is shown below that such phenomena as thixotropic levels can be maintained during viscometry measurements. The design of the present instrument is such that these levels can be obtained and their consistency factors measured. The only requirement is to have an instrument that can be operated quickly and smoothly while the speed of cup rotation is being constantly changed except for the brief interruptions necessary for reading the scales. With the viscometer shown in Figure 1, thirty changes of speed together with the r. p. m. and scale (M) readings can be made in 3 minutes or even less.

OPERATIONAL PROCEDURE. The ink (or other material) is placed in the cup to a depth of about 0.5 inch, and the cup is keyed into the viscometer and allowed to come to temperature. Additional holes are provided in the top plate of the bath for extra cups, saving time for temperature maintenance. The bob is lowered into the cup, but is prevented from going too far by a stop on the movable frame at the back of the instrument. The introduction of the bob forces any excess ink up over the edge of the cup where it is scraped off.

The cup is now started rotating at the lowest speed, and the scale readings on M and G are recorded. The handle on G is turned slightly to the next speed and the two scale readings are again recorded. This procedure is repeated until the highest desirable speed is reached, after about 15 speeds have been recorded. As soon as the highest reading is made, the handle on G is set into reverse motion and a downward curve is obtained in a similar manner. This also should comprise about 15 points before the lowest speed is reached.

The up and down curves are now plotted, by plotting r. p. m. *vs.* torque (scale M reading times calibration value for the spring). If the material is an ordinary printing ink, it will be thixotropic and the up and down curves will not coincide. This condition is due to thixotropic breakdown. The two curves plotted together will make a loop which will be referred to as a "hysteresis loop". A large loop means considerable breakdown and, conversely, a small loop, small breakdown. No loop at all would indicate no detectable thixotropy as far as the viscometer is concerned. Materials that possess a yield value but give no hysteresis loop are considered by some investigators to be highly thixotropic. In this case complete thixotropic breakdown upon agitation is assumed to take

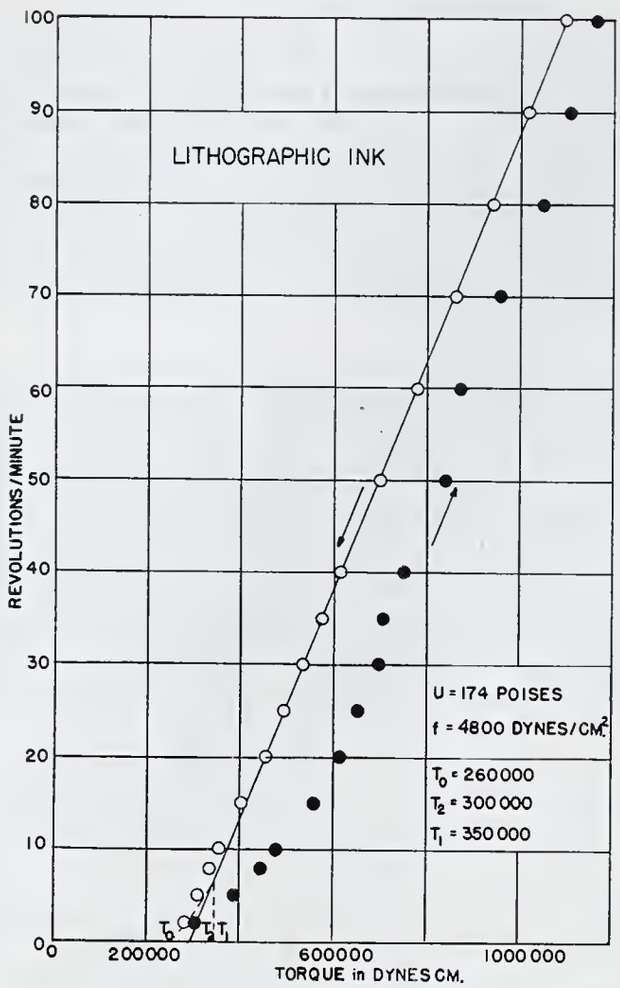


FIGURE 11. TYPICAL CURVE FOR THIXOTROPIC PLASTIC
Down Curve. T_2 = 300,000 Dynes per Cm.

Torque, T	$R.\ p.\ m.$	$(T - T_2)/r.\ p.\ m.$
1090 × 10³	100	7.90 × 10³
1018	90	7.98
942	80	8.03
868	70	8.11
780	60	8.00
702	50	8.04
622	40	8.04
585	35	8.14
538	30	7.93
485	25	7.40
458	20	7.90
408	15	7.20
360	10	7.00
340	8	5.00
318	5	3.60
288	2	..

place so rapidly that a loop cannot be recorded by the viscometer. If such were the truth, a material of this kind would show, after standing unagitated for awhile, a very marked change in consistency when suddenly touched or stirred with a spatula, but nothing to indicate a consistency change can be observed or felt. In addition, one would have to argue that the larger the hysteresis loop, the smaller the amount of thixotropy, which is contrary to common sense.

When the down curve is compared with the up curve, a marked difference will be noticed. The up curve is bow-shaped and lacks smoothness. The down curve is smooth and linear except at its extreme lower end. The linearity of the upper section of this curve is in conformity with Reiner's prediction and, therefore, indicates constant yield value and constant plastic viscosity in that region. After the experimental data obtained with the viscometer are plotted, a straight line is drawn through the maximum number of points on the down curve that will fall upon it. This line intersects the torque axis at T_2. From T_2 and the slope of this line, yield value and plastic viscosity are calculated by Equations 4 and 5.

The values for U and f, determined in the manner described above, hold for the straight part of the curve. What happens to these values in the curvilinear section is still a debatable question. Reiner states that if f and U are constant $T_0 = 2\pi R_b^2 hf$ and $T_1 = 2\pi R_c^2 hf$. However, when f, determined from Equation 5, is substituted in these equations the resulting T_0 appears to be too large and T_1 too small. The reason for these discrepancies is not known at present and, in view of the scarcity of pertinent data, hasty conclusions would appear dangerous.

Thixotropic Levels

The main purpose of this paper has been to describe the viscometer (Figure 1) and its application to the Reiner equation. Although an extended treatment of the rheology of thixotropic materials may not be called for at this time, thixotropy cannot be ignored entirely, for it produces a decided effect on the experimental data.

The up curve exemplifies the continuous breakdown of the thixotropic structure as the rate of flow (r. p. m.) is gradually

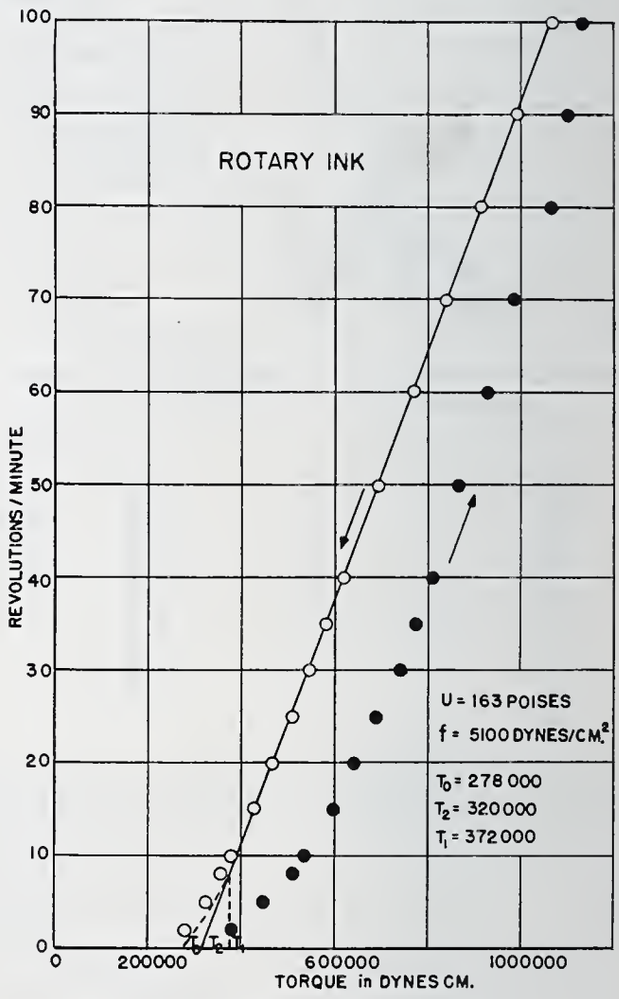


FIGURE 12. TYPICAL CURVE FOR THIXOTROPIC PLASTIC
Down Curve. T_2 = 320,000 Dynes per Cm.

Torque, T	$R.\ p.\ m.$	$(T - T_2)/r.\ p.\ m.$
1062 × 10³	100	7.42 × 10³
1990	90	7.44
905	80	7.31
838	70	7.40
770	60	7.50
688	50	7.36
615	40	7.38
580	35	7.43
545	30	7.50
502	25	7.28
460	20	7.00
420	15	6.67
378	10	5.80
358	8	4.75
325	5	1.00
280	2	..

increased. The down curve is maintained linear without any conscious effort on the part of the operator—i. e., there are no requirements other than continuous uninterrupted work. Obviously if the viscometer is stopped long enough, thixotropic buildup will beeome effective and linearity will cease. In order to obviate this possibility the viscometer has been designed to make quick and easy operation possible; hence the dozen or more measurements on the down curve can be accomplished in less than a minute.

When the above procedure is followed, linearity of the down curve results if pseudoplasticity is not involved, indicating that there exist at that time a constant yield value and plastic viscosity. The constancy of these factors shows that a temporary stability is maintained in the thixotropic structure; hence, a "thixotropic level" has been reached, the exact position of which is influenced by the pretreatment which the material has received. This includes handling and special treatments such as milling, etc., that might be given to the material prior to the test. Pretreatment covers the entire history of the material before commencing the down curve. The thixotropic breakdown received during the measurement of the up curve is one of the most important factors involved here. If the up curve is stopped at 200 r. p. m. the material will have received more breakdown than if 100 r. p. m. had been selected for the upper limit; therefore the curve will be shifted toward the ordinate. Consequently, for plant control purposes, the procedure for obtaining rheological data on thixotropic systems must be rigidly standardized (Figure 4).

The temporary maintenance of linearity is probably due to two factors. Normally a thixotropic substance breaks down more quickly than it builds up. On the down curve the speed of flow is being decreased, so that no further breakdown can be expected; also an appreciable buildup evidently takes more time than is employed for the completion of the down curve. Consequently the down curve can show no change in consistency, and linearity results.

The lack of coincidence of the up and down curves has been pointed out in the literature (*9*, *11*, *13*) at various times, but apparently nowhere have thixotropic levels been emphasized or discussed. This could be explained on the assumption that a suitable commercial viscometer had not been built for the purpose. These levels, however, are of great importance to investigators who are compelled to work with thixotropic systems, for they constitute something far more substantial than the usual one-point measurements made on unstable materials.

The hysteresis loop criterion for thixotropy must not be confused with the Goodeve (*4*) "coefficient of thixotropy". Goodeve's coefficient is the intercept which the curve makes on the force axis—a function of yield value. From the viewpoint presented in this paper the magnitude of yield value is not quantitatively related to the area of the hysteresis loop and, therefore, bears no quantitative relation to thixotropic breakdown and buildup.

The following questions are often asked: How far can the down curve (Figure 4) be shifted toward the ordinate by increasing the agitation—i. e., by raising the upper limit of the r. p. m.? Can all thixotropic materials be reduced to Newtonian liquids if sufficiently high rates of flow are used? It is doubtful if experimentation can give a conclusive answer to either question, for no matter how high the r. p. m. employed, it can always be argued that if higher r. p. m. had been available the results might have been different. There is, however, experimental evidence showing that the rate of shift toward the ordinate decreases as the r. p. m. is uniformly increased. This indicates that in the case of pigment suspensions, like heavy printing inks, an ultimate position for the curve might be attained without the yield values actually becoming zero. On the other hand, thixotropic materials unquestionably exist that can be reduced by agitation to a state where they give complete Newtonian flow.

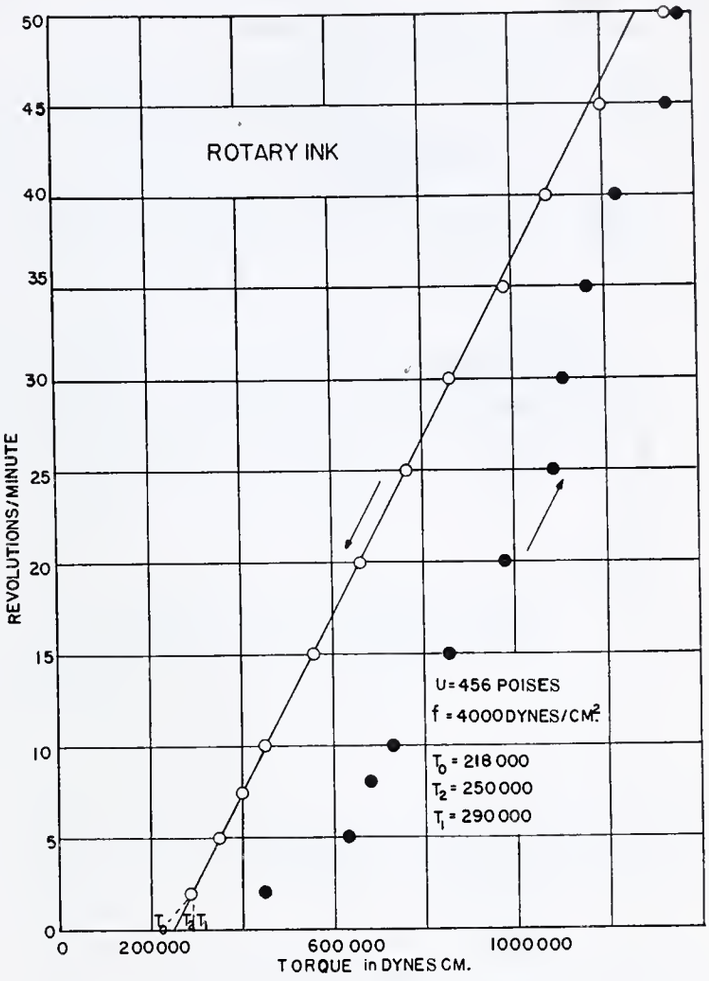


FIGURE 13. TYPICAL CURVE FOR THIXOTROPIC PLASTIC

Down Curve. T_2 = 250,000 Dynes per Cm.

Torque, T	$R.\ p.\ m.$	$(T - T_2)/r.\ p.\ m.$
1342 × 10³	50	21.8 × 10³
1200	45	21.1
1080	40	20.8
990	35	21.1
855	30	20.1
752	25	20.1
645	20	19.8
550	15	20.0
452	10	20.2
400	8	18.8
350	5	20.0
288	2	19.0

Slippage

One of the great virtues of the rotational viscometer is that it reduces slippage in most cases to a negligible amount. Exceptions will be found where syneresis occurs, but this, an abnormal condition, is not likely to be found in the field of materials covered by this article. Slippage arises from the viscous flow (Newtonian) of the lubricating layer of vehicle which always exists where the material contacts the wall of the viscometer. For any given material the thickness of this layer is the same, whether a capillary tube or rotational viscometer is employed. In the case of the capillary tube, the thickness of this layer often constitutes a substantial percentage of the radius; consequently, the flow curve contains a substantial proportion of slippage flow. This condition completely changes the shape and position of the curve, so that plastic viscosity calculated from its tangent in the customary manner might easily contain an error of 50 per cent or over. Angle α is too great by an amount equal to angle β. Unfortunately, β is very difficult to determine with sufficient

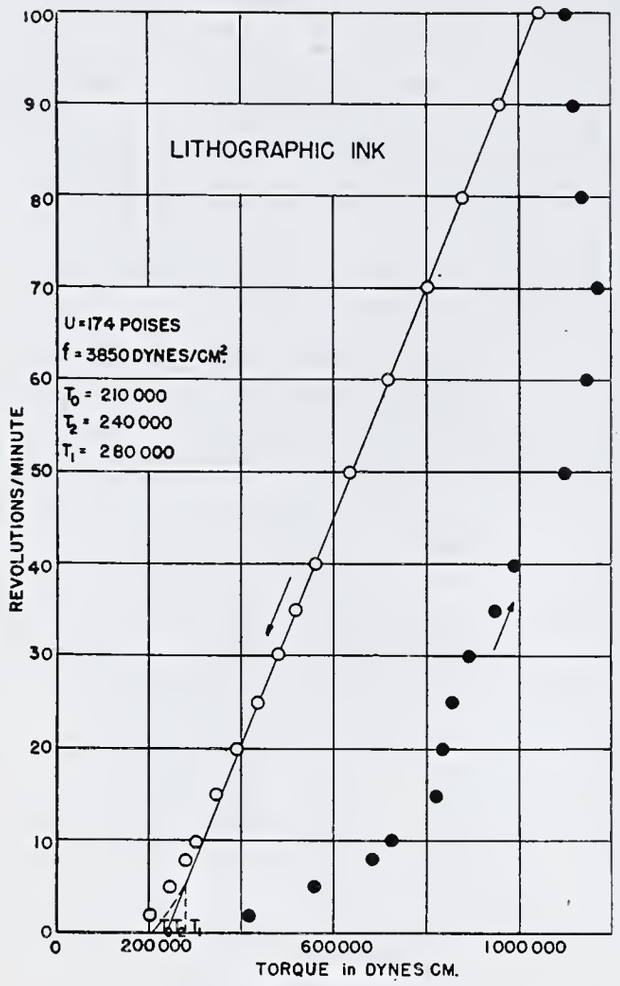


FIGURE 14. TYPICAL CURVE FOR THIXOTROPIC PLASTIC

Down Curve. T_2 = 240,000 Dynes per Cm.

Torque, T	$R.\ p.\ m.$	$(T - T_2)/r.\ p.\ m.$
1045 × 10³	100	8.05 × 10³
953	90	7.92
863	80	7.79
788	70	7.83
717	60	7.95
633	50	7.86
560	40	8.00
515	35	7.88
478	30	7.93
438	25	7.92
390	20	7.50
348	15	7.20
302	10	6.20
280	8	5.00
248	5	1.60
203	2	..

accuracy, and consequently corrections for slippage are not usually made (Figure 5).

In the rotational viscometer the slippage layer is relatively thin compared to the distance between the bob and cup, thus reducing slippage flow to an undetectable amount. This can be demonstrated experimentally. The capillary tube is smooth-walled and therefore ideal for permitting slippage. The wall of the bob and cup of the rotational viscometer, however, can be roughened, grooved, or altered, to reduce slippage. If the plan shown in Figure 6 is carried out with both the bob and cup walls grooved, slippage should be reduced considerably, if not eliminated altogether. If the width of the groove is equal to half the distance between the grooves, the area over which slippage can take place is reduced 33 per cent. Such a scheme has been carried out with printing inks and with similar pigment suspensions known to produce slippage when run in capillary tubes. The results were negative—i. e., no differences in yield values and plastic viscosities could be detected whether the material was run in a grooved or ungrooved viscometer. It is fairly safe to conclude, therefore, that if a reduction of at least 33 per cent of the slippage area does not produce measurable results, the entire slippage flow is negligible.

Pseudoplasticity

Certain types of materials, such as solutions of rubber, resins, and cellulose esters, give nonlinear curves, when sufficiently concentrated. Bingham has called these substances pseudoplastics and Ostwald refers to them as having "structural viscosity". Reiner has made an extensive study of the subject and states that the rheological characteristics of a pseudoplastic are a variable coefficient of viscosity and an absence of a real yield value.

From a practical viewpoint there is no convenient way of determining the controlling factors (constants) of the pseudoplastic curve. The initial viscosity at the beginning of the curve near the origin can be obtained approximately by taking a one-point measurement at the lowest r. p. m. This viscosity value is one of the constants of the equation. "Apparent viscosities" can also be calculated at any desirable r. p. m., if such calculations have a practical technical value. A pseudoplastic curve is shown in Figure 8.

Accuracy and Precision

In determining the accuracy of the viscometer, it is necessary to employ stable substances like Newtonian liquids, preferably using liquids of 20 poises or under. Such materials can be measured satisfactorily with a capillary-tube viscometer and so can be used for ascertaining the accuracy of any other type of viscometer.

One of the standard oils measured and sold by the National Bureau of Standards was obtained and its viscosity determined with the rotational viscometer. The torsion member was calibrated by the weight method described here. It was found that the Bureau of Standards' value could be checked with the rotational viscometer readily to within ±1.0 per cent. Measurements of plastic viscosity of thixotropic inks, using a definite r. p. m. for the upper limit, can normally be duplicated to within ±5.0 per cent. For practical routine work in industrial plants this degree of precision is satisfactory, especially where the viscosity range covered is as wide as in the oil, paint, and printing ink industries. In regard to checking yield values, the instrument shown in Figure 1 gives satisfactory results in the neighborhood of 1000 dynes per sq. cm. Yield values lower than 100 dynes per sq. cm. require for satisfactory precision a more delicately constructed instrument of greater sensitivity. Such a rotational instrument, built by the Research Laboratories of the Interchemical Corporation, can detect yield values as low as 5 dynes and differences in viscosities of as little as 0.001 poise.

To the rheologist who is accustomed to working with capillary-tube viscometers and materials of relatively low viscosity, the degree of precision given above will seem fairly low. However, in general, the technologist who makes viscosity measurements in an industrial plant almost invariably uses a one-point method. This will make impossible the calculation of yield value and plastic viscosity. The viscosity of a plastic determined by a one-point method is a variable, depending on the rate of shear and therefore of doubtful rheological value. To such an investigator a precision of ±5 per cent in plastic viscosity and ±20 per cent in yield value constitutes a very substantial improvement.

Experimental Data

The examples shown in Figures 9 to 14 were taken at random from about 5000 similar curves, and are entirely

typical. They certainly corroborate Reiner's prediction of linearity, and also prove the existence of definite thixotropic levels. The fact that the down curves are linear above T_1 means that U and f are constant above this point and can readily be determined from the simple linear equations, 4 and 5. These facts, in the opinion of the writer, justify the use of a properly constructed rotational viscometer and the application of the Reiner equation for industrial rheological measurements and calculations.

Acknowledgment

The author is indebted to Interchemical Corporation for permission to publish this work, to Ruth N. Weltmann who supervised the production of the laboratory data and gave valuable advice on the construction of the viscometer, and to Daniel Smith.

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Simplified Apparatus for Catalase Determination

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A METHOD of simply and rapidly determining the extent of inactivation of the enzyme catalase, after the blanching process and before dehydration or freezing of vegetables, is presented.

Norgarrd (*2*), Knott (*1*), Pack (*3*), and Tressler and Evers (*4*) have described methods for catalase assay based on the amount of oxygen liberated by the action of the enzyme on hydrogen peroxide. While these methods are accurate, Norgarrd's requires too long a time to complete the reaction, Knott and Pack's both require specialized apparatus, while the method devised by Tressler and Evers, though satisfactory upon the whole, will not give accurate results with all vegetables.

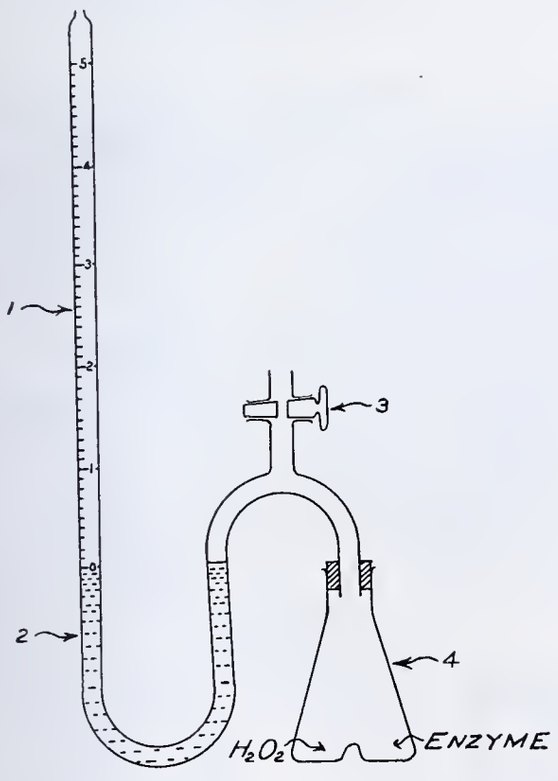

FIGURE 1. CATALASE APPARATUS

1. 5-cc. pipet graduated in 0.1 cc., fused to U-piece of tubing of same diameter
2. Water in tube, at 0 cc. level of pipet at start of reaction
3. Stopcock to adjust level of water after flask is attached
4. 50-cc. Erlenmeyer flask with divided bottom

By using a modification of the Tressler procedure, eliminating the fermentation tube and substituting the apparatus illustrated in Figure 1, the extent of inactivation can be determined accurately in 2 minutes, and the apparatus is simple to construct and inexpensive. Such a method should have value, especially in food-processing plants where it is necessary to make catalase determinations in a minimum period of time. The Canadian Government requires catalase tests run on all vegetables after blanching as a quality control measure for the dehydrated products.

PROCEDURE. An accurately weighed 1.0 gram sample is ground in a mortar with 0.6 gram of calcium carbonate and 1.0 gram of fine sand. Ten milliliters of water are added and the grinding is continued for about 2 minutes. One milliliter of this mixture is pipetted into one half of the special divided flask, and 2 ml. of hydrogen peroxide (Dioxygen) are placed in the other half. The flask is then attached to the manometer and the whole apparatus is suspended in a thermostatically controlled water bath at 20° C. When the apparatus reaches the bath temperature, the stopcock is closed (water level in U-tube set at 0 ml.) and the apparatus is shaken for 2 minutes. A reading of the pipet is then made to determine the amount of oxygen liberated. The catalase may be reported as milliliters of oxygen liberated by 0.1 gram in 2 minutes. For general use the calculations put forth by Tressler and Evers have been found adequate.

This method gives only relative values but is very useful for the estimation of a series of comparisons. It was designed for rapid factory laboratory use, but can be used where more quantitative values are desired.

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Simplified Dropping Mercury Electrode for Polarographic Analysis

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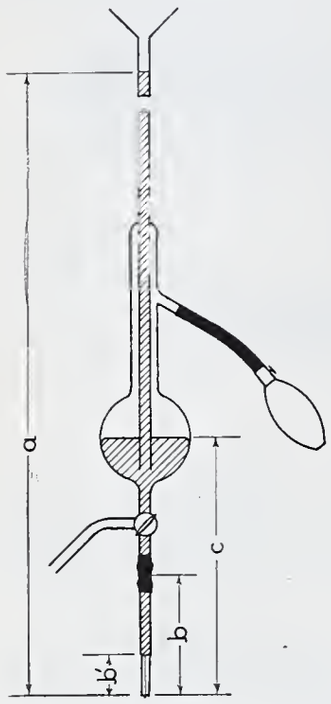

FIGURE 1. DROPPING MERCURY ELECTRODE

a. Adjustable mercury column, 24 to 70 cm.
b. Detachable capillary and tube, 11 cm.
b'. Actual capillary, 3 cm.
c. Minimum mercury level, 24 cm.

MANY different types of dropping mercury electrodes have been described since the simple mercury reservoir and capillary connected by rubber tubing were first used by Heyrovský (*1, 3, 4, 7–10*). All these, however, require the use of rubber tubing, which eventually causes contamination and plugging of the capillary, or are so complex that cleaning is difficult. Furthermore, the more elaborate ones require cumbersome mountings which make them difficult to use in connection with cells immersed in a constant-temperature bath. The most suitable arrangement should consist of a single piece of apparatus supported by one easily adjusted clamp and made entirely of glass. One such electrode, described by Mueller (*6*), operates on the Mariotte flask principle. This electrode was tried, but was found to have one disadvantage: In order to maintain the constant mercury pressure the apparatus must be evacuated to lower the mercury level to the end of a tube located below the mercury surface in the reservoir. This immersed end cannot be seen, and consequently a manometer must be employed to check against leaks and variations in pressure through room temperature and barometric changes.

The following apparatus is capable of supplying any desired mercury pressure in such a way as to allow accurate visual measurement and adjustment. It fulfills the requirements of simplicity of construction, is sturdy in spite of being made almost completely of glass and is suitable for simple and flexible mounting. It is easily cleaned and provides for reversing the direction of mercury flow through the capillary. This is often useful in removing contamination in the upper part of the capillary without dismantling the assembly. The apparatus is composed entirely of glassware to be found in any laboratory and exposes the mercury to only 0.6 cm. (0.25 inch) of neoprene tubing.

This apparatus, shown in Figure 1, is made of a 250-ml. distilling flask, a 2-mm. three-way T-stopcock, 40 cm. of 7-mm. tubing, and a rubber pressure bulb with a release valve of the type used on a baumanometer. Pulleys and counterweight (not shown) facilitate raising and lowering the system.

The operation is very simple. A capillary of the type de-

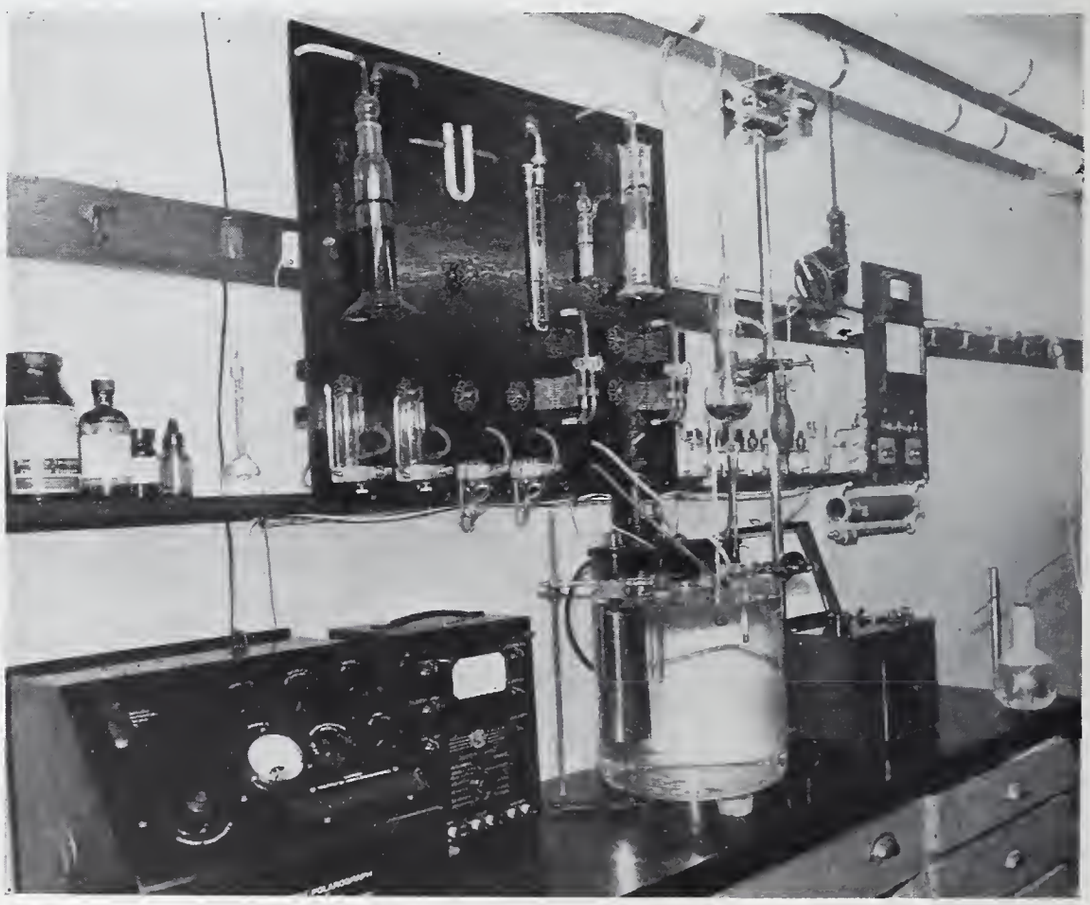

FIGURE 2. LABORATORY ARRANGEMENT FOR ROUTINE POLAROGRAPHIC ANALYSIS

Heyrovský XI polarograph, vacuum tube microammeter, constant-temperature bath, dropping electrode with counterbalance, vacuum tube potentiometer, and pH meter. Against the wall can be seen control board for degassing and part of equipment for bias current and damping condensers.

scribed by Maas (5) is attached to the lower end of the stopcock by means of a short length of neoprene tubing. The reservoir is half filled through the top funnel and the air space between the stopcock and the capillary is filled by turning the cock repeatedly. As the cock is opened, connecting the reservoir to the capillary, a few drops of mercury flow into the air space until the pressure is equalized. The cock is then turned to the side position, exposing the air space above the capillary to atmospheric pressure through the mercury drain arm. This process is repeated until the column is full and no air bubbles remain. The cock is left in the position connecting the capillary and the reservoir until it is necessary to drain the mercury from the reservoir.

With the ordinary capillary only one drop of mercury will flow, as long as no pressure is added, but the capillary must not be immersed in any solution except distilled water, for it will tend to be contaminated. The tip should not be immersed in mercury, as this will start the capillary flowing.

When the electrode is to be used, pressure is first applied through the rubber bulb until a few centimeters of mercury in excess of what is desired are obtained in the column. This is gradually reduced to the desired mark, usually 50 to 60 cm. by adjusting the release valve. The tip is now washed with distilled water and dried, and the entire asembly is lowered until the tip is suitably immersed in the electrolysis vessel. The 0.6 cm. (0.25 inch) of exposed neoprene tubing allows lateral movement and adjustment of the tip and prevents breakage.

At the conclusion of operation the assembly is raised, the tip is washed and dried, the mercury is dropped by opening the release valve, and the apparatus is left as it is. The tip may be protected from dust by a rubber policeman, or immersed in distilled water.

The apparatus is easily cleaned. This is important, for clogging of the capillary and erratic drop rate through partial contamination have caused much lost time in polarographic analysis. It has been found wise to remove all mercury from the reservoir and capillary every 40 days and thoroughly clean both it and the apparatus. If the capillary becomes clogged or the drop time becomes fast, slow, or erratic the capillary must be cleaned. Trouble near the tip may often be removed by placing the tip, with mercury flowing, in a solution of aqua regia and washing with distilled water (2). However, if the contamination is in the upper part of the capillary it may be removed by immersing the tip in mercury, attaching a vacuum line to the drain arm, and reversing the direction of flow by turning the stopcock. Should the capillary still remain clogged, it should be removed and cleaned thoroughly.

The fastest and most reliable way to assure absolute cleanliness is to attach a pump to the capillary and evacuate the latter while it is being heated on a hot plate. When the passage is cleaned, it is treated, still under vacuum, with concentrated nitric acid, with dichromate-sulfuric acid solution, with distilled water, and then dried for 1 to 2 hours.

This electrode, the polarograph, and auxilary equipment for routine analysis are shown in Figure 2.

Acknowledgment

The author wishes to express his indebtedness to J. Rud Nielsen for several helpful suggestions.

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A Laboratory Temperature and Humidity Cabinet

For Studying the Hygroscopic Properties of Tobacco

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A laboratory cabinet is described which furnishes a circulating atmosphere of regulated temperature and humidity. Means of humidifying, dehumidifying, heating, and cooling are provided. Using this cabinet, the equilibrium moisture characteristics of five types of leaf tobacco important in cigaret manufacture have been studied over a range of atmospheric conditions.

A CIRCULATING atmosphere of regulated temperature and humidity is needed to study the equilibrium moisture characteristics of tobacco samples of various kinds. A laboratory cabinet to provide such an atmosphere can be built by installing readily available control instruments in a second-hand refrigerator of the side-icing type. Such cabinets have for some time been in satisfactory operation in the two laboratories reporting the present work. They are readily adjustable and are capable of covering a range of temperatures and relative humidities. The features provided are humidification, dehumidification, heating, and cooling.

Assembly of Instruments

Humidification operates continuously. Clay flower pots up to 25 cm. (10 inches) in diameter are used, filled with water, and when necessary are bound around the outside with cloth, fed by small wicks leading inside the pot.

Dehumidification is intermittent. Laboratory compressed air, controlled by a humidistat and a solenoid valve, is injected into the cabinet through the drain in the bottom. A strainer protects the solenoid and a needle valve regulates the pressure. Air is conducted by 1-cm. (0.375-inch) copper tubing extending about 20 cm. inside the cabinet. The humidistat controls relative humidity within ± 1.5 per cent where the temperature is regulated to a range of 1° F. (0.55° C.).

Heating, which is intermittent, is controlled by a direct thermostat operating over a differential of 0.5° F. The heater is made of 16.75 meters (55 feet) of Chromel A, 20-gage wire wound on a slatted Transite cylinder 10.8 cm. in diameter and 14 cm. long. The wire coils are separated and held in place between turns of

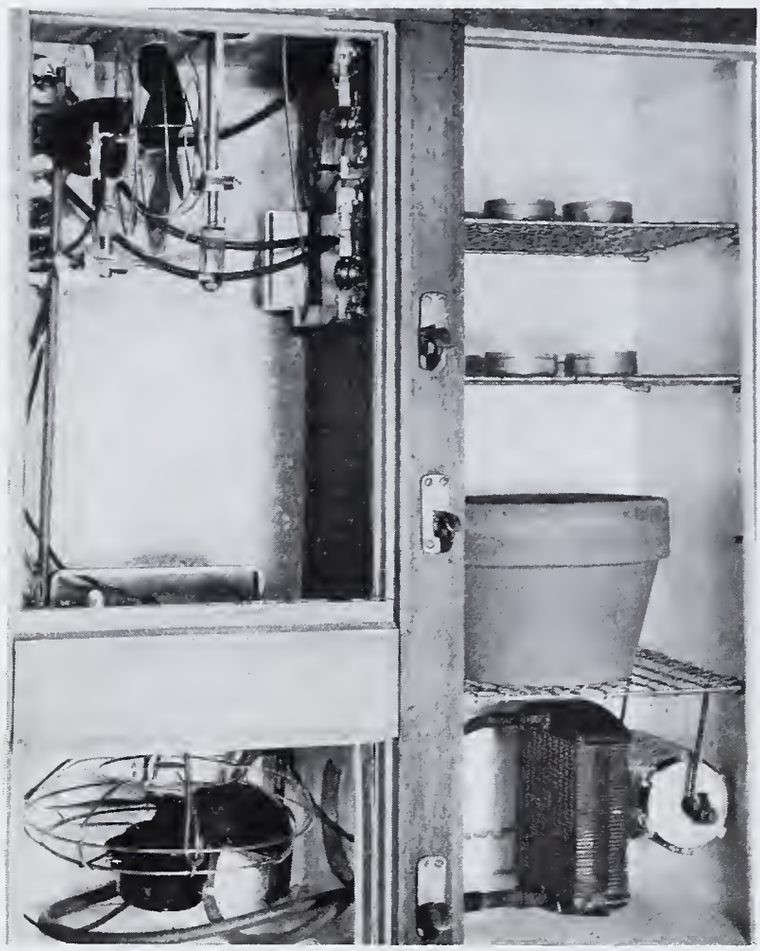

FIGURE 1. INSIDE VIEW OF CABINET

Upper left compartment. On right wall, humidistat and two thermostats, with attachments for outside adjustment; on back wall, panel with electric outlets for fans and control instruments. Thermometers are shown in operating position. Booster fan at bottom.

Right compartment. Sample shelves, unglazed clay pot for humidifying, cooler, and heater.

asbestos string wound about the individual slats. There is also some heat produced by the fans used to circulate air.

Cooling is intermittent. A direct thermostat and relay (or an inverse thermostat) actuates a 11.4-liter (3-gallon) per minute pump circulating water between an auto heater core inside the cabinet and 8 turns of coiled 1.25-cm. (0.5-inch) copper tubing in a 11.4-liter (3-gallon) insulated cooling reservoir, located outside. The circulating system is vented to an aspirator bottle on top of the cabinet, which acts as an injector.

To obtain wet- and dry-bulb readings, a hole 7 cm. in diameter, cut through the top of the ice compartment of the cabinet, is fitted with a wooden disk having a floor flange fastened on the bottom. A pipe from the flange supports a tube of water to wet the wet-bulb thermometer. The thermometers pass through holes 3.8 cm. apart, drilled through the disk and flange.

For adjustment of control instruments from outside the cabinet, a thrust or pull is transmitted to the instrument-setting levers by means of auto choke wires, in flexible cables, attached to rods passing through the box above the door. The rods are threaded, with a narrow groove cut through the threads, and to prevent torsion, a small fixed lug rides in the groove. Thrust or pull is caused by turning brass knobs with holes tapped through the center. Knobs and fixed lugs are held in place by a double yoke fastened to the outside of the cabinet. The flexible cables, wires, fixed lugs, and grooved rods (unthreaded) are the more recent type of dash control attachments obtainable at any large auto supply house. The yoke to hold the fixed lugs, the yoke in which the brass knobs ride, and the tapped brass knobs are added. Control instruments are mounted on a panel in the ice compartment and around the air passage.

The position of the instruments and working parts in the cabinet is shown in Figures 1 and 2. In Figure 1 the cooler and heater are displaced to the right of their operating positions, for purposes of photography. The electrical hookup is shown in Figure 3.

The cost of a cabinet of this type is approximately $290, distributed about as follows: small laboratory pump, $23; Minneapolis-Honeywell humidistat, $15; two Minneapolis-Honeywell bimetallic thermostats, $16; second-hand refrigerator, $10; two Taylor thermometers, $8; two fans, $15.25; cooling system, $12.25; outside instrument controls, $5.50; solenoid valve and air line fittings, $14.75; heater, $2.75; electrical fittings, $10.50; labor $159.50.

Operation of Cabinet

To attain an air velocity of 270 meters (900 feet) per minute past the wet-bulb thermometer (*2*), the thermometers are placed as close to the fan as possible. In addition, the mercury bulbs are located at points about the same radial distance from the center of the fan. These points are indicated by agreement of dry-bulb temperatures at the two positions. To obtain this agreement it may also be necessary to adjust the positions of the cooling and heating units. If anemometer readings are low, a second fan to promote circulation is advisable. The size of air ports between compartments in the cabinet affects air velocity.

As in sling psychrometer measurements, the wet-bulb wick should be wetted at intervals rather than continuously. The authors find it convenient to lower the wet bulb into a tube of water and raise it again to its fixed position for reading. With the thermometer positions in satisfactory adjustment and the control instrument settings adjusted to maintain a particular range, gravimetric determinations of water vapor can be made occasionally to check the relative humidity found by wet- and dry-bulb thermometry (*1*). For the gravimetric determinations, a tube passing from the cabinet through the thermometer

FIGURE 2. OUTSIDE ATTACHMENTS OF CABINET

Water-cooling system and pump, with aspirator bottle to keep system free of air; insulated bath removed to show coil. Compressed air line to cabinet against wall at left. On back top of cabinet, front panel with pilot light on lead to solenoid, connection to house current, and lead to pump. At rear of top, heater lamp bank and relay that controls pump.

well cover leads to a tared U-tube of anhydrous magnesium perchlorate thence to a guard tube containing the same reagent, and finally to an aspirator bottle. A 5-liter sample of air is drawn from the cabinet in 30 minutes, during which time a record of the thermometer readings is kept to obtain the mean observed relative humidity to be compared with that found by calculation from the weight of water vapor, as in Table I.

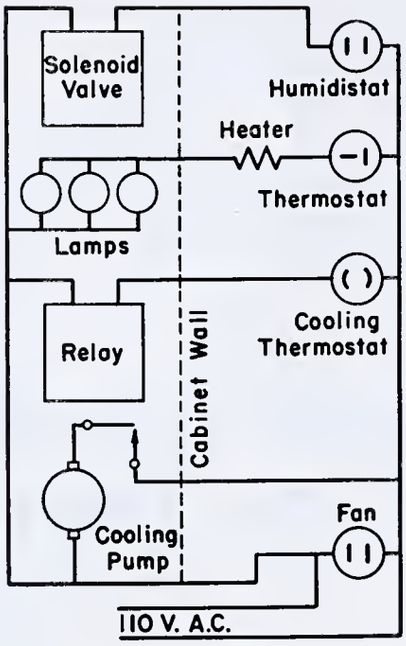

FIGURE 3. WIRING DIAGRAM

Without cooling continuously, temperatures can be maintained from room temperature to the upper limit of the thermostat range. Relative humidities range up to about 50 per cent above that of the room. For low humidity conditions the range is limited by the dew point of the compressed air, which may vary seasonally. The range of conditions studied is given in Table I. For the authors' purpose, lower temperature ranges could be obtained most conveniently by moving the cabinet to an unheated room in which compressed air is available.

The humidifying capacity chosen (size and number of clay pots of water) is only a little more than that necessary to maintain the desired humidity. In case of a sudden drop in outside humidity, the humidifying capacity chosen may prove insufficient. Air circulation, of course, accelerates exchange between outside and inside air and also raises the temperature several degrees, thus putting an additional load on the cooling and humidifying systems. Operating at high humidities, the inside cooling coil may condense moisture at the end of a cooling cycle and depress the humidity. (To attain very high humidities, a practicable device would be an atomizer operating on compressed air previously humidified by passing it through water contained in a vertical column of iron pipe of convenient diameter.) To overcome this depression, cooling water at a slightly higher temperature is obtained by putting the ice used in the external cooling reservoir into a bucket. Sufficient water is left in the reservoir to make contact with the bucket and cover two or three turns of the coil.

Moisture Equilibria of Leaf Tobaccos

In order to illustrate the kind of experimental work that can be done in this cabinet, the moisture-retaining properties of five types of leaf tobaccos commonly used in cigaret manufacture were studied: Turkish (Smyrna); Burley, U. S. type 31, grade C5F; Maryland, U. S. type 32, mixture of grades X2L and X2F; North Carolina flue-cured, U. S. type 12, mixture of grades C4L, C4F, and X2F; South Carolina flue-cured, U. S. type 13, mixture of grades C4L, C4F, and X2F.

The tobaccos were cut to shreds of about the width used for cigarets. Samples of approximately 6 grams each, contained in ointment tins, were exposed in the cabinet in duplicate until substantially constant weight was reached. This required about 6 hours, and was determined by capping the tins at intervals, withdrawing from the cabinet, and weighing. The moisture content of the samples was then determined by means of a Brabender semiautomatic moisture tester. The experiments were all made at the same temperature (80° F., 26.67° C.), while a 30 to 70 per cent range of relative humidity was covered.

The equilibrium moisture contents of the tobaccos at the several conditions studied are given in Table II. The significance of these data is emphasized in Figure 4, which shows that of the types studied, the flue-cured tobaccos are the most responsive to changes in humidity, being the most hygroscopic of all types at the higher humidities and the least hygroscopic of all types at the lower humidity. In sharp contrast is Maryland tobacco, whose moisture content is affected least of the five types by changes in humidity. The behavior of Burley and of Turkish lies between the two extremes.

TABLE I. RELATIVE HUMIDITY DETERMINED SIMULTANEOUSLY BY TWO METHODS

Wick	By Thermometers (A)	By Water Vapor Determination (B)		A − B
	%	*Grains/cu. ft.*	%	%
Silk	30.4	3.497	32.0	−1.6
	29.7	3.279	30.0	−0.3
	41.7	4.546	41.6	0.1
	40.8	4.590	42.0	−1.2
	51.2	5.596	51.2	0
	50.85	5.464	50.0	0.85
	58.4	6.259	57.2	1.2
	57.75	6.225	56.9	0.85
	68.25	7.485	68.5	−0.25
	69.5	7.676	70.2	−0.7
Cotton	38.9	4.293	39.3	−0.4
	51.7	5.753	52.6	−0.9
	58.6	6.478	59.25	−0.65

TABLE II. EQUILIBRIUM MOISTURE CONTENTS OF FIVE TYPES OF TOBACCO AT 80° F. AND RELATIVE HUMIDITY INDICATED[a]

	30%	40%	50%	60%	70%
	%	%	%	%	%
Turkish (Smyrna)	7.85	8.35	9.65	11.60	14.45
Burley, type 31	8.15	8.55	9.70	11.75	13.50
Maryland, type 32	8.50	9.10	10.35	11.60	13.10
North Carolina flue-cured, type 12	7.25	8.40	10.15	12.80	15.10
South Carolina flue-cured, type 13	7.50	8.75	10.45	13.25	15.10

[a] Each moisture percentage is the average of duplicate determinations.

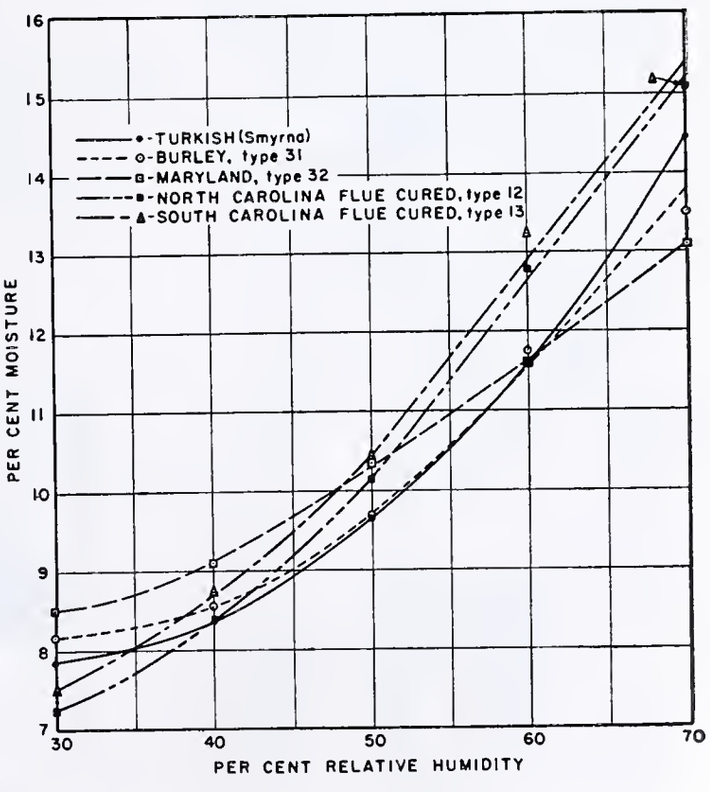

FIGURE 4. EQUILIBRIUM MOISTURE CONTENT OF CIGARET-TYPE TOBACCOS AT 80° F.

The success of this type of cabinet in dealing with tobacco problems involving moisture suggests that it can also be used in determining the moisture characteristics of a variety of other materials of plant and animal origin.

Acknowledgment

The authors wish to acknowledge the assistance and contributions of R. M. Cone of the Chemical Warfare Service, U. S. Army, and H. E. Foote and G. H. Alexander of Mellon Institute, all of whom have aided in the development of the type of cabinet reported here.

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Presented before the Division of Agricultural and Food Chemistry at the 103rd Meeting of the American Chemical Society, Memphis, Tenn.

A Temperature- and Humidity-Controlled Dryer for a Chemical Engineering Laboratory

ROBERT M. SCHAFFNER[1] AND JAMES COULL, University of Pittsburgh, Pittsburgh, Penna.

DRYING is one of the important unit operations, and it is necessary that a chemical engineering laboratory provide suitable equipment for undergraduate experiments on drying and for carrying out research work. These two requirements present slightly different problems. Small commercially constructed dryers enable students to become familiar with the general operations and performance of the larger commercial units. However, unless the apparatus is constructed to special specifications, which increase the cost of the equipment, it is not entirely satisfactory for research work. On the other hand, small dryers which have been designed especially for research work (*2*) frequently have structural features quite different from commercial dryers.

The writers designed and constructed a humidity- and temperature-controlled dryer that meets most of the requirements for research and instructional purposes at a total cost of less than $700 for materials, heater, fan, and control instruments.

The details of the design were determined from the writers' experiences and from recently published papers on drying (*1–3*).

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Design

The over-all size of the dryer is 5.5 feet long, 3 feet wide, and 5 feet high. The angle iron frame shown in Figure 1 was cut and welded and provisions for holding the trays, heater, fan, and pipe lines were made as shown. A 24 × 24 inch air heater consisting of horizontal steam pipes and vertical aluminum fins was purchased and attached to the frame as shown in section *A-A*. A 20-inch fan driven by a shaft and pulley was bought and attached to the frame, and a series of 1-inch angle iron pieces was bolted to the upper middle section of the frame to provide rests for the trays. The humidity supply line consisted of a manifold steam pipe with six outlets for sprays and was attached in front of heater. Upon completion of the framework, it was placed in position and bolted on a foundation of concrete 8 inches thick.

The walls of the dryer were constructed in small sections to enable any one of the panels to be removed without dismantling

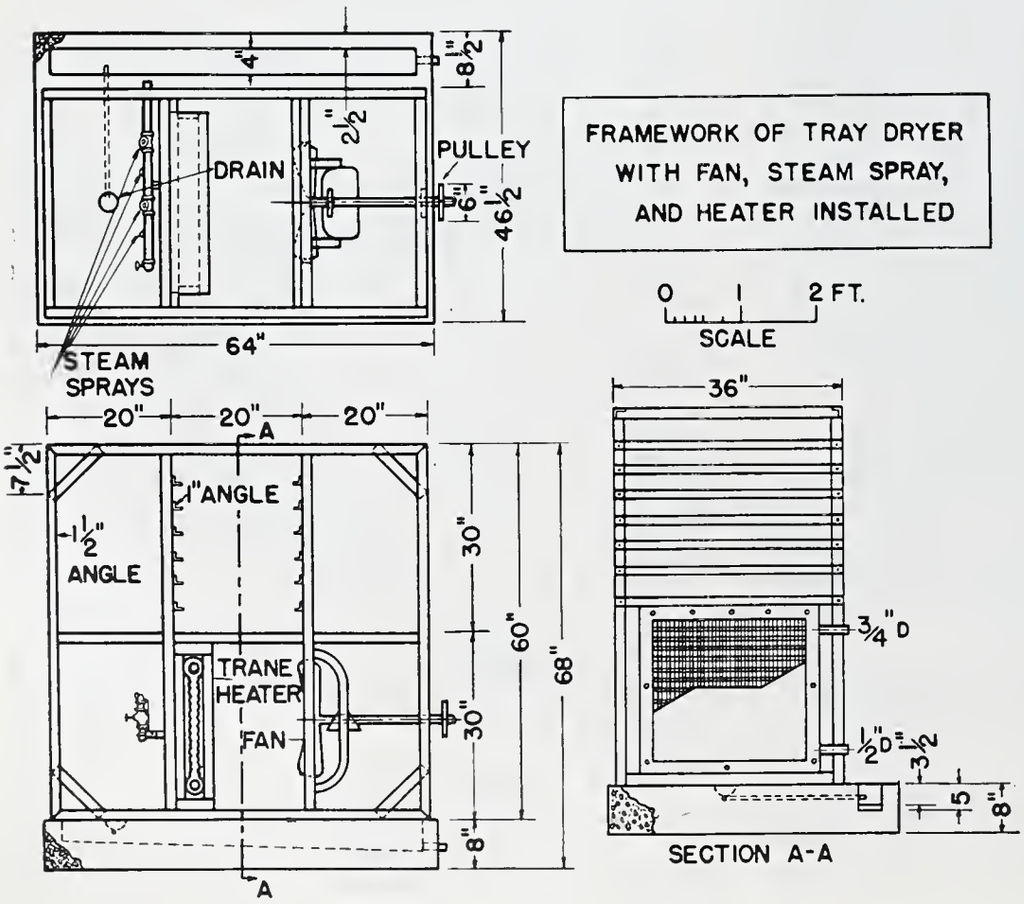

FIGURE 1

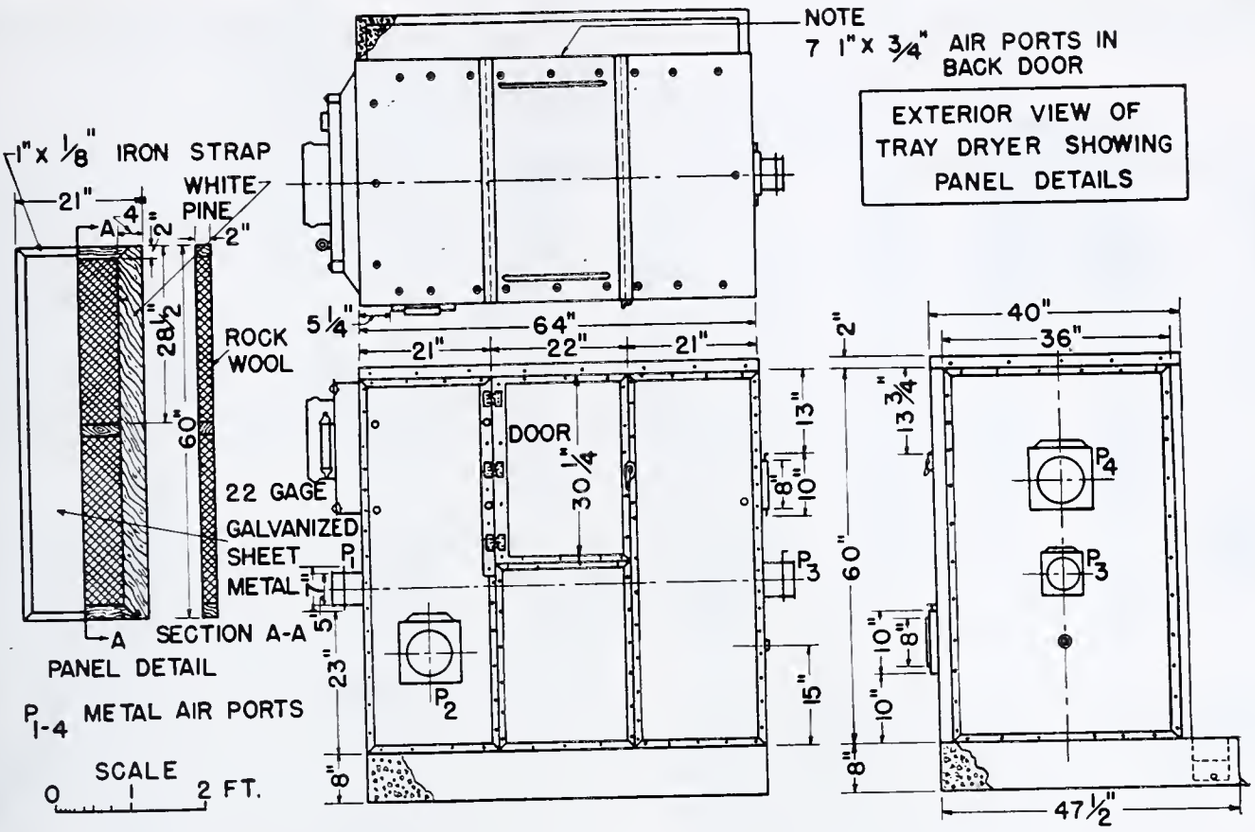

FIGURE 2

any of the others. The panels consisted essentially of white pine frames which surrounded the rockwool insulation. Each panel was then covered on all surfaces with galvanized sheet iron. All the overlapping edges of sheet metal were soldered together to prevent moisture from reaching the interior of the walls. Section *A-A*, Figure 2, illustrates the structural features of the panels. The ports, P_1 and P_3, for the air inlet and outlet and the observation ports, P_2 and P_4, were regular sheet metal air ducts with dampers. The top view of the roofing panels shows two slits which were used for inserting the weighing rods. Two door panels, one on each side of the tray compartment, were used on the dryer apparatus.

The automatic control apparatus was purchased and attached to the dryer as shown in Figure 3. Wet and dry-bulb vapor-type controllers open and close the steam lines to the sprays and heaters. When the dry-bulb temperature drops below the set condition, air opens a diaphragm valve attached to the heater steam line, and a similar setup is used for the steam

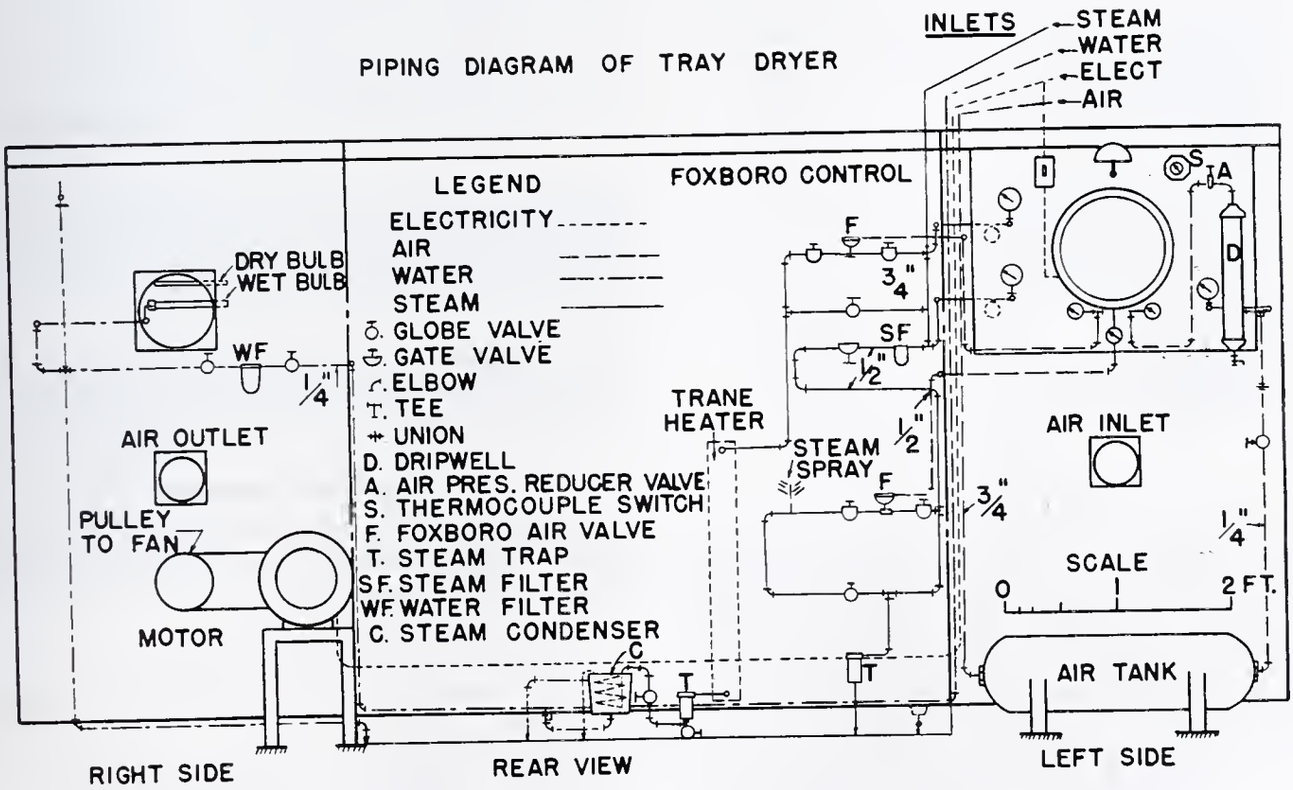

FIGURE 3

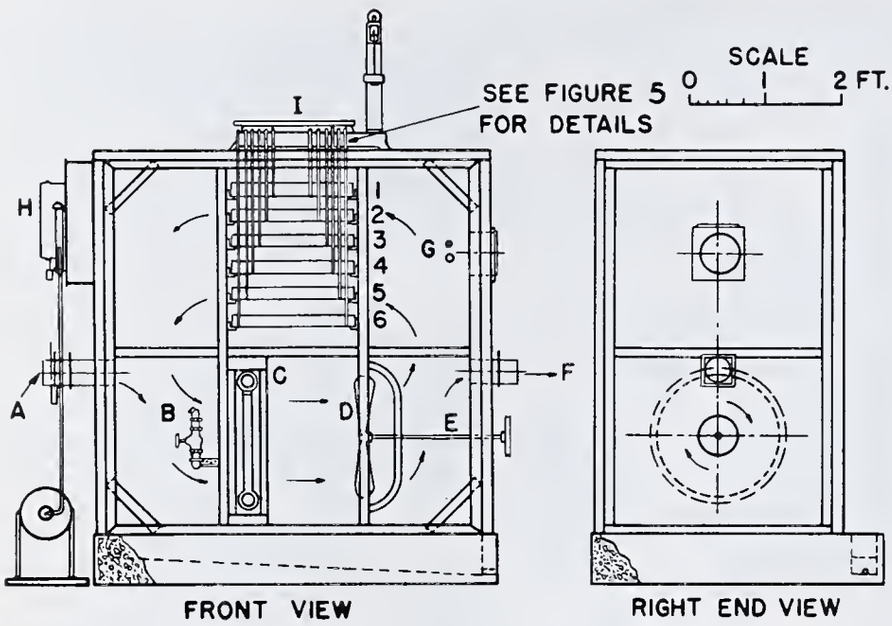

FIGURE 4 (*Left*). OPERATION OF TRAY DRYER

A. Air inlet
B. Steam spray
C. Heater
D. Fan
E. Shaft and pulley
F. Air outlet
G. Dry- and wet-bulb thermoregulators
H. Foxboro control
I. Platform scale

spray and wet-bulb controller system. The controller and supply lines and the motor for the fan are shown on Figure 3.

Copper trays having a drying surface of 4 square feet were constructed. In order to be able to weigh the trays without taking them out of the dryer, the writers designed the apparatus shown in Figure 4. This weighing mechanism consists essentially of a rectangular frame which is placed on a scale on the roof of the dryer, directly over the trays. To each tray, vertical rods (shown in detail in Figure 5) are bolted and extended up through the slots in the roof to the scale. The rods of a particular tray are hooked over the weighing frame when the weight of the tray is to be determined. By this method each tray can be individually weighed without disturbing the drying and without any loss of heat. Thermocouples were also placed on each tray and at various points in the dryer.

The itemized cost of the dryer for the price conditions prevailing in Pittsburgh during the fall of 1939 and spring of 1940 is as follows:

Unit	Cost
Angle iron and strip iron	$ 8.75
Galvanized sheet steel	11.85
White pine	2.88
Rock wool and weather stripping	2.79
Sheet metal ducts	10.00
Door fasteners and hinges	2.75
Paint	4.56
Cement and sand	3.25
Welding rod and solder	7.35
Pipes, valves, gages, etc.	72.01
1 air heater (Trane) 24 × 24 inches	49.75
Controller and valves (Foxboro)	325.80
Thermocouple wire and switch	2.62
Instrument panel, aluminum sheet	6.72
Thermometer	2.00
Bolts and other fastening	1.70
Fan (Breeze)	28.00
Pulleys	7.97
Motor for fan	37.00
Copper for trays and weighing rods	39.00
Fairbanks scale	34.20
Electrical fixtures and labor	27.45
	$688.40

Operation

In a typical experimental procedure the trays are filled with the testing materials, mixed with the desired amount of water, and placed in the dryer. The front and rear doors are opened, the weighing rods are bolted to the trays, and the thermocouple wires are placed on the surface of each tray. The temperature and humidity conditions are set on the control instrument and the steam, air, and water are turned on. The fan and pulley are set for a desired velocity and the motor is started. When the drying conditions have been reached, each tray is periodically weighed with the weighing apparatus.

The surface temperature of the materials on each tray may be determined with a potentiometer. The air velocity over each

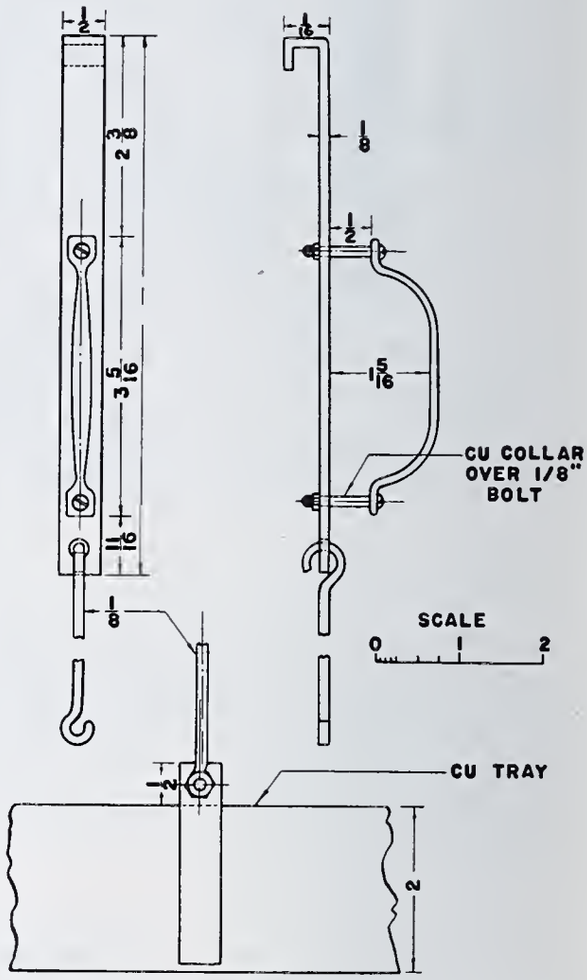

FIGURE 5. DETAIL OF A WEIGHING ROD

tray is obtained by using a duct jet and a velometer. These data are recorded periodically, and the drying is continued throughout the constant and falling rate periods. From the data obtained, the various drying calculations and correlations can be made for each material.

Reproducible drying data for a wide range of conditions have been obtained and studies of drying rates for various types of materials are being continued at the university.

Acknowledgment

The authors wish to thank the chemical engineering students for their assistance in constructing the dryer and for making the experimental runs.

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A Simple Viscometer for Research and Control

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The viscometer described consists of the better features of those described to date, assembled into one through adaptation of interchangeable ground joints. Rapidity is combined with rather low cost and accuracy equal to any of the accepted kinematic viscometers. A variation suitable for use with opaque liquids is presented.

THE routine determination of the viscosity of petroleum products has undergone great improvement in recent years as a result of the adoption of capillary-tube instruments in A. S. T. M. standard methods. Errors as high as ±0.5 per cent are permissible in the Saybolt Universal viscosity determination, but now kinematic viscosity may be measured to ±0.2 per cent. This improved accuracy has made possible very much closer determinations of viscosity index, particularly in the lighter grades of motor oil, toward which there has been a strong trend of late. The importance of more precise knowledge of viscosity index values occurs particularly in meeting specifications in commercial production. Table I shows the effect on viscosity index (*2*) of small errors in viscosity at 210° F. (98.89° C.). The viscosity index error resulting from the permissible Saybolt Universal viscosity error amounts to several units in the S. A. E. 10 range. This becomes somewhat less in the heavier oils, as shown by the examples of S. A. E. 50 grade.

A number of capillary-tube viscometers have been described in the literature as contributions toward improved viscosity measurement, including the designs of Cannon and Fenske (*1*), FitzSimons (*3*), Ruh *et al.* (*4*), Ubbelohde (*5*), and Zeitfuchs (*6*, *7*). All possess certain individual advantages and disadvantages. It was believed that practically all the advantages could be incorporated in one viscometer which would be equally usable in routine or research work. Consequently the instrument described below was designed with the following requirements as its basis.

ACCURACY. An improved viscometer should be capable of determinations as precise as obtainable with any of those recognized by A. S. T. M. Method D445-39T. It might suitably utilize the essential parts of one of the instruments described therein. It should be capable of still greater precision in research work, where time may be of less importance than in control laboratories.

SPEED. It should be possible to measure viscosities rapidly and to bring the equipment to proper temperature rapidly. Many of the smaller control laboratories operate only during the daytime and, therefore, it is of some value to them to be able to bring their viscometers to working temperatures within approximately 20 minutes.

COST. Many laboratories still using Saybolt viscometers would find it desirable to convert to capillary instruments in order to take advantage of their greater accuracy, if their price could be reduced. This, then, was considered to be an important requirement.

FLEXIBILITY. This is also associated with speed. A single laboratory may have occasion to determine the viscosities of oils ranging from S. A. E. 10 to bright stocks at 210° F. and from Diesel fuels to S. A. E. 60 or 70 oils at 100° F. (37.78° C.). It should be possible to make any of these measurements with an efflux time of 100 to 600 seconds. Any change of capillaries to meet this requirement should be accomplished quickly.

RUGGEDNESS. A viscometer which is easily broken is of little use in the long run and may become expensive. It is not believed that glass instruments per se are fragile, but their construction should be such that it will not be necessary to handle them often.

To satisfy all the above requirements, the vapor type of thermostat, essentially as described by FitzSimons (*3*), was used. This constant-temperature device has proved to be highly satisfactory, not only in research and inspection laboratories but also in the control rooms of process units where operators are enabled to check product quality at frequent intervals. The ability of this sort of thermostat to reach equilibrium temperature rapidly is often of considerable value. The absence of cyclical temperature changes is also an advantage. In these respects it differs radically from constant-temperature baths employing liquid media.

The Zeitfuchs viscometer (*6*, *7*) combines the advantages of simplicity, low cost, and freedom from temperature corrections, and was therefore chosen as the actual measuring device. To allow rapid interchangeability of viscometer parts, particularly with different sizes of capillaries, these were mounted in the inner halves of 45/12 interchangeable ground joints, the outer half forming the top of the vapor jacket. Thus a change from one viscometer to another requires but a few seconds.

The construction of the instrument is shown in Figure 1. Each viscometer part, *A*, of whatever capillary bore, is constructed exactly according to the directions of Zeitfuchs and is sealed into the inner half of a 45/12 interchangeable ground joint, *B*, at such a point that the top of the overflow tip is about 2 cm. below the ground portion of the joint. The vapor thermostat consists of the jacket, *C*, with an outer half of a 45/12 joint at the top. Just above the enlarged portion at the bottom, a side arm joins the circulation tube, *D*, the lower end of which carries the liquid returning from condenser *E* to the main body of liquid, filling 0.5 to 0.7 of the enlarged portion of the jacket. The condenser is constructed preferably as shown, vapors being condensed in an annular space between cool surfaces. This is particularly important when operating at 100° F. with cooling water temperatures as high as 70° to 75° F. (21° to 24° C.). The pressure inside the thermostat is reduced and maintained constant by the method described by FitzSimons, connection being made to the top of the condenser.

Zeitfuchs has given the dimensions of six viscometers designed to cover the normal range of viscosities encountered

TABLE I. VISCOSITY INDEX ERRORS RESULTING FROM SMALL VISCOSITY ERRORS

True Viscosity at 210° F. *Cst.*	Measured Viscosity at 210° F. *Cst.*	Viscosity Error at 210° F. %	Viscosity Index
Kinematic viscosity at 100° F. = 20.550 cst.			
4.000	4.000	0.0	100.0
4.000	4.010	0.25	101.3
4.000	4.020	0.50	102.7
Kinematic viscosity at 100° F. = 26.820 cst.			
4.000	4.000	0.0	0.0
4.000	4.010	0.25	2.0
4.000	4.020	0.50	4.0
Kinematic viscosity at 100° F. = 249.31 cst.			
20.00	20.00	0.0	100.0
20.00	20.05	0.25	100.3
20.00	20.10	0.50	100.6
Kinematic viscosity at 100° F. = 551.07 cst.			
20.00	20.00	0.0	0.0
20.00	20.05	0.25	0.8
20.00	20.10	0.50	1.6

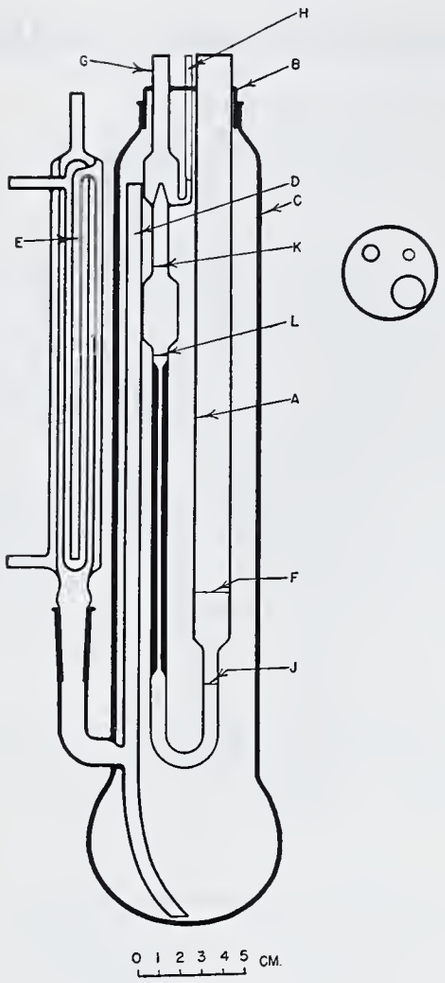

FIGURE 1. VISCOMETER

in petroleum products. Since viscometer constants somewhat different from these may be desired for special cases, the following equation is given for calculating capillary-bore radius, all other dimensions being held as described by Zeitfuchs:

$$\log r = \frac{\log C_1}{4} - 0.97169$$

where r = capillary bore radius in cm. and C_1 = viscometer constant when viscosity is in centistokes and time is in seconds. In general, the required capillary radius for any kinematic viscometer operating under the influence of gravity can be closely approximated by the following equation:

$$\log r = \frac{\log C_1 - \log\left(\frac{8V}{100\,\pi g}\right)}{4}$$

where V = volume in ml. between marks, g = gravitational constant in cm. per second per second, and other symbols are as in the preceding equation. The simplifying assumption that the average hydrostatic head is within a few per cent of the capillary length, is employed.

Viscometers with simple calibration constants, such as 0.1000 or 0.2500, have been advocated by some, but it is believed that in most applications little time is saved by this refinement. The Saybolt Universal units are still so firmly entrenched that it is usually necessary to convert from centistokes to S. U. seconds by use of a table. Furthermore, the kinetic energy correction often enters to a significant degree, thus requiring an added tabular reference. All conversions may be incorporated in one table, for each viscometer, giving the relation between efflux time and viscosity in S. U. seconds at the three commonly used temperatures.

To operate the viscometer, the appropriate liquid is added to the thermostat to a point about 1 to 2 cm. below the side arm. The most suitable liquids are methylene chloride at 100° F. (37.78° C.), acetone at 130° F. (54.44° C.), and tertiary amyl alcohol at 210° F. (98.89° C.). The proper viscometer is set in place, well lubricated with a material which is not too stiff at room temperature. An A.S.T.M. kinematic-viscosity thermometer hangs from the drain tube of the viscometer. The liquid is heated by means of an electric flask heater of approximately 250 watts at 100° and 130° F. or 350 watts at 210° F. For maximum flexibility a 550- to 660-watt heater, controlled by an autotransformer, may be used when alternating current is the source of power. In hazardous locations steam heat has been successfully employed.

Cool water is passed through the condenser. Pressure inside the thermostat is reduced by means of a water aspirator or other type of evacuating pump. The pressure will fall until air enters through the water column, the height of which determines the pressure and therefore the temperature inside the apparatus. The temperature is adjusted to the exact point desired by raising the water column to lower the temperature or by lowering the water column to raise the temperature. As described by Zeitfuchs, the liquid whose viscosity is required is poured into the large tube of the viscometer to mark F, and is raised by application of suction to G, with H closed, until the liquid level falls to J. The amount which has not overflowed through the tip is, then, the standard volume with which the viscometer is calibrated and with which viscosity determinations are made thereafter. Excess liquid is withdrawn through H. When the thermostat is installed, one of the viscometer parts is inserted and the orientation of the jacket adjusted so that the capillary will be truly vertical. Since in their manufacture the capillaries are placed parallel to the axis of the ground joint, all viscometer parts will be satisfactorily aligned when they are in place in the thermostat.

After filling the viscometer with the correct amount of liquid, viscosities are measured by merely noting the time required for the meniscus to fall from K to L. This, times the constant found in calibrating, less the kinetic energy correction, gives the kinematic viscosity of the liquid. Calibration is accomplished preferably by the use of water as a primary reference. Alternatively the viscosity standards of the A. P. I. or of the National Bureau of Standards may be used.

The average control and inspection laboratory would probably set up two vapor thermostats for each inspector giving his full time to viscosity measurements. One of these would operate at 210° F., the other at 100° F. A series of viscometer parts would be available, so that the viscosities of the various samples could be determined with an efflux time of 100 to 600 seconds, thus conserving the operator's time. If the viscosity at 210° F. of an S. A. E. 50 motor oil is required immediately after running an S. A. E. 10 oil, a quick change from a viscometer with a constant of approximately 0.025 to one with a constant of about 0.100 would provide efflux times of 150 to 200 seconds for each.

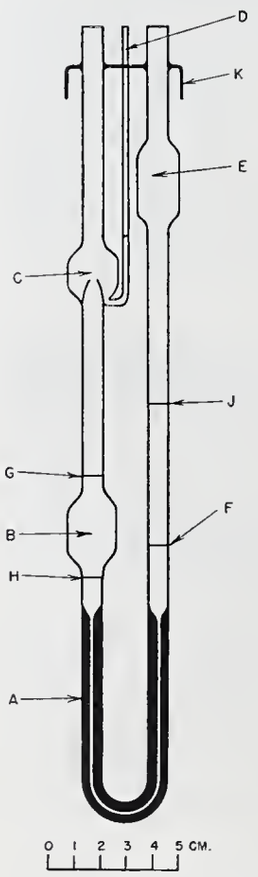

FIGURE 2. VISCOMETER FOR OPAQUE LIQUIDS

It is difficult to use any of the kinematic viscosity instruments, so far described, with opaque liquids such as residual oils and solvent extracts. For such applications, the instrument shown in Figure 2 was designed. It is a modification of the Zeitfuchs viscometer, possessing all its advantages, including convenience of proper filling.

In use, the viscometer is filled approximately to mark J. When the liquid has attained operating temperature, the level is depressed from J to F, the excess overflowing at C. To start a viscosity determination, the liquid level is depressed to a point a little below mark H. The efflux time is that elapsing during the passing of the meniscus between points H and G under the sole influence of gravity. Since the body of the liquid advances over the marks, there is no difficulty in the measurement of the efflux time even when its color is very dark. This instrument is suitable for use with residual oils, the less viscous solvent extracts, and similar materials; however, it is not designed for highly viscous materials of any type. Perhaps 2000 centistokes would represent the maximum practically measurable viscosity.

Acknowledgment

The author wishes to express his appreciation to R. Shutt for his continued interest during the course of this work.

Grateful acknowledgment is made to the Battelle Memorial Institute for permission to publish this material.

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Presented before the Division of Petroleum Chemistry at the 102nd Meeting of the American Chemical Society, Atlantic City, N. J.

An Electromagnetic Densitometer

A. R. RICHARDS, Trinidad Leaseholds, Ltd., Pointe-à-Pierre, Trinidad, B. W. I.

An apparatus is described in which the principle of balancing the attraction of a magnetic field against the upthrust on a submerged armature is used as a rapid method of measuring liquid densities. The precision of the determinations depends on the range of the instrument and is of the order of 1 part in 800 for densities between 0.62 and 0.82 gram per ml.

CONSTRUCTION of the densitometer described below was undertaken in order to facilitate density determinations on some two hundred 5-ml. samples every 8 hours. Ordinary hydrometers were unsuitable, since they require at least 20 ml. of sample, while pycnometer measurements would have been too slow.

The essential parts of the instrument consist of a small glass float held under the surface of the sample by an adjustable stop and a small coil surrounding the lower portion of the sample tube. If current passing through the coil is gradually increased, the resulting magnetic field will eventually exceed a critical value, such that the force on the armature will become sufficient to draw the float away from the stop. If suitable precautions are taken, the density of the sample may be determined accurately by measurement of the critical current.

Theory

The theoretical considerations affecting the design of the apparatus can be expressed in the following manner:

Let the pole strength induced in the armature be m and the magnetic potential at these poles be V_N and V_s. It follows that the potential energy, E, of the armature in the field is given by:

$$E = m(V_N - V_s) = \frac{m\Delta s\ (V_N - V_s)}{\Delta s}$$

where Δs is the distance between the poles.

Now $(V_N - V_s)/\Delta s = H$, the strength of the field, and $m\Delta s = M = vI$ where M, v, and I represent the magnetic moment, volume, and intensity of magnetization of the armature, respectively. Thus

$$E = vIH$$

The work done during a small displacement, ds, of the armature is given by the equation which follows:

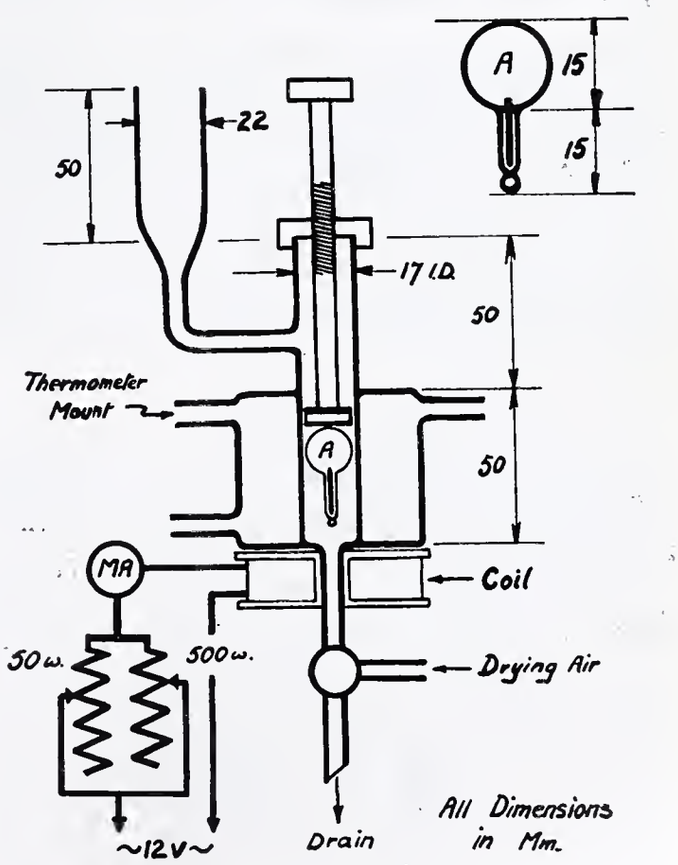

Figure 1. Diagram of Apparatus

$$Fds = vd(IH)$$

where F is the force acting on the armature along the direction of displacement. Then

$$F = \frac{vd(IH)}{ds}$$

The relation between I and H depends on the susceptibility, k, and the geometrical form of the armature. For small values of H, k may be regarded as constant and in general,

$$I = Hf(k)$$

and

$$F = \frac{vd(IH)}{ds} = \frac{vd(H^2f(k))}{ds} = AH\frac{dH}{ds}$$

Now $H = i\psi(s)$ where i is the current flowing in the coil producing the field, and $\psi(s)$ is a function determined by the configuration of the system. Therefore,

$$\frac{dH}{ds} = i\Psi'(s)$$

Hence

$$F = Bi^2\,\Psi(s)$$

Since the float always starts from the same position, the value of $\Psi(s)$ has a fixed value for any given position of the stop. Hence, $F = Ci^2$ where C is a constant.

If the volume of the float is V and the density of the liquid is ρ, the upthrust on the totally immersed float is ρV. When the current in the coil reaches the critical value the opposing force is $F + W$, where W is the weight of the float

$$\rho V = Ci^2 + W$$

or

$$\rho = Ri^2 + P$$

where R is constant, providing the geometrical arrangement of the apparatus is reproducible, and P is the density of the float.

In order to prevent permanent magnetization of the armature and minimize sticking of the float, alternating current from a step-down transformer is used in the field coil. The value of i in the above equation is in practice, therefore, the value recorded by an alternating current milliammeter. A Westinghouse Type PY5 has been found suitable in this service. The sensitivity of the densitometer is increased and the range decreased by increasing the size of the float, reducing the size of the armature, and reducing the number of turns in the field coil. Since the float is submerged, surface tension effects are eliminated.

Experimental

The detailed design of the apparatus is shown in Figure 1. The float, A, constructed from Pyrex glass, encloses an armature made from a short length of iron nail. The mean density of the float is adjusted by adding or removing glass at the tip until it just floats in liquid, the density of which corresponds to the lowest value of the range required. The inside diameter of the float tube should be about 2 mm. greater than that of the float to ensure a free passage without unduly increasing the volume of the apparatus. The float is centered on the bottom of the stop. The lower end of the float tube is sloped to allow easy drainage and the drain tube allows 0.5-mm. clearance around the tail of the float. The three-way tap is sealed on after mounting the coil; the lower limb is used as a drain and the side limb serves to admit air for drying the apparatus. The bearing for the adjustable stop is cemented in place after insertion of the float. The coil is wound on an ebonite former with a thin core and consists of about 28 grams (1 ounce) of double cotton-covered copper wire 23 A. W. G. It is connected in series with a 20-volt alternating current transformer and the variable resistance assembly shown. This circuit gives the most satisfactory control.

A water jacket completely surrounding the float chamber is used for maintaining a standard temperature during determinations. Drift due to rise in temperature of the coil is not noticeable with currents below 600 milliammeters and the insertion of a water jacket between the drain tube and the coil is not necessary. It is easy to maintain a temperature constant to within ±0.2° C. which produces an error of less than 0.0002 gram per ml. in the densities of gasoline fractions.

The instrument can be reset after cleaning by adjusting the position of the stop until the milliammeter reading for a liquid of known density agrees with the original calibration. This setting is checked by the use of a second liquid.

By analysis of the experimental figures given in Table I, it can be shown for this instrument that

$$d^{20}_{vac.} = 0.701\,i^2 + 0.621 \qquad (1)$$

Table I. Density Measurements

Current, Amperes	$d^{20}_{vac.}$ from Pycnometer	$d^{20}_{vac.}$ from Equation 1	Difference, Calcd. and Found
0.085	0.6255	0.626	+0.000 (5)
0.152	0.6370	0.637	+0.000
0.179	0.6429	0.643	+0.000 (1)
0.223	0.6563	0.656	−0.000 (3)
0.302	0.6842	0.684	−0.000 (2)
0.383	0.7230	0.723	−0.000
0.397	0.7309	0.732	+0.001 (1)
0.424	0.7445	0.746	+0.001 (5)
0.425	0.7458	0.747	+0.001 (2)
0.445	0.7598	0.759	−0.000 (8)
0.459	0.7673	0.768	+0.000 (7)
0.475	0.7795	0.778	−0.001 (5)
0.477	0.7811	0.780	−0.001 (1)
0.488	0.7874	0.788	+0.000 (6)
0.500	0.7962	0.797	+0.000 (8)
0.502	0.7974	0.798	+0.000 (6)
0.515	0.8082	0.807	−0.001 (2)
0.521	0.8146	0.813	−0.001 (6)

Although the range of measurement can be adjusted, it is not convenient to construct an instrument with a range of more than 0.2 gram per ml., since the precision is limited by the accuracy with which the milliammeter may be read, which is of the order of ±1 ma. over the 750-ma. range. Substituting the value of this uncertainty, δi, in the following equation

$$d^{20}_{vac.} \pm \delta d = 0.701\,(i \pm \delta i)^2 + 0.621$$

gives the maximum uncertainty in the density, δd, as ±0.0007 gram per ml. for currents not in excess of 500 ma. A similar error arises during calibration, so that the maximum error is not more than ±0.0014 gram per ml. If the instrument has been cleaned and reset but not recalibrated, a further additional error of not more than ±0.0007 gram per ml. must be included, making the greatest uncertainty under the least favorable conditions not more than ±0.0021 gram per ml. or 1.0 per cent of the useful range.

The difference between the observed and calculated densities shown in Table I exceeds in certain cases the estimated precision for given current values. This is due to absolute errors in the milliammeter, since it is stated by the makers that the values indicated may differ from the absolute values by 0.5 per cent of the full-scale readings of both the 250- and 750-ma. ranges. In view of the above, it is recommended that densities be read from an experimental curve determined for a given instrument and milliammeter and covering the entire range.

Since the sensitivity can be increased at the expense of the range, the ultimate precision is limited only by temperature control and the reproducibility of the initial system.

Acknowledgment

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Detection of Orthophosphates by Means of Drop Reactions

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MANY methods for detecting or determining orthophosphates are reported in the literature, but little has been published concerning their detection by means of drop reactions. In the work that has been reported, the studies of interferences have often been incomplete and there is little agreement between the results of various investigators. In order to evaluate the more promising reactions, comparative data concerning interferences and sensitivities were obtained. After an extensive survey of the literature, the strychnine molybdate (*2*), ammonium molybdate–stannous chloride (*3*), and ammonium molybdate–benzidine (*5*, *7*, *8*) tests were selected for study. A modified strychnine molybdate test devised by the authors was also included in the investigation.

Scope of Investigation

The following ions and compounds were studied, using 1 per cent solutions, in regard to the ion or compound in question:

Group I. Li^+, Na^+, K^+, Cu^{++}, Rb^+, Ag^+, Cs^+, Au^{+++}

Group II. Be^{++}, Mg^{++}, Ca^{++}, Zn^{++}, Sr^{++}, Cd^{++}, Ba^{++}, Hg^+, Hg^{++}

Group III. BO_2^-, $B_4O_7^{--}$, Al^{+++}, Ga^{+++}, Y^{+++}, In^{+++}, La^{+++}, Ce^{+++}, Tl^+

Group IV. CO_3^{--}, SiO_3^{--}, Ti^{+++}, Sn^{++}, Sn^{++++}, Pb^{++}, Zr^{++++}, Th^{++++}

Group V. NH_4^+, NO_2^-, NO_3^-, $H_2PO_2^-$, $P_4O_{13}^{------}$, $P_6O_{18}^{------}$, PO_3^-, HPO_4^{--}, $P_2O_7^{----}$, V^{+++}, VO_4^{---}, AsO_2^-, $HAsO_4^{--}$, Sb^{+++}, Bi^{+++}

Group VI. S^{--}, $S_2O_3^{--}$, SO_3^{--}, SO_4^{--}, Cr^{+++}, CrO_4^{--}, SeO_3^{--}, SeO_4^{--}, MoO_4^{--}, TeO_3^{--}, TeO_4^{--}, WO_4^{--}, UO_2^{++}, UO_4^{--}

Group VII. F^-, Cl^-, ClO_3^-, Mn^{++}, MnO_4^-, Br^-, BrO_3^-, I^-, IO_3^-, ReO_4^-

Group VIII. Fe^{++}, Fe^{+++}, Co^{++}, Ni^{++}, Ru^{+++}, Rh^{+++}, Pd^{++}, OsO_5^{--}, Ir^{++++}, Pt^{++++}

Miscellaneous Substances. CN^-, $Fe(CN)_6^{---}$, $Fe(CN)_6^{----}$, CNS^-, acetate, oxalate, malonate, adipate, succinate, phthalate, tartrate, citrate, lactate, gluconate, *i*-inositol, *d*-sorbitol, mannitol, sucrose, dextrose, aniline, pyridine, resorcinol, and catechol

The solutions were made up and the results reported in the manner of West (*10*)—that is, interfering substances which gave a test similar to that given by orthophosphate were listed as "positive" interferences, those substances which inhibited the formation of true tests for phosphate were listed as "negative" interferences, and those substances which were so highly colored or gave such highly colored reaction products that test colors were hidden, were listed as "masking" interferences. The actual investigation of interferences was carried out with a ratio of HPO_4^{--} to interfering substance of 1 to 10. This ratio constituted a stringent test of the method and at the same time indicated what could be expected of it under reasonable working conditions. Where more favorable ratios exist there may be fewer interferences, but the use of the test in the presence of such questionable interferences would entail considerable risk. If ratios less favorable than 1 to 10 are encountered there may be a few more negative interferences than are reported in these studies. However, no additional positive interferences should be encountered unless the concentration of the test solution is increased beyond 1 per cent, which is the highest concentration to be recommended for use with drop reactions.

C.P. chemicals were used throughout the investigation.

Experimental

STRYCHNINE MOLYBDATE TEST. It has long been known that orthophosphate in various complex combinations is a delicate reagent for alkaloids. Denigès (*2*) reversed the reaction, using the alkaloidal combinations for the detection of phosphates.

Reagents. Denigès proposed a reagent made up by dissolving 0.5 gram of strychnine sulfate in water, adding 10 ml. of concentrated nitric acid and 10 ml. of nitro ammonium molybdate solution (Sonnenschein and Eggertz formula), and diluting the solution to 100 ml. Because the concentration of the nitric acid in the reagent has a great influence on the number of interferences, the authors suggest a slight modification in the composition of the reagent. It was found that a reagent prepared as follows gave the best results: To 30 ml. of concentrated nitric acid are added 2 grams of molybdic acid, and it is diluted to 75 ml., and stirred until solution is complete. Then 0.6 gram of strychnine sulfate is added and heated gently until all the strychnine has dissolved. It is diluted to 100 ml. and heated slightly below the boiling point for 3 to 5 minutes until a yellow solution is obtained.

Method of Study. The interference study was carried out on a black spot plate in the following manner:

1. To 1 drop of 1 per cent solution of element under consideration was added 1 drop of strychnine molybdate reagent.
2. To 1 drop of 1 per cent solution of element under consideration was added 1 drop of 0.1 per cent solution of phosphate plus 1 drop of strychnine molybdate reagent.

A yellow precipitate indicated a positive test, and variations in color due to interferences were noted.

Results of Investigation. Positive interferences: Au^{+++}, Be^{++} (very slight), VO_4^{---}, $HAsO_4^{--}$, WO_4^{--}, Pt^{++++} (slight). Negative interferences: V^{+++}, As^{+++}, Sb^{+++}, F^-, $C_2O_4^{--}$, CNS^-, (slight), $P_6O_{18}^{------}$, $P_4O_{13}^{------}$, $P_2O_7^{----}$, Ti^{+++}, Zr^{++++}, Sn^{++}.

Masking interferences: S^{-} (brownish black precipitate), $S_2O_3^{--}$ (reduces the phosphomolybdate to molybdenum blue), I^{-} (brown precipitate), MnO_4^{-} (color of ion), $Fe(CN)_6^{----}$ (brown precipitate), Ir^{++++} (color of Ir^{++++} colors test precipitate brown).

The polyphosphates reduce the sensitivity of the test probably through a competitive reaction with the reagent, but soon begin to revert to orthophosphate when put in solution. Consequently, tests for orthophosphates are obtained from solutions of polyphosphates which are allowed to stand, regardless of the particular test procedure followed.

Table I. Interferences

Test	Limit of Identification (Gram of P)	Limiting Concentration of HPO_4^{--}	Number of Positive Interferences	Number of Negative Interferences	Number of Masking Interferences
Strychnine molybdate	1.6×10^{-8}	100 cu. mm. of 0.005% solution	6	12	6
Ammonium molybdate–stannous chloride	1.6×10^{-8}	100 cu. mm. of 0.005% solution	4	2	10
Ammonium molybdate–benzidine	9.7×10^{-8}	30 cu. mm. of 0.001% solution	13	1	13
Modified strychnine molybdate	9.7×10^{-8}[a]	30 cu. mm. of[a] 0.1% solution	0	7	3

[a] If confirmatory test is run on filter paper, limiting concentration is 30 cu. mm. of 0.01 per cent solution in regard to HPO_4^{--}. This is equivalent to 9.7×10^{-7} gram of phosphorus.

Ammonium Molybdate–Stannous Chloride Test. Denigès (*3*) proposed the ammonium molybdate–stannous chloride test for the detection of phosphates and arsenates. The heteropoly acid formed when ammonium molybdate is added to an acid solution of soluble phosphates or arsenates is readily reduced by stannous chloride. The molybdenum blue which is formed by this reduction is discussed by Denigès (*4*) in regard to stable and unstable forms. Chapman (*1*) showed that the silicomolybdate may also be formed when acidity is low. Once this complex silicomolybdate is formed it is stable, even after the acidity is increased. Interference, when as much as 700 parts per million of silica is present, is avoided if the acid is added first, followed by the molybdate reagent.

Reagents. Ammonium molybdate, 5 grams dissolved in 100 ml. water and poured into 35 ml. of nitric acid (sp. gr. = 1.2).

Stannous chloride, 0.96 gram of stannous chloride dihydrate dissolved in 50 ml. of 1 to 2 hydrochloric acid solution. This solution should be made up fresh.

Hydrochloric acid, approximately 4 *N*.

Method of Study. The test was carried out on a white spot plate.

1. To 1 drop of the 1 per cent solution of the element under consideration was added 1 drop of 4 *N* hydrochloric acid, plus 1 drop of ammonium molybdate solution, plus 1 drop of stannous chloride.
2. To 1 drop of 1 per cent solution of the substance under consideration was added 1 drop of 0.1 per cent solution of orthophosphate, plus one drop of 4 *N* hydrochloric acid plus 1 drop of ammonium molybdate solution, plus 1 drop of stannous chloride. A blue-green coloration was an indication of a positive test for phosphate.

Results of Investigation. Positive interferences: Be^{++} (very slight), Ti^{+++} (fades out quickly), $HAsO_4^{--}$, WO_4^{--}. Negative interferences: $C_2O_4^{--}$, lactate (slight).

Masking interferences: Au^{+++} (blue to black color), SeO_3^{--} (orange precipitate), SeO_4^{--} (orange precipitate), TeO_3^{--} (black precipitate, fades out quickly, so does not interfere with the test), TeO_4^{--} (same as TeO_3^{--}), S^{-} (black precipitate), $Fe(CN)_6^{---}$ (dark brown precipitate), $Fe(CN)_6^{----}$ (dark red color), CNS^{-} (red color), Pd^{++} (modifies test color to a very dark dirty green).

Ammonium Molybdate–Benzidine Test. Feigl proposed (*7*) the use of an acetic acid solution of benzidine hydrochloride as a means of confirming the presence of phosphorus in the yellow precipitates produced by ammonium molybdate. The precipitate, to which benzidine has been added, is held over an open ammonia bottle. If the precipitate is composed of ammonium phosphomolybdate, a blue color is developed. According to Feigl (*8*) a blue coloration will be obtained if 0.00066 mg. of phosphorus pentoxide is present. An even more sensitive test is obtained (*6*) if the ammonium molybdate solution is placed on "blue band" filter paper and dried in an air oven. If a drop of the test solution is added to freshly prepared molybdate paper, followed by a drop of benzidine and a drop of saturated sodium acetate solution, a blue fleck is formed if 0.05 microgram of phosphorus pentoxide is present. It has been claimed (*9*) that interferences with this test from silicates and borates are prevented if the ammonium molybdate solution contains 15 per cent tartaric acid. The use of tartaric acid, however, seems to be impractical, since it was found that it not only prevented reactions with silicates and borates, but also inhibited the reaction with phosphates. The molybdate paper–sodium acetate method was selected for study.

Reagents. "Blue band" filter paper is impregnated with 5 per cent ammonium molybdate solution and dried in an open air oven.

Benzidine, 0.05 gram of benzidine hydrochloride dissolved in 10 ml. of glacial acetic acid and diluted to 100 ml. with water.

Sodium acetate, saturated solution.

Method of Study. The test was run on the impregnated filter paper in the following manner:

1. To the impregnated molybdate paper was added 1 drop of 1 per cent solution of the ion being studied, plus 1 drop of benzidine solution, followed by 1 drop of the sodium acetate solution.
2. To the impregnated molybdate paper was added 1 drop of 1 per cent solution of the ion being studied, plus 1 drop of 0.1 per cent solution of orthophosphate. One drop of benzidine was then added, followed by 1 drop of sodium acetate solution. A blue fleck indicated a positive test for orthophosphate.

Results of Investigation. Positive interferences: Be^{++}, SiO_3^{--}, V^{+++} (color of ion), VO_4^{---}, $HAsO_4^{--}$ (this reaction is much slower than the phosphate test), $S_2O_3^{--}$, Cr^{+++} (color of ion), CrO_4^{--} (purple), SeO_3^{--} (slight), TeO_3^{--} (slight), BrO_3^{-} (slight), I^{-}, Fe^{++}, CN^{-}, $Fe(CN)_6^{----}$.

Negative interference: F^{-}.

Masking interferences: Cu^{++} (brown stain), Au^{+++} (brown to blue stain), Ti^{+++} (blue to black precipitate forms immediately upon addition to paper), Sn^{++} (same as titanium), NO_2^{-} (brown stain), Sb^{+++} (causes immediate formation of blue stain in the presence of phosphate), S^{--} (black stain), MnO_4^{-} (brown stain), Fe^{+++} (slight masking effect due to color of ion), $Fe(CN)_6^{----}$ (brown stain), catechol (brown stain), Ru^{+++} (masking is due to color of ion), Ir^{+++} (brown stain).

Modified Strychnine Molybdate Test. As a result of the investigation of known drop test procedures a modified strychnine molybdate test was developed which the authors believe superior to other tests for phosphate, particularly in regard to specificity (see Table I). The new test is identical to the Denigès strychnine molybdate procedure except that the precipitate formed with strychnine molybdate is treated with a reducing agent in order to establish the presence of phosphorus. Several reducing agents were tried, including stannous chloride, thiosulfate, 1-amino-2-naphthol-4-sulfonic acid, and benzidine. Benzidine proved to be the best of those tried.

Reagents. Strychnine molybdate, 2.0 grams of molybdic acid added to 20 ml. of concentrated nitric acid and diluted to 75 ml. with stirring until solution is complete. Strychnine sulfate (0.6 gram) is added and heated gently until all the strychnine has dissolved. It is diluted to 100 ml. and heated slightly below the boiling point for 3 to 5 minutes until a yellow solution is obtained.

Benzidine, 0.05 gram of benzidine hydrochloride dissolved in 10 ml. of glacial acetic acid and diluted to 100 ml. with water.

Sodium acetate, saturated solution.

Method of Study. The test is best carried out on a combination black and white spot plate designed by West (*11*). If a combination spot plate is not available, the strychnine molybdate reagent is first added to the test solution on a black spot plate. If a precipitate forms, a confirmatory test is run on a white spot plate. For detection of phosphate in quantities below 0.01 mg. it is suggested that the confirmatory test be carried out on a good grade of quantitative filter paper.

1. To 1 drop of 1 per cent solution of the ion being studied was added 1 drop of strychnine molybdate reagent. If a precipitate could be seen against the black background of the combination spot plate its identity was established by the further addition of 1 drop of benzidine reagent, followed by enough sodium acetate solution to neutralize the excess acid present.

2. To 1 drop of a 1 per cent solution of the ion under consideration was added 1 drop of a 0.1 per cent solution of phosphate, plus 1 drop of the strychnine molybdate reagent. If a precipitate could be seen against the black background of the combination spot plate its identity was established by the further addition of 1 drop of benzidine reagent, followed by enough of the sodium acetate solution to neutralize the excess acid. A blue-green color, after the addition of the sodium acetate solution, which could be seen against the white background of the combination spot plate, indicated a positive test for phosphate.

Results of Investigation. Positive interferences: None.

Negative interferences: As^{+++} (slight interference in a neutral solution), V^{+++} (no interference in a neutral solution), F^-, $C_2O_4^{--}$ (slight), $P_6O_{18}^{------}$, $P_4O_{13}^{------}$, $HAsO_4^{--}$ (slight).

Masking interferences: S^{--} (brown-black precipitate), MnO_4^-, $Fe(CN)_6^{----}$ (brown precipitate).

The results obtained from the interference study are listed in Table I. Of the tests studied not one was entirely free from all types of interference.

The interference study was run with solutions in which the ion being studied was most stable. This made it necessary to run some of the tests in rather strong acid solutions, others in basic solution. For detecting phosphates in unknown solutions by any of the four methods studied, best results are obtained if the original test solution is nearly neutral or weakly acid.

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Microestimation of Bromide as Pentabromorosaniline

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GUARESCHI (*1*), in 1913, reported that bromine vapor caused paper impregnated with Schiff's reagent to turn violet. Since then there have been a number of unsuccessful attempts to apply this reaction to the quantitative determination of bromide (*2*, *3*, *5*). Although Wikoff, Bame, and Brandt (*4*) claim to have measured the bromide content of normal blood by its use, the present writer has been unable to duplicate their results.

There are described below the conditions under which bromide is quantitatively oxidized to bromine and substituted on rosaniline (reduction unnecessary) to give pentabromorosaniline, which can be separated completely from unreacted pigment by extraction into benzyl alcohol from 7 *N* sulfuric acid and determined photometrically.

Experimental

Preparation of Pentabromorosaniline. Two grams of bromine were added to 1 gram of rosaniline in dilute acetic acid. The purple precipitate was recovered in 92 per cent yield, based on the expected formation of pentabromorosaniline acetate. It was found to be impure, and was separated from chloroform-soluble material by Soxhlet extraction. It crystallized from methanol in dark green prisms with intense metallic luster. There was no sharp melting point. An analysis was conducted by E. R. Duncan, to whom the author is indebted for the following report:

$C_{22}H_{18}N_3Br_5O_2$: Calculated,	C 34.87	Found,	34.91, 34.37
	H 2.42		3.03, 2.83

The salts of pentabromorosaniline are insoluble in water, but are soluble in polar organic liquids. They are strongly adsorbed even on glass. Triaminotriphenylcarbinol pigments are multiple indicators. The pentabromorosaniline may be separated from rosaniline by extraction from 7 *N* sulfuric acid by benzyl alcohol, at which strength of acid the rate of change of color with change of pH of pentabromorosaniline is minimal and its distribution coefficient between alcohol and acid is most favorable. At this concentration, the solubility of the yellow triacid form of rosaniline in benzyl alcohol is very low. The extinction curve of pentabromorosaniline in a benzyl alcohol extract from 7 *N* sulfuric acid is given in Figure 1. In benzyl alcohol, pentabromorosaniline follows Beer's law at 585 millimicrons. The molar extinction coefficient, $\bar{E}_{585} = 1.57 \times 10^5$, is calculated according to the formula:

$$E\lambda = \frac{2.303}{d.c} (\log I_0/I)$$

Oxidation of Bromide to Bromine and Addition of Bromine to Rosaniline. Of the many oxidizing solutions

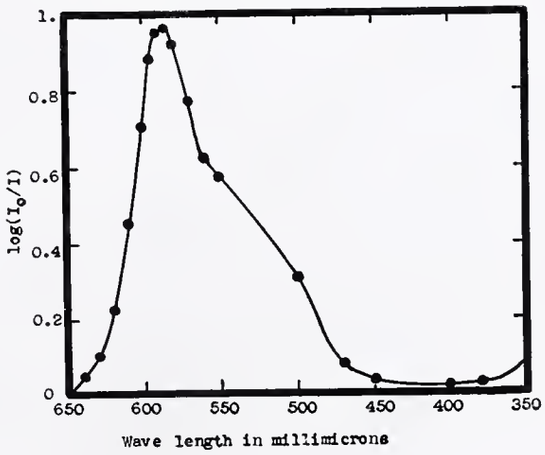

Figure 1. Extinction of Pentabromorosaniline (Diacid Form) in Acid Benzyl Alcohol at Various Wave Lengths

Table I. Spectrophotometric Determination of Bromide in Potassium Bromide Solution

Bromide *Micrograms*		Transmission (Average of 2 Readings)	Bromine Observed *Micrograms*	Error %
4.00		87.60	4.05	+1.00
8.00		71.85	8.42	+5.25
16.00		51.90	15.60	−2.50
24.00		35.25	24.14	+0.58
32.00		25.55	31.24	−2.38
40.00		16.75	40.56	+1.40
	Chloride *Micrograms*		Apparent Bromide Observed	
8.00	35.5	75.30	7.39	−7.6
16.00	35.5	54.10	14.69	−8.2
24.00	35.5	38.60	22.14	−7.8
32.00	35.5	27.50	29.62	−7.4
40.00	35.5	20.00	36.65	−8.4
24.00	10.65	37.50	22.78	−5.1
24.00	35.50	38.60	22.14	−7.8
24.00	177.50	37.65	22.69	−5.6
24.00	355.50	34.15	22.84	+3.5
	Normal Blood			
0.00	1–100	99.40[a]	1.73	..
4.00	1–100	83.35	5.15	+28.8
16.00	1–100	51.35	15.84	− 1.0
24.00	1–100	35.20	24.17	+ 0.7
32.00	1–100	26.30	30.61	− 4.3
40.00	1–100	18.95	37.84	− 5.4

[a] Same reading is obtained with a water blank.

which have been proposed for the liberation of bromine from bromide without the oxidation of chloride, the most satisfactory has been found to be persulfate. It was necessary, however, to transform the peroxydisulfuric acid ($H_2S_2O_8$) into peroxymonosulfuric acid (H_2SO_5) in order to obtain consistent and reliable results. The peroxymonosulfuric acid obtained by the method to be described below liberates bromine at a relatively slow rate, so that time must be allowed for the reaction to occur quantitatively. The solution is unstable and must be made daily from a suitable salt of peroxydisulfuric acid.

The addition of the liberated bromine to rosaniline occurs quantitatively, but at the dilutions employed it requires several minutes for completion. In contact with the oxidizing solution, the pigments are bleached quickly at room temperature, so that the reaction is best carried out as quickly as possible at 0° C.

Procedure and Analytical Results

Reagents. Powdered potassium persulfate ($K_2S_2O_8$), and 28 *N* sulfuric acid.

Solution of rosaniline base, 60 milligrams per liter of aqueous solution, made up hot.

Commercial reagent grade benzyl alcohol. (It is not necessary to redistill this grade of benzyl alcohol, although some may contain as much as 2 per cent of either benzyl chloride or benzyl benzoate.)

Procedure. One hundred milligrams of potassium persulfate are covered with 25 ml. of 28 *N* sulfuric acid in a 50-ml. volumetric flask. After standing 30 minutes at room temperature the solution is diluted to volume with cooling.

The sulfuric acid solution (2.0 ml.) is introduced into a 25 mm. × 200 mm. test tube, and 1.0 ml. of the bromide solution is added without cooling. After standing 3 minutes it is placed in an ice bath for 1 minute. A maximum of 1.0 ml. of rosaniline solution is then added dropwise over an interval of 2 minutes. With small amounts of bromide 1.0 ml. of rosaniline would contain a large excess which would introduce an error in the photometric determination, since a small amount of rosaniline is extracted by the benzyl alcohol. The addition of rosaniline is therefore terminated when an orange tint indicates that an excess is present in the solution. The volume is then brought to 4.0 ml. with the required amount of distilled water.

Three minutes after the addition of the first drop of rosaniline, 10.0 ml. of benzyl alcohol are added and the tube is thoroughly shaken. The contents are poured into a centrifuge tube and spun for a few moments to clear and separate the phases. The red-purple supernatant alcohol phase is poured off and its transmission is measured in the photometer. In his determinations, the author has employed both the Beckman Model D spectrophotometer and the Coleman DM spectrophotometer with equally good results. A standardization is first carried out with known quantities of bromide. In the case of the determinations with the Coleman instrument, using a slit 5 millimicrons wide, at 585 millimicrons, square cuvettes 10.0 mm. deep, and with the temperature of the benzyl alcohol solution 26° C., he obtained the following formula in the standardization of the method against bromide:

$$\text{Micrograms of bromide} = 50.82\ (2\text{-log } T) + 1.13$$

For this equation, $\sigma_{(\text{est. } \gamma\ \text{Br})} = 0.51$.

When measurements must be made with a visual color comparator it is best to work with bromide levels of 50 to 400 micrograms, and a rosaniline solution containing 450 mg. per liter. A color filter of yellow cellophane has been found satisfactory.

By the use of the Coleman DM spectrophotometer, results such as are given in Table I are readily obtainable.

One set of determinations made with a visual colorimeter on a sample of blood to which known amounts of bromide were added is given in Table II, using a rosaniline solution containing 450 mg. per liter.

The method is inapplicable in the presence of iodide, but is apparently not influenced by the presence of thiocyanate.

Table II. Colorimetric Determination of Bromide Added to Blood

Bromide Added *Micrograms*	Standard	Unknown	Bromide Found *Micrograms*	Error %
60	10	25.6	62.4	+4
80	20	40.3	79.4	−0.8
160	20	20.4	157	−2
240	20	13.2	242	+1
320	20	10.1	317	−1
400	20	8.6	372	−7

Standard: 160 micrograms of Br per milliliter.

Summary

A simple, rapid, inexpensive, and reliable method is described for the determination of bromide in solutions including biological fluids without preliminary separation from chloride. It is based on the reaction of bromine with rosaniline, and colorimetric or photometric determination of the color of the pentabromorosaniline extracted by benzyl alcohol from 7 *N* sulfuric acid solution.

Acknowledgment

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Correction: The photomicrograph on page 434 of the May issue represented cadmium iodide, and was furnished through the courtesy of Mary L. Willard.

Detection and Quantitative Determination of 4-Amino-2-methyl-1-naphthol

A Synthetic Vitamin K

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THE hydrochloride of 4-amino-2-methyl-1-naphthol has been introduced to the medical profession as a water-soluble therapeutic agent which possesses vitamin K activity. Increased interest in this compound has necessitated the development of a chemical method for its detection and quantitative determination in preparations now on the market. The procedure described has been found suitable for the assay of preparations containing 4-amino-2-methyl-1-naphthol and yields consistent results with quantities as low as 0.02 mg. Hitherto, the assay of solutions and dry mixtures of 4-amino-2-methyl-1-naphthol hydrochloride has been dependent on the curative effect of the compound on vitamin K–depleted chicks (*4*). It is hoped that the proposed chemical method may supplant, in part, the more complicated biologic assay procedure.

Of possible chemical methods to be investigated, it was felt that a colorimetric method should take precedence over volumetric or gravimetric procedures because of the small quantities of material to be dealt with and the relative ease of colorimetric manipulation. Accordingly, a reagent was sought which would

Possess a high degree of sensitivity

Produce a color which would obey Beer's law of light absorption and be sufficiently stable to allow time for accurate color comparisons

Not be adversely affected by the presence of sodium bisulfite, since this compound is used to stabilize aqueous solutions of 4-amino-2-methyl-1-naphthol

Possess sufficient specificity to permit differentiation of the compound in question from its possible decomposition products

The latter characteristic is of importance because in the biologic method of assay such differentiation is not possible. One of the oxidation products of 4-amino-2-methyl-1-naphthol is 2-methyl-1,4-naphthoquinone (menadione), which possesses the same antihemorrhagic activity when measured on a molecular basis. Investigation of sodium pentacyanoammineferroate, $Na_3[Fe(CN)_5NH_3]$, as a possible reagent revealed that this compound possessed the desired characteristics.

Anger (*1*) described a colorimetric test for the detection of primary aromatic amines which required sodium pentacyanoaquoferriate, $Na_2[Fe(CN)_5H_2O]$, as the reagent. This test provides a basis for the procedure followed herein. In a discussion of Anger's test, Feigl (*2*) attributed the production of color to the substitution of the coordinately bound water molecule, in the pentacyanoaquoferriate complex, by a molecule of the aromatic amine according to the following reaction:

$$[Fe(CN)_5H_2O]^{--} + R{-}NH_2 \longrightarrow [Fe(CN)_5R{-}NH_2]^{--} + H_2O$$

During the course of this investigation, sodium pentacyanoaquoferriate as employed by Feigl (*2*) and sodium pentacyanoammineferriate were tested but were found to possess no advantage over the more readily prepared sodium pentacyanoammineferroate; consequently, the latter compound was chosen for the quantitative determination of 4-amino-2-methyl-1-naphthol. The procedure finally adopted depends on the interaction of sodium pentacyanoammineferroate in alkaline solution with 4-amino-2-methyl-1-naphthol to produce an intense blue color and the comparison of the intensity of this color with that produced by known quantities of the aminonaphthol.

The production of a blue color through the interaction of sodium pentacyanoammineferroate, $Na_3[Fe(CN)_5NH_3]$, and the aminonaphthol may be attributed to a reaction analogous to that which has just been shown—i. e., the replacement of the coordinately bound molecule of ammonia by a molecule of the aminonaphthol.

Curve *A* in Figure 1 represents an absorption spectrum typical of the colored solutions employed in the quantitative determination of 4-amino-2-methyl-1-naphthol. The solution employed to determine the curve shown was prepared by the addition of 1.0 mg. of 4-amino-2-methyl-1-naphthol to 1 ml. of the sodium pentacyanoammineferroate reagent solution. This mixture was allowed to stand for 15 minutes and was then diluted to a concentration corresponding to 0.004 mg. of aminonaphthol per ml. The solution exhibited but little absorption in the blue region of the spectrum.

Curve *B* illustrates the contribution to the total absorption made by the yellow colored reagent solution. The reagent exhibits no appreciable absorption above 5000 Å. For this reason, visual matching of the blue color of the solution is simplified, and more precise spectrophotometric measure-

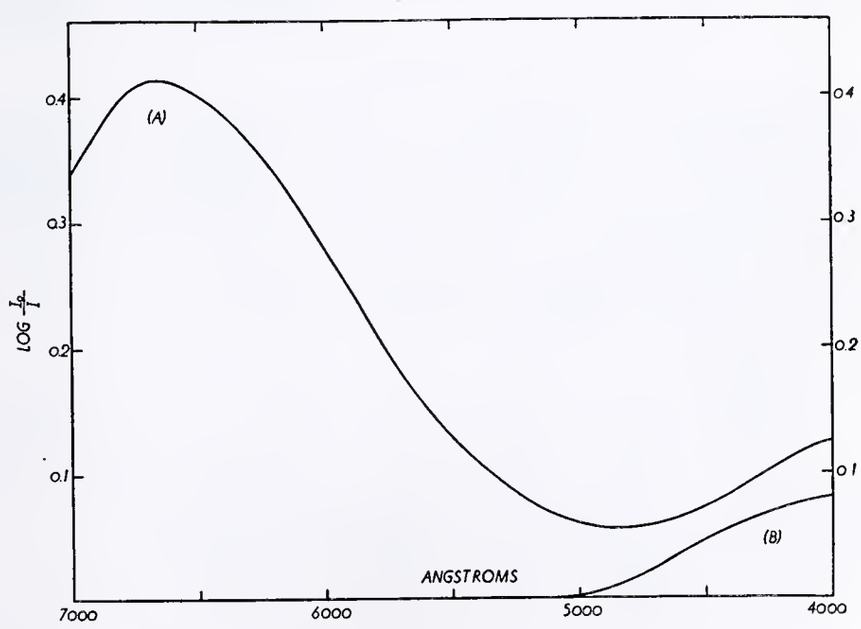

FIGURE 1. DETERMINATION OF 4-AMINO-2-METHYL-1-NAPHTHOL

A. Absorption of solution resulting from interaction of 4-amino-2-methyl-1-naphthol hydrochloride with sodium pentacyanoammineferroate

B. Absorption of sodium pentacyanoammineferroate reagent solution

ments of the absorption maximum at 6650 Å. would provide a highly accurate determination.

To test the applicability of Beer's law $\left(k = \frac{1}{c} \log \frac{I_0}{I}\right)$ to this solution, the values of $\log \frac{I_0}{I}$ were determined at 6500 Å. for various dilutions. It is obvious from the values of k (Table I) that the solution satisfies Beer's law within the limits of experimental error.

Accuracy of Method

The data reproduced in Table II were obtained by visual colorimetric assay of synthetic mixtures of known composition.

The greatest error occurred with the solutions of lowest aminonaphthol content because the concentration of the standard solution was maintained at 1.0 mg. per ml., while the synthetic mixtures varied in aminonaphthol content. The error is due to the difference in color caused by the presence of excess reagent in the solutions of low aminonaphthol content and may be eliminated by the use of a standard of appropriate strength.

The effect of bisulfite on the developed color was determined by adding known amounts of sodium bisulfite to solutions of equal aminonaphthol content, then adding the reagent and comparing the resulting colors. From the data obtained it was apparent that amounts of sodium bisulfite up to 5 mg. per milligram of 4-amino-2-methyl-1-naphthol had no effect on the color; quantities above this amount caused the color to develop more slowly and resulted in a reduction of the final color intensity.

Table I. Applicability of Beer's Law to Colored Solution Obtained by Interaction of Sodium Pentacyanoammineferroate with 4-Amino-2-methyl-1-naphthol

$\log \frac{I_0}{I}$	c, Concentration of Aminonaphthol *Mg./ml.*	k, $\frac{1}{c} \log \frac{I_0}{I}$
1.000	0.010	100
0.508	0.005	101
0.402	0.004	100
0.192	0.002	96
0.098	0.001	98

The presence of 2-methyl-1,4-naphthoquinone, an oxidation product of 4-amino-2-methyl-1-naphthol, was found to have no effect on the development and final intensity of the color.

Specificity of Reaction

Sodium pentacyanoammineferroate in alkaline solution reacts with other primary aromatic amines to yield blue colors, with aliphatic and aromatic nitroso compounds to yield green colors, and with hydrazine and its derivatives to produce red to yellow derivatives. Such compounds might interfere in the application of this method; however, it is believed that there is little likelihood of their presence in dosage forms of 4-amino-2-methyl-1-naphthol intended for oral or parenteral use. Aliphatic and secondary or tertiary aromatic amines do not interfere.

Determination of 4-Amino-2-methyl-1-naphthol

The reagent, prepared as described below, may be employed for the detection of this drug in aqueous solution. One drop of the unknown solution is placed in the depression of a spot plate and one drop of reagent is added. After a few minutes the appearance of a blue or green color indicates the presence of 4-amino-2-methyl-1-naphthol. Used in this manner, the test will detect 0.0005 mg. of the aminonaphthol. (This test must be employed with due regard to the interferences mentioned above.)

Reagents. Standard 4-amino-2-methyl-1-naphthol solution: 60.53 mg. of pure 4-amino-2-methyl-1-naphthol hydrochloride and 50.0 mg. of sodium bisulfite dissolved in 50.0 cc. of distilled water contained in a dark glass-stoppered bottle. Under conditions of normal usage this solution is stable for from 4 to 6 hours; when allowed to stand overnight it may decrease in strength as much as 10 to 15 per cent.

Sodium pentacyanoammineferroate solution: 250 mg. of sodium pentacyanoammineferroate and 500 mg. of anhydrous sodium carbonate dissolved in 25 cc. of distilled water. This solution is stable for about one week.

Table II. Analysis of Samples of Known Composition

4-Amino-2-methyl-1-naphthol Added *Mg.*	Quantity Found *Mg.*	Error *Mg.*
1.00	1.00	0.00
1.00	1.00	0.00
0.95	0.97	0.02
0.93	0.94	0.01
0.90	0.93	0.03
0.85	0.87	0.02
0.80	0.84	0.04

The crystalline reagent $Na_3[Fe(CN)_5NH_3]$ was first described by Hofmann (*3*). It may be prepared as follows:

Ten grams of sodium nitroprusside are ground to a fine powder and added to 30 ml. of concentrated ammonium hydroxide solution. The mixture is shaken to dissolve the salt, and the resulting solution is left overnight at from 0° to 10° C. The crystals which form are removed by filtration, washed several times with 95 per cent ethanol and once with absolute methanol, and dried over sulfuric acid in vacuum. (Additional crystalline material may be obtained in a finely divided state from the mother liquor by adding 95 per cent ethanol, filtering, and washing in the manner described. The material thus obtained is of lower purity than the crystalline substance which forms overnight, but it may be used effectively as a reagent.)

Procedure. For the assay of ampouled solutions, appropriate dilutions are made, so that the resulting solution contains approximately 1 mg. of 4-amino-2-methyl-1-naphthol per ml. Powdered mixtures which contain the drug may be extracted with distilled water containing 0.1 per cent of sodium bisulfite, added to inhibit decomposition of the drug. The extract should be diluted to contain about 1 mg. of 4-amino-2-methyl-1-naphthol per ml. of solution, and the final solution may be filtered if necessary.

The unknown solution (1 cc., calculated to contain about 1 mg. of 2-methyl-4-amino-1-naphthol) is transferred to a 50-ml. volumetric flask, 1.0 ml. of the standard aminonaphthol solution is placed in a second 50-ml. flask and, if necessary, the contents of the flasks are adjusted to approximately equal volumes with distilled water. Then 1.0 ml. of sodium pentacyanoammineferroate reagent is added to each flask, mixed, and set aside in the dark for 15 minutes. Finally, distilled water is added to the mark of each flask and mixed, and the solutions are compared in a colorimeter. If the solutions to be compared are found to vary more than 10 per cent in aminonaphthol content, it is best to carry out a second determination after adjusting the concentration of the standard to approximately that of the unknown. Consistently accurate results may be obtained in this manner.

Summary

A method developed for the quantitative determination of 4-amino-2-methyl-1-naphthol depends on the interaction of sodium pentacyanoammineferroate in alkaline solution with 4-amino-2-methyl-1-naphthol to produce a blue color.

The absorption spectrum of a typical solution obtained in this determination has been determined.

The reagent described will detect the presence of 0.0005 mg. (0.5 microgram) of 4-amino-2-methyl-1-naphthol.

Acknowledgment

The data for the absorption curves were obtained by means of a Cenco-Sheard spectrophotelometer through the courtesy of Thorfen R. Hogness, in the laboratories of Spectroscopic Biological Investigations, University of Chicago.

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Microbiologic Assay of Natural Pantothenic Acid in Yeast and Liver

Influence of Clarase Digestion

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MICROBIOLOGIC assays of natural pantothenic acid have often given much lower values than chick assays, because of the existence of part of the vitamin in combined form, in which it is unavailable to the test organism. That this combined form of pantothenic acid could be freed by enzyme digestion was noted in 1934 by Rohrmann, Burget, and Williams (*2*). Using yeast as the test organism, they obtained much higher assays with extracts from autolyzed tissue, especially liver, than from fresh tissue. Pennington, Snell, and Williams (*1*) advised autolysis of samples, wherever suitable, previous to microbiologic assay with *Lactobacillus casei*. Strong, Feeney, and Earle (*3*) also using *L. casei*, found that clarase digestion of yeast and animal tissues satisfactorily increased the pantothenic acid values obtained.

[Since the submission of this manuscript for publication a report by Waisman, Henderson, McIntire, and Elvehjem (*4*) has appeared, comparing the use of pepsin, clarase, and pancreatin in preparation of meats for such an assay. Pancreatin was found more effective than clarase in treatment of muscle tissue, and gave about the same results with softer tissue such as liver and kidney. Both were more effective than pepsin.]

Since accounts of the use of clarase have been rather lacking in detail, it seems worth while to record here experiences with this enzyme in digestion of yeast and liver preparations used in pharmaceutical manufacturing. These materials have consistently given the authors much lower values for pantothenic acid by the microbiological method than by the chick method. After adequate clarase digestion, however, the values obtained by the microbiologic method are higher, and compare favorably with those obtained by the chick method. In their assays the authors have followed the method outlined by Strong, Feeney, and Earle (*3*).

Test materials were digested at pH 5.0 and 37° C. under toluene. To 0.5 gram of the test sample were added 1.0 gram of the clarase powder and water to 10-cc. volume. If necessary, coarse particles, and particularly yeast cells, were broken up in a small glass colloid mill before incubation of the mixture. After digestion the toluene was removed, and appropriate dilutions of the mixture were made for testing.

Clarase is available as a dry powder in crude and concentrated forms, and from the standpoint of expense, it was of considerable interest to know whether or not the crude material would be satisfactory. In Table I are shown comparative results with the two forms. Only 10 per cent mixtures of the concentrated clarase are considered since, in the authors' experience, lower concentrations were inadequate with 48 hours' digestion time. In no instance was the recovery of pantothenic acid as great after digestion with crude clarase as with the concentrated enzyme, even though the crude material was used in as much as 20 per cent concentration. Possibly longer digestion would release more of the vitamin but, for control of manufacturing, 48 hours is about the limit of convenience, and some of the results suggest a possibility of loss of pantothenic potency with prolonged incubation.

In the light of this information, a number of different lots of yeast powder, yeast extract, and liver extract were tested, and one lot of Solvamin (Commercial Solvents Company). Assays were made without digestion, and after digestion of the samples with 10 per cent concentrated clarase for 48 hours at 37° C. Independently, assays on the same samples by the chick method were made by Mrs. F. Peirce Dann of the company's bioassay laboratories. Table II shows the results. The assays for pantothenic acid were much higher after digestion than before, and generally approximated the values reported by the animal method.

The clarase itself contains an appreciable amount of pantothenic acid which must be considered in evaluation of the results. Since the different lots contain varying amounts, a sample of each lot should be assayed after self-digestion when it is first used and occasionally thereafter.

The digestion of these materials, especially yeast, apparently produces, or releases, a substance into the digest that interferes with the metabolism of the test organism. The evidence of this is the progressively lower values obtained at

TABLE I. COMPARISON OF CRUDE AND CONCENTRATED CLARASE IN PANTOTHENIC ACID ASSAY[a]

(Clarase digestion, 48 hours)

Test Material	Crude 10%	Crude 15%	Crude 20%	Concentrated 10%
	Micrograms per gram			
Yeast powder	154	167	...	170
Yeast extract	346	...	...	358
Liver paste No. 1	324	323	...	470
Liver paste No. 2	...	178	...	213
Liver paste No. 3	...	...	470	565
Liver powder No. 1	...	...	130	198
Liver powder No. 2	...	...	388	548
Liver powder No. 3[b]	...	...	...	338
	...	...	...	335
	...	...	...	331
	...	...	...	327

[a] Assay values shown as micrograms of pantothenic acid per gram of test sample.
[b] Values of 338, 335, 331, and 327 were obtained after digestion 48 hours, 72 hours, 4 days, and 6 days, respectively.

TABLE II. PANTOTHENIC ACID VALUES OBTAINED BY MICROBIOLOGIC AND BY CHICK METHODS

(Expressed in micrograms per gram of test material)

Test Material	Microbiologic: Not digested	Microbiologic: Digested[a]	Chick Method
Yeast powder No. 1	55	137	Slightly over 165
Yeast powder No. 2	55	170	About 200
Yeast extract No. 1	223	321	360
Liver extract paste No. 1	357	370	Slightly over 300
Liver extract paste No. 2	165	213	Not over 300
Liver extract paste No. 3	456	565	600
Liver extract paste No. 4	288	448	450
Liver extract powder No. 1	219	338	360
Liver extract powder No. 2	204	357	...
Liver extract powder No. 3	124	198	300 or more
Liver extract powder No. 4	377	548	600 or more
Solvamin	426	1240	1200
Clarase (crude)	4.8	..	...
Clarase (concentrated), Lot 1	11.5	..	...
Clarase (concentrated), Lot 2	10.0	16.0	...
Clarase (concentrated), Lot 3	...	27.0	...

[a] Digested 48 hours with concentrated clarase 10%.

Table III. Calculations at Various Test Levels of Pantothenic Acid in a Yeast Powder after Clarase Digestion

Test Solution Added per Culture	Digestion with Concentrated Clarase 10%
Cc.	
0.5	188
0.75	173
1.0	177
1.5	153
2.0	133

the higher levels tested (Table III). More difficulty has been encountered with yeast than with liver preparations, perhaps because the lower potency does not permit sufficient dilution of the test sample beyond the range of activity of the inhibitor. With such a progressive decrease in values for pantothenic acid as the test level is increased, it is sometimes difficult to decide which, if any, of the figures are correct. Usually an average of calculations from the two or three lowest test levels has approached fairly closely to the potency obtained by the chick assay. It is probable, of course, that materials of lower potency would present a real difficulty because of the stronger dilutions required for testing.

The assays of the clarase itself also show the presence of an inhibiting substance, with some lots more than with others, but in none has this been enough to account for the effect in the digested samples of yeast or liver, which are tested in much weaker dilutions than the clarase alone.

Obviously, if this inhibition phenomenon could be overcome, less difficulty would be encountered in making calculations and the dependability of the method would be increased. The authors have not studied this problem except to try extraction of the digested material with hexane and with ether before testing. The interfering substance was not removed.

Summary and Conclusions

Digestion of 0.5-gram samples of yeast and of liver preparations with 1.0 gram of concentrated clarase in a total volume of 10 cc., for 48 hours at 37° C., has proved adequate for release of combined pantothenic acid. After such treatment the assay values by the microbiologic method compared favorably with those obtained by the chick method, although averaging somewhat lower.

Digestion with double this amount of crude clarase powder was inadequate.

Digestion of these materials with clarase apparently produces or releases a substance which interferes with the metabolism of the test organism, *L. casei*. This does not interfere seriously in testing materials of higher potency where test levels are out of range of the inhibiting factor. If this effect can be overcome, the dependability and usefulness of the method will be increased.

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Direct Determination of Sulfur

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An ammoniacal cuprous sulfate reagent is prepared by reducing a solution of cupric ammonium sulfate with hydroxylamine hydrochloride. The reaction of this reagent upon elemental sulfur in acetone solution to form colloidal cuprous sulfide has been made the basis of a turbidimetric method for the direct determination of sulfur.

The reagent was found to be capable of detecting 1 microgram of sulfur in 10 ml. of solution. The determination is fairly specific and accurate. The rapidity with which a large number of samples can be analyzed makes it especially useful for routine work. The method has been applied particularly to the analysis of sulfur in spray residues.

A RAPID method of analysis for sulfur in spray residues is much needed in the practical evaluation of commercial fungicides.

The usual procedure (*3*, *11*) has been to oxidize the elemental sulfur to sulfate, which is then precipitated and weighed as barium sulfate. The gravimetric method is so time-consuming that various volumetric (*6*, *8*, *13*, *14*) and photometric (*15*) procedures for the estimation of sulfate ion have been proposed. However, for work with spray residues, the oxidation itself requires a considerable amount of time, no matter whether the sulfur is first extracted from the plant material (*11*) or the whole sample is oxidized in an alkaline fusion (*1*). The same objection would apply to methods based upon preliminary reduction of sulfur to sulfide (*5*).

Thus a direct determination of sulfur in the solvent used for extraction from the plant material would be the most desirable procedure. A direct iodometric method has already been proposed by Fleck and Ward (*4*), and an alkalimetric method first suggested by Kühl (*7*) has been adapted to the analysis of spray residues by Small (*10*), but large errors inherent in these methods make them of little use for accurate work.

In 1929 Pierce (*9*) found that von Nagy Ilosva's reagent (*12*) could be used as an extremely sensitive test for carbon bisulfide in adulterated olive oil and noted that elemental sulfur also gave a test with the reagent.

After considerable experimentation the authors have modified the ammoniacal cuprous sulfate reagent so that it reacts quantitatively with elemental sulfur in solution to give cuprous sulfide which can be determined turbidimetrically.

Reagents

Stock Solution A. Cupric sulfate pentohydrate, 4.0 grams; ammonium hydroxide (28.3 per cent ammonia by weight), 48.0 ml.; distilled water to make 100 ml.

Stock Solution B. Hydroxylamine hydrochloride, 20.0 grams; distilled water to make 100 ml.

Reagent Solution. To 5 ml. of solution A add 5 ml. of solution B, then slowly add water to make 50 ml. and shake vigorously. The reagent is unstable and should be freshly prepared from the stock solutions each day. The rate of decomposition is increased by the action of light, of heat, and especially of oxygen. The reagent should thus be kept cool and out of direct sunlight, and the container should be closed with a Bunsen valve or a loosely fitting stopper, since nitrogen is slowly evolved from the solution. The complete decomposition of the reagent may take several days and is indicated by the sudden return of the blue color of ammoniacal cupric sulfate. The quality of the reagent solution may be tested by adding 1 ml. to 20 ml. of a 50 per cent solution of acetone in water. If no blue color appears on standing 3 minutes, the reagent is satisfactory for use. In practice the authors discard all solutions which have been made up more than 12 hours, since no trouble has been experienced with the stability of the reagent up to that time.

Table I. Precision of Results

Sulfur Taken	Average Deviation of 6 Determinations
Micrograms	*Microgram*
5	0.3
10	0.2
20	0.2
40	0.3
60	0.4

Preparation and Solution of Sample

A sufficient quantity of leaves or other plant material is taken to ensure a representative sample. The necessary number may vary from two or three hundred leaves from at least three trees for field samples to as few as five or ten leaves when a precision laboratory sprayer is employed. With large samples several hundred disks of known area are cut from the leaves and the sulfur is dissolved by stirring them for 2 minutes each in a series of beakers containing 250, 200, and 200 ml. of acetone. Further rinsing may be necessary with some samples, and in any case the final rinsing should be tested for sulfur by mixing 5 or 10 ml. of the acetone extract with an equal quantity of distilled water and adding 1 ml. of the cuprous sulfate reagent. The combined acetone extracts and washings are made up to a definite volume with more acetone and a convenient aliquot is taken for analysis.

Used acetone can be recovered by a single distillation, since extensive tests have shown that sulfur is not carried over with boiling acetone.

The acetone extracts and particularly the standard solutions of sulfur should be protected from direct sunlight. Investigation has shown that elemental sulfur undergoes a photochemical reaction when dissolved in organic liquids such as acetone, dioxane, and butyl carbitol. Ultraviolet light was found to be most efficient in producing this reaction, while the longer wave lengths had little effect. Thus, in a solution containing 200 micrograms of sulfur in 100 ml. of acetone, this reaction was 85 per cent complete in 20 minutes when exposed to radiation from a quartz-enclosed mercury vapor lamp 20 cm. above the center of the liquid and operating at 4.2 amperes and 120 volts. A similar solution required 11 days to reach the same stage of reaction when enclosed in Pyrex glass and exposed to indirect winter daylight.

The product of this photochemical reaction does not react with the cuprous sulfate reagent. The formation of combined sulfur can be prevented by wrapping the container in black paper and keeping it in a dark cabinet. A standard solution used periodically for the determination of calibration curves and repeatedly exposed for short periods of time to indirect daylight was found to have undergone no change in elemental sulfur content over a period of 4 months when the above precautions were observed.

Experimental Procedure

An aliquot containing not more than 75 micrograms of sulfur is pipetted directly into the photometer tube and diluted if necessary with pure acetone to a volume of 10 ml. Ten milliliters of distilled water are added and mixed by inverting the tube five times. The tube is carefully cleaned with a soft cloth and inserted in the photoelectric photometer and the instrument is adjusted to the maximum scale deflection. One milliliter of cuprous sulfate reagent is then added and the centents of the tube are thoroughly mixed by inverting five times. The tube is again wiped off and inserted in the photometer, and the scale deflection exactly 60 seconds after the addition of the reagent is recorded.

The galvanometer readings are related to micrograms of sulfur by means of a calibration curve. The experimental points on this curve are obtained by adding known increments of sulfur from a standard solution to a series of photometer tubes containing the blank acetone extracts. The sulfur in these solutions should be determined in exactly the same way as in the unknowns. A convenient concentration of sulfur for the standard solution is 400 to 500 mg. per liter, which may be diluted further if desired.

The sulfur for the standard solutions was recrystallized four times from thiophene-free benzene and dried in a desiccator.

An Evelyn photoelectric colorimeter was used in this work, but any good photometer could be used. For this determination a direct-reading type of photometer is to be preferred to the photoelectric null-point type or the neutral-wedge type, both of which must be manually adjusted to a drifting end point which is to be recorded at a definite time.

Interfering Materials

The light green color of the leaf pigments in the acetone extracts affects the galvanometer readings slightly, but this effect can be practically eliminated by using a filter having a 95 per cent light transmission between 5500 and 5850 Å. with a maximum transmission at 5650 Å.

A more serious source of interference is caused by the plant waxes and oil residues which affect the formation of the colloidal cuprous sulfide. Although correct results may be obtained by using a calibration curve obtained with a blank extract of the same materials, the reproducibility of the results is generally adversely affected.

In working with a laboratory sprayer glass plates coated with cellulose nitrate are sometimes used as a standard surface (*2*). A white turbidity is produced when water is added to the acetone extract, so that the final light transmission might be expected to be decreased. Actually, however, the formation of cuprous sulfide may be so inhibited that more light is transmitted than if the cellulose nitrate were absent.

Carbon disulfide, inorganic sulfides, and xanthates react with the reagent but sulfates, sulfites, thiosulfates, thiocyanates, organic sulfides and disulfides, and sulfones, mercaptans, and thiophenols do not. Carbon tetrachloride produces a red coloration with the reagent.

Table II. Recovery of Sulfur from Leaves and Glass Plates Sprayed with a Laboratory Sprayer

Type of Surface	Concn. of Sulfur	Weight of Spray Deposit	Calcd. Sulfur in Deposit	Sulfur Found
	Mg./g. spray solution	*Gram*	*Micrograms*	*Micrograms*
Glass	0.13	0.0403	5.2	5.9
Leaf	0.26	0.0411[a]	10.7	10.9
Glass	0.39	0.0414	16.1	15.8
Leaf	0.52	0.0403[a]	21.0	23.0
Glass	0.52	0.0403	21.0	22.8

[a] Deposit on leaves was not weighed. Weights given are averages of values determined on glass plates before and after leaves were sprayed.

Results

The reproducibility of results obtained by this method depends to a great extent upon careful technique. The formation of a colloidal precipitate is often affected by slight variations in the procedure. The precision attained in this laboratory is indicated in Table I. The deviation of a single determination should not exceed 1 microgram.

The accuracy of the method is shown in Table II. Apple leaves and glass microscope slides coated with cellulose nitrate were sprayed with known concentrations of sulfur. In a given time a nearly constant amount of spray is deposited on a given area of surface, so that the total amount of sulfur can be calculated. The agreement of the last two columns is satisfactory.

Table III. Solubility of Commercial Sulfur Products in Butyl Carbitol at 25.0° C.

Product	Equilibrium Concentration Approached from	Solubility of Sulfur G./l.
Recrystallized C. P. sulfur	Supersaturated soln.	2.18
	Unsaturated soln.	2.21
Mike sulfur	Supersaturated soln.	2.14
	Unsaturated soln.	2.26
Koppers flotation paste (dried at 90° C.)	Unsaturated soln.	2.34
Stauffer's paste (dried at 90° C.)	Unsaturated soln.	2.15
Stauffer's dry magnetic 70 paste	Unsaturated soln.	2.20

To determine whether commercial sulfur products contained appreciable quantities of interfering materials, the solubility of several of these products in the monobutyl ether of diethylene glycol was determined. This solvent, known commercially as butyl carbitol, may be substituted for acetone throughout the analytical procedure and the amount of sulfur in solution determined by this method. The results in Table III show that no appreciable interfering materials occur in the commercial fungicides tested and that the solubility of the sulfur in them is equivalent to that of recrystallized C. P. sulfur.

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A New Microtest for Iodide

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ALTHOUGH most chemical analyses depend upon the possibility of chemically converting the constituents to be detected into another characteristic form, the sensitivity of these chemical tests which depend upon a characteristic precipitate, gas, or colored or fluorescent solution is necessarily limited by the physical constants involved and the personal equation of the observer. However, by making use of catalyzed reactions, extremely small amounts of substances can be detected. Thus, in the detection of iodide, the use of catalyzed reactions has been very successful.

Feigl and Frankel (*4*) developed a method for detecting the presence of 0.05 microgram of iodide in a concentration of 1 part per million, based on the conversion of *o*-nitroaniline, its isomers, or other primary aromatic amines to form a diphenyl derivative with bromobenzene in the presence of cuprous iodide or a mixture of copper and potassium iodide. Using the catalytic effect of iodide upon the ceric-arsenite oxidation-reduction reaction, Kolthoff and Sandell (*6*) showed that 0.05 microgram of iodide can be detected in a concentration of 1 part in 500,000 (*3*). The authors have also presented a method (*5*) for detecting iodide by its catalytic effect upon the nitrite-arsenite oxidation-reduction reaction, whereby 0.2 microgram of iodide can be detected in a concentration of 1 part in 500,000.

Continuing their work on the detection of halides, the authors have developed a new, simple, and rapid microtest for iodide, which is based upon the catalytic effect of iodide on the nitrate-arsenite oxidation-reduction reaction and may be carried out in a small test tube or on a spot plate. A search of the literature revealed no mention of this effect, except where it was applied to the manufacture of arsenates by the oxidation of arsenious oxide with nitric acid. Smith and Miller (*8*), investigating the inhibitory effect of mercury on this reaction, showed that hydrochloric, hydrobromic, and hydriodic acids will act as catalysts. Shortly before this, Behse was granted a patent (*1*) for the manufacture of arsenic acid by means of nitric acid using hydrochloric acid as a catalyst. Later, Latimer (*7*) obtained a patent in which iodide was used as a catalyst. He also claimed that the other halogens, chloride, bromide, and fluoride, could be used as catalysts but that much greater concentrations of these were required.

Since both bromides and chlorides catalyze this reaction, conditions had to be obtained under which moderate amounts of these anions would not interfere in the detection of relatively small amounts of iodide. Using the procedure described below, the authors were able to detect 1.0 microgram of iodide in a concentration of 1 part in 50,000. The presence of 500 micrograms of either thiocyanate, bromide, chloride, or a mixture of these anions does not catalyze this reaction in this procedure. Other anions of the silver nitrate group which are likely to interfere—cyanides ferricyanides, ferrocyanides, and sulfides—are removed in the usual way (*2*) with cobalt nitrate.

Procedure for Detection of Iodide

To a small test tube (or a spot plate) containing 1 drop of the solution to be tested, add 1 drop of *M* sodium arsenite and 2 drops of water and mix thoroughly. Add 2 drops of concentrated nitric acid and allow to stand for 3 minutes after complete mixing. Next add 3 drops of 0.5 *M* silver nitrate, stir the mixture thoroughly, and add 1.5 *M* sodium carbonate dropwise. The formation of a red-brown precipitate of silver arsenate shows the presence of iodide. If the precipitate is yellow (due to silver arsenite), iodide is absent.

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Photoelectric Color

Description and Mensuration of the Color of Petroleum Products

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Photoelectric color is a new system for the measurement of the color of lubricating oils. The color is measured directly in technologically significant terms. Only two readings are required: brilliance and characterization of the hue.

The system uses a photoelectric colorimeter, designed for accuracy and easy standardization for the measurement of oil colors. The colorimeter has a continuous scale from 1 to 100 for the definition of the intensity of such colors.

Deviation of hue of petroleum color from normal is given directly in magnitude and direction.

The color definition is independent of the visual response of the operator and of the particular instrument.

I. C. I. data can be calculated from photoelectric color, if desired. However, this is rarely desirable in petroleum technology. Photoelectric color can be correlated with the Union system.

The system is easily learned and its standardization is based on colored solutions which are reproducible, easily prepared, and sensitive.

THE petroleum industry has employed a number of systems for the measurement and specifications of the color of petroleum products, each of which depends upon a visual matching of the color of the sample with that of a standard. Those systems commonly in use involve arbitrary standardization and cannot be correlated with each other by fundamental means. Furthermore, they afford no practical means for matching both brilliance and hue and, in lighter colors, purity as well—essential factors for a visual match.

Two methods in particular use are specified by the American Society for Testing Materials. The Union colorimeter (*1*) is used for determining the color of lubricating oils, while light-colored products are measured with the Saybolt chromometer (*2*), which bears no relation to the Union colorimeter except that both depend upon a series of yellow disks and in addition the Saybolt color involves variation in depth of sample. These methods have several disadvantages. The instruments themselves are not entirely uniform. The glass standards are difficult of duplication and are subject to temperature changes, and their optical systems are not completely standardized. The disks correspond only to certain selected points in the range of possible oil colors. Anomalous results have been noted on so-called abnormal samples, when the brilliance or hue of the oil differs from that of the disk. These methods lack any relation to accepted systems of color definition (*3, 8, 9, 10, 12, 13, 15, 17, 20*), a brief description of which is given by Gardner (*7*).

Another method uses the Tag-Robinson colorimeter (*22*). This is a combination of the Union and Saybolt methods and involves similar difficulties.

The Lovibond system (*11*) has a theoretical advantage in that it attempts to overcome differences in hue by virtue of series in several hues of standard glasses, more closely spaced. In a limited sense the Lovibond system supplies the equivalent of a spectral curve. However, it suffers from the remaining difficulties of the other systems and, indeed, its added complexity results in an aggravation of the instrumental disadvantages to the point where the actual results obtained are far removed from the theoretical possibilities.

A more novel method which has been used occasionally in the petroleum industry is known as "true color" (*16*). Here, an oil or solution of an oil is chosen as standard either arbitrarily or in accordance with its match to a given Lovibond glass. Oils or their solutions are compared with the standard by variation of the thickness of the unknown. Other investigators have pointed out that the standard oil must be similar in color to that being measured, that the diluent introduces anomalies, and that Beer's law holds for some samples but not for others. Consequently, a general correlation of true color with Union or Lovibond color is almost impossible, and widely varying values have been published (*14, 18, 23, 24*). An attempt to correct some of the faults of true color was made in the "optical density" method (*6*), which uses a neutral filter as standard and a green light source. The improvement is not substantial and the technique is further complicated.

All these systems are subjective and dependent on the observer and to a greater or lesser extent on the spectral selectivity of his eye at the time of observation. The instruments are difficult to standardize, different instruments giving different readings to the same observer. Differences in spectral distribution between standard and sample particularly aggravate the difficulties of the subjective observer. Where a thickness is varied as in the Saybolt, Tag-Robinson, true color, and optical density methods, dependence is placed on the applicability of Lambert's law. Occasionally petroleum products follow Lambert's law; often, probably because of fluorescence, they do not.

The use of a photoelectric colorimeter has the potentiality of eliminating the effects of the subjective color response of individual observers or their equivalents. A step in this direction was proposed by Story and Kalichevsky (*21*). Their proposal is limited to a single reading, which cannot express fully the color of an oil. Multiple-reading photoelectric colorimeters have been proposed which by the use

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of either three or four filters yield values that may be related to the various International Commission on Illumination quantities (*19*).

These systems yield the basic color information but in terms which lack immediate significance in petroleum technology. For oil colors they are unnecessarily complex and fail to take advantage of the fortunate fact that the colors of petroleum oils vary only over a limited range.

The spectrophotometric curves of petroleum products are substantially smooth, have the same general shape, and invariably show higher transmission as the wave length increases. For any given brilliance, oils of various origins and methods of refining may be represented by a family of curves intersecting in the general region of 545 mμ. Some of these curves will show a higher red transmission than others, and a correspondingly lower blue transmission—that is, all oils of a given brilliance have the same transmission at the point of intersection, but some of the curves passing through this point are steeper than others. Thus for any oil, complete characterization can be obtained by measurements of brilliance and of one other transmission at a wave length remote from that of the intersection. This may be at either the red or blue end of the spectrum, but for reasons described below, it is made at a certain point in the red region.

This paper presents a new system for the mensuration and description of the color of petroleum oils by means of a specially developed photoelectric colorimeter, so that it is objective, avoids any unnecessary complexity, and is descriptive of the oil color in terms of direct physical significance.

The method is simple to handle and permits rapid determination. Provision is made for checking the instrument's standardization by means of suitable aqueous solutions which may be easily prepared by any chemist. The results are convertible to the standard I. C. I. terminology (*10*). The system has been correlated with the Union method. A correlation with the Saybolt method is expected to be furnished shortly. Within the limits of experimental error, the system also lends itself readily to being "additive".

The general method here proposed should have broad application in color mensuration, particularly for classes of materials which have smooth, substantially unidirectional spectrophotometric curves of the same general shape. However, the present study concerned itself only with petroleum products. This system has also been applied to fatty products, with a view to Lovibond correlation (*5*).

Description of Method

The method, in its bare essentials, consists in focusing a stable beam of light into the sample which is held in a standard container. The light is modified by an optical filter and falls on a photoelectric cell. Readings are taken directly from the apparatus scale. One reading is required and a second is optional. The former is taken with the aid of a filter designated as "North Sky" or, in the case of very light oils, with a filter designated as "Violet". The second reading is taken with a filter designated as "Red". These readings can be converted directly, if desired, into I. C. I. values or Union color numbers.

The present system measures (1) the brilliance and (2) the steepness of the spectral curve. The first factor expresses the appearance of oil of a given thickness to a standard I. C. I. observer looking at C illuminant; and the second factor is best expressed in terms of the deviation of the steepness of the spectral curve from that of the average or normal at the given intensity. In this manner a deviation in one direction would show that the oil is greenish and in the other direction that it is reddish, with the magnitude of the figure giving the extent of deviation.

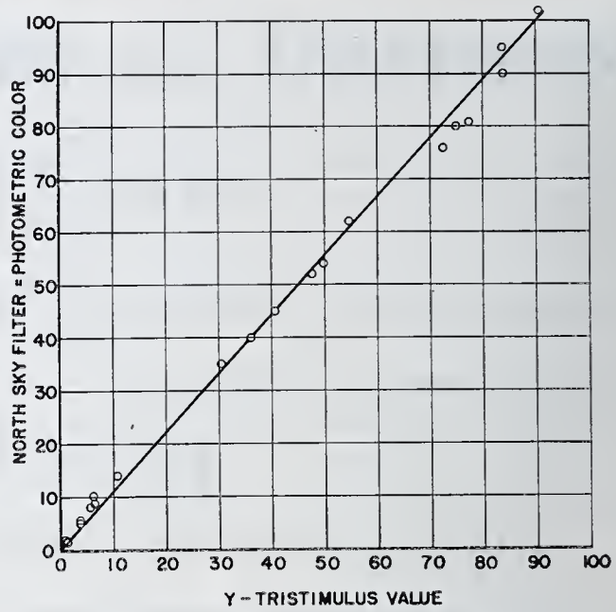

FIGURE 1. CONVERSION CHART OF NORTH SKY READINGS TO BRILLIANCE

It is expected that as a result of a future accumulation of data involving this second reading, it will become possible to use it as a refining guide as well as for the evaluation of finished oils.

The first of these factors is taken with the North Sky filter in position. This filter was so chosen that the instrument (not the filter) would respond as an average observer looking through the oil at the north sky, but was later modified, as the N. P. A. 8 oil gave too low a reading for practical use. By changing the filter to make the instrument correspond to a standard observer and C illuminant, the entire range of the Union system was best accommodated on the scale without reducing the length of light path through the sample. This intensity or brilliance factor is read on a scale 1 to 100 and the reading is reported in "photoelectric units". The North Sky filter is a broad-band filter, but should not be confused with daylight glass.

(The North Sky reading would otherwise have differed from the combination of C illuminant and standard observer, in that the former provided for some near ultraviolet. This is ordinarily regarded as invisible and is therefore discounted. Such disregard is erroneous when it is considered that the eye is not "totally" blind to near ultraviolet light. Some fraction, indeed, a very small fraction, does affect the visual response. On the other hand, the absorption of near ultraviolet by a yellow substance such as oil is great as compared with the absorption of the more visible region, so that while the visual response in this region is small, such effect is disproportionately felt.)

The second factor is determined with a narrow-band filter on either end of the spectrum. For convenience, the red end is preferred. At the extreme red end, slight anomalies, otherwise unimportant, may arise, and for this reason the filter was so chosen that the instrument would respond to an average wave length of 610 mμ.

While "color response" appears to be based primarily on brilliance, the eye is also influenced by the hue or dominant wave length of the oil. For this reason, difficulty is encountered in measuring many oils by the Union colorimeter whose comparison disks are fixed in hue which may differ markedly from that of the sample. In order to express this property, two other parameters such as the X and Z tristimulus values appear to be necessary.

A third filter is provided, designated as V, which causes the instrument to respond to an average wave length of 390 mμ. This filter is suitable for "magnifying" the North Sky reading on the very light oils of the Saybolt range, so that the thickness of the sample does not need to be increased. The spectral range of this filter was particularly chosen so that it could be converted to the equivalent of North Sky reading by the simple equation given below.

The correlation of violet with Saybolt and the handling of petroleum colors in the lighter ranges will be taken up in detail in a future paper, but for the present the authors wish to point out that in the very light range, though not in the darker ranges, readings with one filter are equivalent except

for magnitude to readings on oil with any other filter, narrow or broad.

By this method, a continuous color scale has been provided covering the entire range from the equivalent of Union No. 8 to Saybolt +30+.

Experimental

THE COLORIMETER. The photoelectric colorimeter developed in the course of this work is based on Diller's (*4*).

One of the features of the optical system of the instrument is that it enables the use of test-tube sample containers without substantial error resulting from variations in the refractive index of the samples. The filters are arranged so that the effects of oil fluorescence are minimized. The light path is fixed. The lamp is of the prefocused type with a bar-shaped filament, and it is operated from a compensating transformer which supplies a constant voltage. In this manner, changes in spectral distribution of the filament emission are avoided. Such changes cannot be overcome by an attempted balancing of photocells. A single cell of the barrier-layer type is used. The sample container is gripped in a wedge by a scratchproof roller, to maintain further the immobility of the optical system. The calibration has been found to be constant over a period of six years. It is not subject to atmospheric variations or fatigue. The instrument is heavily constructed and its galvanometer is both rapid and damped.

The filters are mounted in a roulette with additional provision for miscellaneous filters, so that the instrument can also be used for chemical analysis. The optical path through the sample is approximately 18 mm. The instrument containing the photometric color filters is adjusted and standardized as a complete unit, so that readings are the same on any of the instruments within the tolerance described below.

Approximately 1000 samples of oil have been used, representing a wide variety of crude source, and of method of refinement. Many of these samples were carefully chosen to illustrate extremes of viscosity index and of treating, but the majority were merely chosen at random from the samples passing through the laboratory for routine testing. The preliminary work consisted of measuring fifty oils, using ten narrow-band glass filters, with dominant wave length ranging from 390 to 660 mμ.

REFERENCE STANDARD. For such measurements, the colorimeter must be set to some standard reading. This necessitates the use of a standard reference liquid, preferably as light or lighter in color than any of the samples. Since the range of measurement was to include that covered by the Saybolt chromometer, a generally available water-white liquid of reproducible color was sought.

For closest similarity to the samples to be measured, a hydrocarbon solvent was indicated. Xylene, toluene, and mineral spirits of various distillation ranges were studied. The colors were found to vary appreciably with redistillation, distillation over caustic, acid treatment, clay treatment, etc., and after such treatments, the colors changed on storage. Consequently, a hydrocarbon standard would require carefully specified preparation, at short time intervals. Distilled water, however, is easily prepared, and is stable and reproducible and was, therefore, chosen as the standard. The colorimeter was set to a scale reading of 100.0 per cent transmission with water in the cell, with any filter in the light path.

NORTH SKY FILTER. Twenty-two oils, of widely varying origins and methods of refinement, were submitted to the Electrical Testing Laboratories for measurement on a Hardy spectrophotometer. The North Sky filter also was measured. The spectrophotometric data were handled by the methods described by Hardy (*8*), using the 30 selected ordinates. The North Sky filter was found to have the following characteristics:

Tristimulus Values	Trichromatic Coefficients
$X = 0.3768$	$x = 0.2766$
$Y = 0.4502$	$y = 0.3305$
$Z = 0.5351$	$z = 0.3929$

Dominant wave length 496 mμ
Relative brightness 45.0%
Excitation purity 11.5%

The filter described by these data is for use with a 15-c. p., tungsten bar filament bulb, operating at 3100° K. behind a heat filter. The photocell is of the barrier-layer type and the optical path is shown by Diller (*4*). It cannot be overemphasized that the instrument must be standardized as a complete unit rather than through standardization of the filter. The procedure for checking the standardization is described below.

The tristimulus values calculated from the spectrophotometric curves of the 22 oils are given in Table I, which also indicates the nature of each oil sample, and gives the photoelectric color or North Sky reading. Columns R and V are readings obtained with other filters, described below.

TABLE I.

Oil No.	Nature	Spectrophotometer Each Value × 100 X	Y	Z	Photoelectric Color R	N. S.	V
1	Naphthenic	1.7	0.74	0.0	10.0	1.1	0
2	Heavy clay	2.3	1.0	0.0	13.0	1.9	0
3	Compounded	2.4	1.0	0.0	12.0	1.3	0
4	Pennsylvania	8.3	3.8	0.0	33.0	5.5	0.8
5	Acid, mid-continent	7.4	3.9	0.0	27.0	5.0	0.3
6	Pennsylvania	11.6	5.8	0.0	42.0	8.0	0.8
7	No. 14 + black oil	8.4	6.2	0.2	26.0	10.0	0.6
8	Naphthenic	11.1	6.7	0.0	34.0	8.5	0.3
9	Solvent-Pennsylvania	19.3	10.9	0.6	56.5	14.0	1.1
10	Acid-naphthenic	34.7	30.4	0.6	70.0	35.0	0.8
11	Solvent, clay	42.9	36.2	0.4	81.0	40.0	0.9
12	Compounded	44.8	40.7	1.2	84.0	45.0	1.0
13	Acid, mid-continent	52.8	47.9	1.9	89.5	52.0	2.0
14	Acid, mid-continent	50.7	50.0	3.8	84.0	54.0	2.0
15	Solvent, mid-continent	50.6	54.9	8.7	88.5	62.0	3.9
16	Naphthenic	65.4	72.6	10.6	97.0	76.0	3.0
17	Naphthenic	66.3	75.0	18.8	99.0	80.0	6.5
18	Solvent, mid-continent	71.4	77.5	40.4	97.0	81.0	20.0
19	Naphthenic—heavy acid	78.1	83.8	71.0	100.0	95.0	44.0
20	Naphthenic—heavy clay	75.0	83.9	46.3	100.0	90.0	23.0
21	Kerosene	88.2	90.3	105.5	101.0	102.0	100.0
22	White oil	88.4	90.4	106.2	101.0	102.0	100.0

The Y tristimulus value is identical with relative brightness, or visual efficiency, commonly referred to as brilliance. The spectrophotometric response of the instrument with the North Sky filter in position is similar in shape to the Y stimulus. The North Sky readings were plotted against the Y values as shown in Figure 1. Photoelectric color is seen to be directly related to Y:

$$\text{Brilliance} = 0.9 \times \text{photoelectric color}$$

Distilled water is used as the standard for photoelectric color, whereas brilliance is based on a pure, theoretical white. The brilliance of distilled water is approximately 90 per cent, accounting for the factor 0.9 in the above equation.

RED FILTER. Petroleum oils have the peculiar property of varying in the relation between brilliance, hue, and saturation in a substantially fixed manner and only one other parameter—to determine the steepness of the transmission curve—is required. The parameter could be similar to either X or Z. The Z values, as shown in Table I, remain very small until the brilliance exceeds 50 per cent (Union No. $2^1/_2$) while the X values are of conveniently measurable magnitude over the entire range with the same liquid stratum used for the North Sky measurement. Consequently, red or orange glasses were preferred.

Dyes of various colors were also added to the oils to give products whose colors differed intentionally from those of normal petroleum products. One of the filters of this series was found to be most satisfactory in distinguishing between all of these samples. Oils which were measured had viscosity indices approximating 0 and 100.

The spectral absorption of water is reasonably uniform

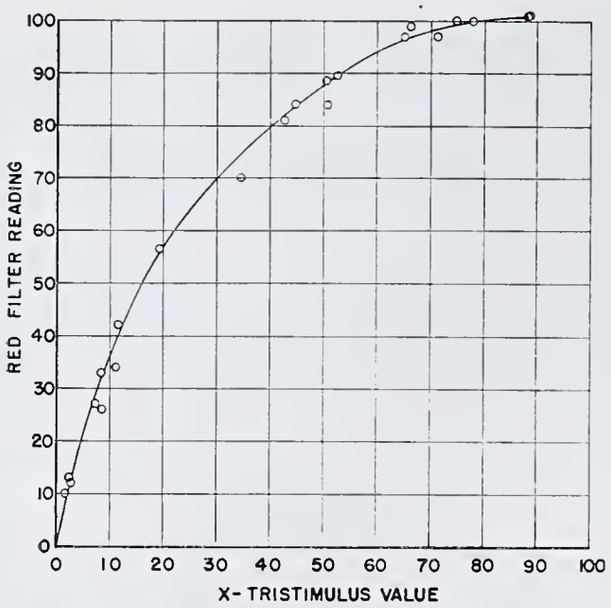

FIGURE 2. CONVERSION CHART OF RED READINGS TO X

throughout the visible region except at the extreme red end (greater than 700 mμ) where the absorption increases. Thus, in this region of the spectrum, the use of water as the standard results in readings which are higher than the corresponding readings at 610 mμ by too great a difference. The oils themselves also sometimes exhibit slight absorption peculiarities in this region, so that anomalous results may be obtained. A consideration of these and the other factors led to a choice of 610 mμ as the average response equivalent of the instrument with the R filter. The characteristics of the particular filter used in order to have an instrument response equivalent to 610 mμ is:

Tristimulus Values	Trichromatic Coefficients
$X = 0.1039$	$x = 0.6992$
$Y = 0.0447$	$y = 0.3008$
$Z = 0.0000$	$z = 0.0000$

Dominant wave length 624 mμ
Relative brightness 4.4%
Excitation purity 100%

Readings taken with the instrument when using this filter are shown in Table I under R where the correspondence to X stimulus may be observed. The red filter readings of Table I were plotted against the respective X values. The resultant curve (Figure 2) shows the relationship of R readings to X values.

RELATION BETWEEN NORTH SKY AND R READINGS. Approximately 300 oils have been measured with both the North Sky and red filters, in order to establish the relation between the two readings. Since oils containing blooming or deblooming agents must be excluded from this work, only oils of known origin and treatment were used. This limited the choice of samples. Figure 3 shows this variation, the two lines denoting the widest variation found. It is possible that oils from unusual sources or methods of treatment will be encountered which lie outside this lens; but, it is not to be expected that such samples will change the essential narrowness of the lens. An average line is drawn through the lens, so that the R readings can be reported as plus or minus deviations from normal.

In general, oils of low viscosity index lie near the lower line. However, the method of refinement also affects the position, acid treatment tending to move the color toward the upper line, and clay treatment toward the lower.

VIOLET FILTER. The Saybolt range (−16 to +30+) corresponds to North Sky readings of 96 to 100. This is an insufficient spread and must be "magnified". This may be done by a substantial increase in sample depth. However, this would involve other factors such as slight turbidity and fluorescence, which would lead to error. Another means of magnification of the spread lies in the use of a violet filter which in this range of light colors results in readings parallel to North Sky readings but of greater spread. The latter means is preferable. The filter was prepared so that for kerosenes in the Saybolt range the instrument gave exactly 10 times the sensitivity as with the North Sky filter. This filter is designated as the violet filter.

The violet filter used has the following characteristics:

Tristimulus Values	Trichromatic Coefficients
$X = 0.0622$	$x = 0.1597$
$Y = 0.0048$	$y = 0.1023$
$Z = 0.3226$	$z = 0.8280$

Dominant wave length 446 mμ
Relative brightness 0.48%
Excitation purity 100%

The spectrophotometric curve of the violet filter corresponds roughly to the Z stimulus, as is shown in Figure 4,

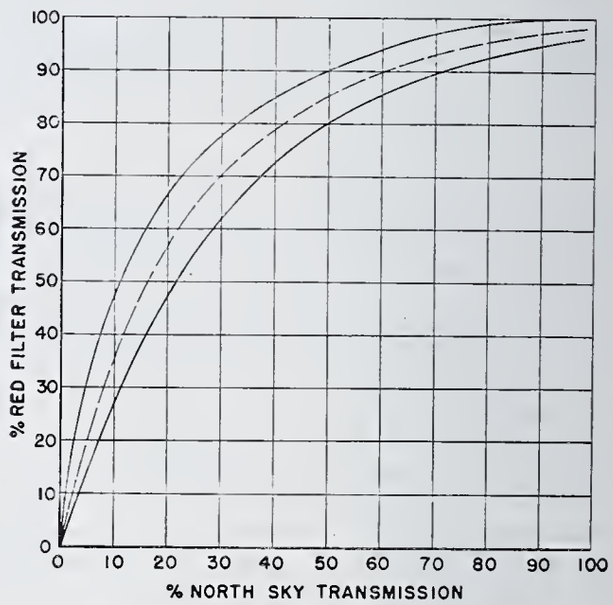

FIGURE 3. NORMAL DEVIATION OF R

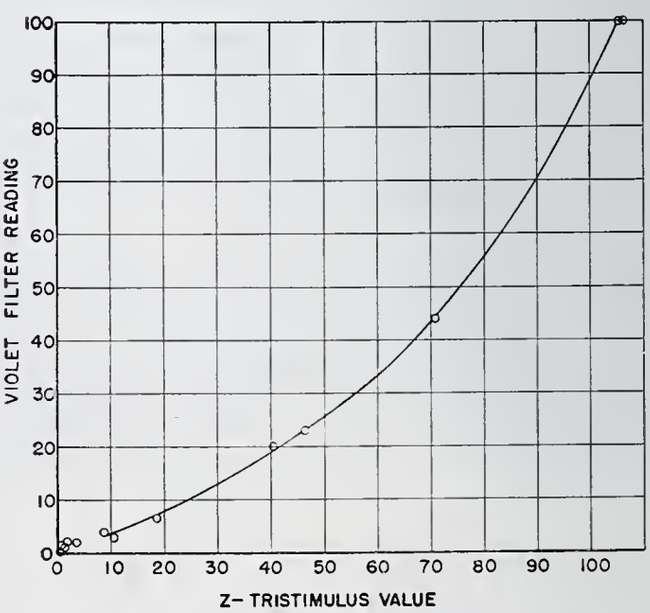

FIGURE 4. CONVERSION CHART OF VIOLET READINGS TO Z

TABLE II. TRISTIMULUS VALUES

Sample	$X \times 100$ Spectral[a]	$X \times 100$ Photoelectric[b]	$Y \times 100$ Spectral	$Y \times 100$ Photoelectric	$Z \times 100$ Spectral	$Z \times 100$ Photoelectric
1	1.7	2.1	0.74	1.0	0.0	0.0
2	2.3	3.0	1.0	1.7	0.0	0.0
3	2.4	2.7	1.0	1.2	0.0	0.0
4	8.3	8.8	3.8	4.9	0.0	0.0
5	7.4	7.8	3.9	4.5	0.0	0.0
6	11.6	12.0	5.8	7.2	0.0	0.0
7	8.4	6.2	6.2	9.0	0.2	0.0
8	11.1	9.0	6.7	7.6	0.0	0.0
9	19.3	19.5	10.9	12.6	0.6	0.0
10	34.7	30.0	30.4	31.5	0.6	0.0
11	42.9	41.0	36.0	36.0	0.4	0.0
12	44.8	44.5	40.7	40.5	1.2	0.0
13	52.8	52.5	47.9	46.8	1.9	0.0
14	50.7	44.5	50.0	48.6	3.8	0.0
15	50.6	51.0	54.9	55.8	8.7	10.0
16	65.4	67.0	72.6	68.4	10.6	9.0
17	66.3	65.5	75.0	72.0	18.8	16.5
18	71.4	72.0	77.5	72.9	40.4	42.0
19	78.1	79.0	83.8	85.5	71.0	71.0
20	75.0	73.0	83.0	81.0	46.3	46.5
21	88.2	88.2	90.3	91.8	105.5	105.5
22	88.4	88.2	90.4	91.8	106.2	105.5

Mean deviation of photoelectric from spectral ±1.0

[a] Computed from Hardy spectrophotometric curves.
[b] Converted from readings on photoelectric colorimeter.

TABLE III. TRICHROMATIC COEFFICIENTS

Sample	x Spectral	x Photoelectric	y Spectral	y Photoelectric	z Spectral	z Photoelectric
1	0.697	0.677	0.303	0.323	0.0	0.0
2	0.696	0.638	0.304	0.362	0.0	0.0
3	0.706	0.692	0.294	0.308	0.0	0.0
4	0.686	0.647	0.314	0.353	0.0	0.0
5	0.655	0.624	0.345	0.376	0.0	0.0
6	0.667	0.625	0.333	0.375	0.0	0.0
7	0.569	0.408	0.420	0.592	0.011	0.0
8	0.624	0.589	0.376	0.411	0.0	0.0
9	0.627	0.607	0.354	0.393	0.019	0.0
10	0.528	0.488	0.463	0.512	0.009	0.0
11	0.540	0.532	0.455	0.468	0.005	0.0
12	0.517	0.523	0.469	0.477	0.014	0.0
13	0.515	0.529	0.467	0.471	0.018	0.0
14	0.485	0.478	0.478	0.522	0.037	0.0
15	0.443	0.437	0.481	0.478	0.076	0.085
16	0.440	0.464	0.489	0.474	0.071	0.032
17	0.414	0.425	0.468	0.468	0.118	0.107
18	0.377	0.385	0.409	0.390	0.214	0.225
19	0.335	0.335	0.360	0.363	0.305	0.301
20	0.365	0.363	0.409	0.403	0.226	0.234
21	0.311	0.309	0.318	0.322	0.371	0.369
22	0.310	0.309	0.317	0.322	0.373	0.369

Mean deviation of photoelectric from spectral ±0.020

where the violet filter readings of Table I, column V, were plotted against the corresponding Z values.

RELATIONSHIP OF V AND NORTH SKY READINGS. When the North Sky reading is 96 or higher, the violet filter is used, its reading converted to North Sky, and the equivalent reading reported as photoelectric color. The violet filter readings on petroleum products are related to the North Sky readings by the following equation:

$$N.S. = 90 + \frac{V}{10}$$

CORRELATION WITH I. C. I. VALUES. To test the accuracy of the smoothed curves shown in Figures 1, 2, and 4, and of the authors' instrument, the values of X, Y, and Z corresponding to the red, North Sky, and violet readings of Table I were read from them. Table II gives a comparison of results from the present colorimeter, and from the spectrophotometer readings. The three photoelectric readings made with the colorimeter used in this work require less than 2 minutes, and X, Y, and Z are obtained directly from the curves. The spectrophotometric curve using a Hardy spectrophotometer requires about 10 minutes, and the required subsequent calculation of X, Y, and Z from that curve occupies a minimum of another 30 minutes. For red readings above 97, where Figure 2 flattens, a more accurate graph than Figure 3 was used to find X.

From the tristimulus values, the trichromatic coefficients may be calculated. Table III shows the results, again from the spectral curves and from the photoelectric readings. Finally, from x and y, the hue and purity of the oil color may be obtained (Table IV).

Table V gives data on the reproducibility of the readings for one of the oils, using spectrophotometric curves and photoelectric readings obtained on the two dates given. The photoelectric colorimeter used in this work is at least as stable as is the recording spectrophotometer.

The above may be summarized as indicating that photoelectric color readings, in conjunction with Figures 1, 2, and 4 will yield tristimulus values agreeing with spectrophotometric data to ±0.01, trichromatic coefficients to ±0.02,

TABLE IV. BRILLIANCE, HUE, AND PURITY

Sample	Brilliance Spectral	Brilliance Photoelectric	Hue, mμ Spectral	Hue, mμ Photoelectric	Purity Spectral	Purity Photoelectric
1	0.74	1.0	624	614	100	100
2	1.0	1.7	622	603	100	100
3	1.0	1.2	628	620	100	100
4	3.8	4.8	617	605	100	100
5	3.9	4.7	607	600	100	100
6	5.8	7.2	610	600	100	100
7	6.2	9.0	590	565	97	100
8	6.7	7.6	599	592	100	100
9	10.9	12.6	604	596	95.0	100
10	30.4	31.5	583	576	96.0	100
11	36.2	36.0	585	583	99.0	100
12	40.7	40.5	582	581	96.0	100
13	47.9	46.8	582	582	96.0	100
14	50.0	48.6	579	575	90.0	100
15	54.9	55.8	576	574	80.0	78
16	72.6	68.4	574	577	82.0	84
17	75.0	72.0	572	574	69.0	71.5
18	77.5	72.9	573	578	43.0	40.0
19	83.8	85.5	570	569	18.0	19.0
20	83.9	81.0	571	571	40.0	37.5
21	90.3	92.0	555	560	1	1
22	90.4	92.0	555	560	1	1

Mean deviation of photoelectric from spectral 5 mμ, 2%

TABLE V. COMPARATIVE REPRODUCIBILITY

	April 30 Spectral	April 30 Photoelectric	June 20 Spectral	June 20 Photoelectric	Difference Spectral	Difference Photoelectric
Red reading	...	97.0	...	97.0	...	0.0
N. S. reading	...	82.0	...	81.0	...	1.0
Violet reading	...	20.0	...	20.0	...	0.0
X	72.4	72.0	71.4	72.0	1.0	0.0
Y	78.9	73.8	77.5	72.9	1.4	0.9
Z	40.0	42.0	40.4	42.0	0.4	0.0
x	0.378	0.383	0.377	0.385	0.001	0.002
y	0.412	0.393	0.409	0.390	0.003	0.003
Hue	573	577	573	578	0	1
Purity	44	40	43	40	1	0

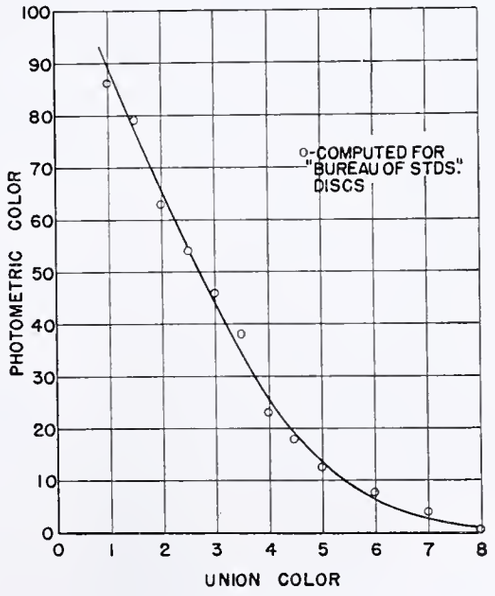

FIGURE 5. CONVERSION CHART OF NORTH SKY TO UNION

Table VI. Colors of Union Disks

National Bureau of Standards representative disks, corrected to 18-mm. sample depth

No.	Brilliance	Hue	Purity
	%	mμ	%
1	75.1	571	28
1½	65.4	573	59
2	44.3	578	86
2½	36.5	582	88
3	28.7	585	91
3½	21.1	589	94
4	9.6	594	98
4½	6.5	599	99
5	3.6	606	100
6	1.7	613	100
7	0.66	617	100
8	0.20	634	100

hue to ±5 mμ, purity to ±1 per cent, and brilliance to ±1 per cent. These results are obtained in less than 5 minutes, in contrast to the half hour or more required for spectrophotometric measurement and calculation. In addition, the former method requires considerably less operative skill and is substantially less expensive. This correlation has been based only on petroleum oils, whose spectral curves are substantially smooth and of the same generic shape.

Correlation with Union Color. The smooth curve of Figure 5 shows the correlation of photoelectric color with Union color, obtained by measurement on over 1000 samples, using one Union colorimeter. Other laboratories which examined the photoelectric colorimeter found that this curve did not apply at all points to their Union color readings. This cannot be avoided, in view of the inherent limitations of the Union method. However, a correlation may be made between Union color and photoelectric color through the medium of the I. C. I. system and the National Bureau of Standards representative disks. Spectrophotometric data obtained by the bureau on a set of Union disks believed to be representative (*19*) were converted to brilliance, hue, and purity (Table VI). North Sky readings were then converted to Union readings through the brilliance relationship after due correction for difference in sample depth.

The hue and purity of the Union disks are fixed, but at a given brilliance the hue and purity of oils vary over a considerable range. While the photoelectric colorimeter, through the North Sky reading, is capable of measuring the brilliance factor alone in complete disregard of the other factors, the eye normally cannot make such a separation.

Visual matching of color, such as is demanded by the Union colorimeter, involves matching the hue, the brilliance, and purity, or any combination of these three properties. The human eye has difficulty in estimating the relative brilliances of two colors if the hues also are dissimilar. Differences in purity cause an additional complication. In Figure 6, the brilliances of the twenty-two oils and of the representative disks are plotted against their respective hues.

Sample 6 has a hue and brilliance corresponding to a Union No. 6 disk. Sample 7 has the brilliance of a Union No. 6 disk and the hue of a No. 3½ disk. Five operators with normal visual response and one who was totally color blind in the red region of the spectrum compared these oils visually and took Union colors on them. The five normal operators said Sample 6 was darker than No. 7 but all gave a reading of 5½ or light 6 to Sample 7 and a reading of 6 on Sample 6. The color-blind observer rated both samples as Union No. 6 and could barely distinguish between them. This and other observations of a similar nature indicate that in the region of Union No. 1 to Union No. 6 the brilliance is used, consciously or unconsciously, as the basis for "color" matching. Hue influences the designation to a small extent. However, in the region corresponding to Union No. 6 to Union No. 8, it appears that hue is of increasing importance and the designation is made on the basis of the redness of the oil. Brilliance is not ignored entirely but is of decreasing importance in this range.

Figure 6 compares the brilliance with the respective hues, both for the representative disks and some typical oils. As will be seen at a glance, the brilliance does not fix the hue and the range of possible hues for a given brilliance is extensive. Indeed, hue and brilliance do not vary uniformly or smoothly among the representative disks themselves (Figure 7). On the other hand, photoelectric color (North Sky) is actual transmittance and does, therefore, vary uniformly with brilliance.

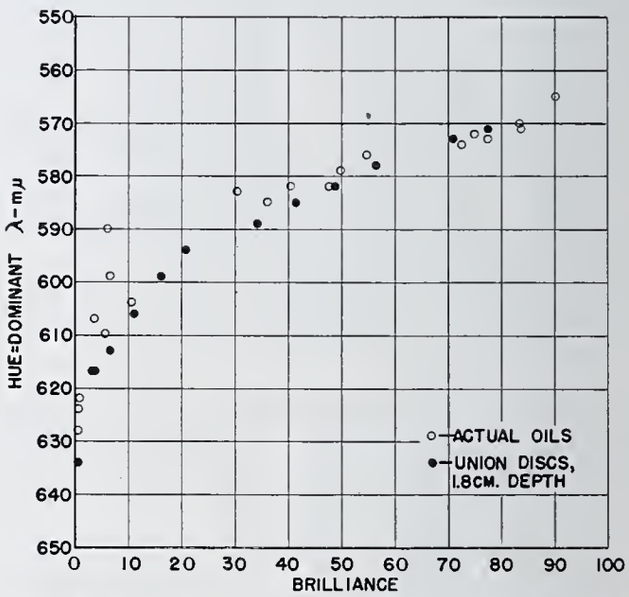

Figure 6. Combinations of Brilliance and Hue of Oils and Disks

In view of the above considerations, a really smooth correlation curve between North Sky and Union cannot be expected. However, the deviations from the authors' smooth curve of Figure 5 are not greater than the customary differences between various sets of Union glasses. The deviation of the Bureau of Standards disks from the smooth curve is shown by the circles in Figure 5. Since the variations among Union colorimeters usually exceed this deviation, the correlation curve for photoelectric color *vs.* Union color might as well be drawn smoothly as in Figure 5. The disparity between the data of Table VII may be attributed to the difference between the authors' Union instrument and that of the bureau.

Table VII. Correlation of Union with Photoelectric–North Sky

Union Color	Photoelectric Color–N. S. from Figures 1 and 6	Smoothed Correlation Curve From Figure 5
1	86	89
1½	79	77
2	63	65
2½	54	54
3	46	44
3½	38	34
4	23	26
4½	18	19
5	12.5	13
6	7.7	6
7	4.0	2.5
8	0.8	1

Standardization of Colorimeter. The reading obtained on any oil depends upon the lamp used, the voltage at which it is operated, the filter used, the light path, the spectral response of the photocell, and its circuit. Many of these variables can be changed by limited amounts and other variables changed to compensate, so that the same oil

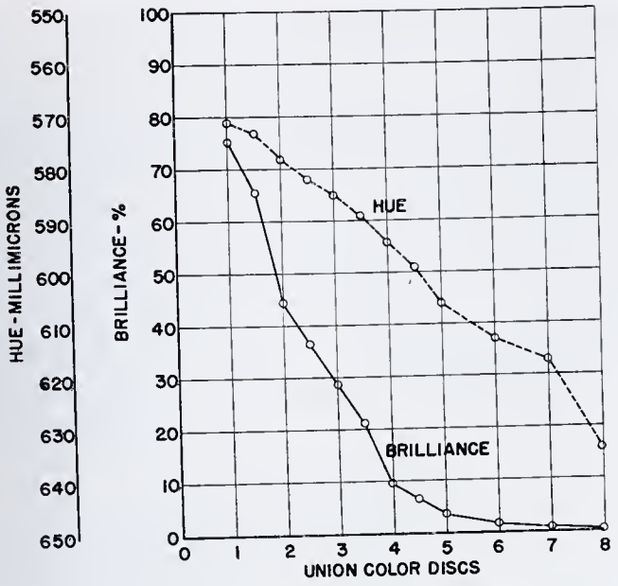

FIGURE 7. HUE AND BRILLIANCE OF REPRESENTATIVE DISKS

readings can be obtained under differing conditions. For this reason, it is necessary to specify the entire combination of parts, not each part. The I. C. I. data given above for the three filters need not be adhered to strictly, provided compensating adjustments are made. Readings taken on oils by different operators on six different instruments of the same type agreed within one unit.

The Union colorimeter uses a specified source of illumination, and disks of specified Y, x, and y. However, the user of one of these instruments is rarely equipped to determine how closely his colorimeter conforms to the standards or what changes, if any, have taken place.

The standardization of the colorimeter used in this work may be readily checked by using standard, reproducible, and easily prepared colored solutions in the same cell as that used for the sample. To accomplish this with the North Sky, red, and violet filters in position, the solutions described below have been chosen.

Checking of Standardization

SOLUTION 1, 300 grams of concentrated sulfuric acid (sp. gr. about 1.84) in 1 liter of solution.

SOLUTION 2, 250 ml. of concentrated ammonium hydroxide (sp. gr. about 0.90) diluted to 1 liter with distilled water.

SOLUTION 3, 10 grams of borax ($Na_2B_4O_7.10H_2O$) and 30 grams of potassium hydroxide dissolved in water and diluted to 1 liter with water.

SOLUTION 4a. Weigh approximately 2.5 grams of nickel sulfate ($NiSO_4.6H_2O$) and dissolve in Solution 2, making up to approximately 100 ml. with Solution 2. Analyze by accepted analytical procedure. Dilute with Solution 2 to give 0.500 gram of nickel per 100.0 ml. of solution. Restandardize, and readjust if necessary.

SOLUTION 4b. Dilute 25.0 ml. of Solution 4a to 100.0 ml. with Solution 2.

SOLUTION 5a. Weigh exactly 10.000 grams of dry, C. P. potassium dichromate. Dissolve in Solution 1, making up to exactly 100.0 ml. with Solution 1.

SOLUTION 5b. Dilute 10.0 ml. of Solution 5a to 100.0 ml. with Solution 1.

SOLUTION 6a. Weigh 0.0200 gram of pure, dry potassium dichromate. Dissolve in Solution 3, and make up to 100.0 ml. with Solution 3.

SOLUTION 6b. Dilute 10.0 ml. of Solution 6a to 100.0 ml. with Solution 3.

If these solutions are to be kept, they should be stored in glass-stoppered, alkali-resistant glass bottles. Even with this precaution, the solutions may develop a very fine precipitate. The photoelectric color reading is affected by turbidity too slight to be visible to the eye. All these solutions, therefore, should be filtered through clean, dry asbestos before measurement. This filtration does not noticeably affect the content of the colored ion (see below).

To standardize the instrument, the above solutions are handled by exactly the same technique as is used for the measurement of the color of a sample. Under these conditions, the scale readings obtained on the standard solutions, with the appropriate filters, should agree with the following values to ± 1 unit:

Standard Solution	Scale Reading (% Transmission)
North Sky Filter	
4a	48
5a	32
5b	49
6a	94
Red Filter	
4a	44
4b	80
5a	99
6a	100
Violet Filter	
4a	79
5b	1
6a	24
6b	70

These particular solutions were chosen because the potassium dichromate solutions are sensitive to variations in the blue and green regions of the spectrum, the potassium chromate solutions to variations in the violet, and the nickel ammino sulfate solutions to variations in the red and blue. In this way variations are covered throughout the entire visible spectrum.

These solutions were chosen to be readily reproducible, sensitive to variations in the colorimeter, but relatively insensitive to slight errors in their preparation. The concentration of the colored ion must, of course, be rigidly fixed but variations in the other components of these solutions have little effect. This is illustrated in the following table:

Solution	Variation	Change in Filter Reading North Sky	Red	Violet
4a	10% change in NH_4OH concentration	0.7	1.5	1.0
5a	80% increase in H_2SO_4 concentration	1.3	1.8	0.0
6a	20% change in borax concentration and 16.7% change in KOH concentration	1.0	0.5	0.3

Changes in the more dilute solutions would be even smaller. In order to check the reproducibility of the potassium dichromate, solutions 5a, 5b, 6a, and 6b were made up using potassium dichromate from four sources: Merck, Baker, Eimer and Amend, and Mallinckrodt. The variation was less than 0.5 scale division in all cases. Nickel sulfate from the same four sources was used in making up solutions 4a and 4b and the variation was again less than 0.5 scale division in all cases. Readings on six different instruments of the same type were reproducible to ± 1.0 scale division. Sets of solutions were prepared by three different operators and agreed to ± 1.0 scale division. Thus, these solutions give an accurate, easily prepared, and reproducible set of standards for the whole system.

Conclusion

This paper has defined a system, designated as photoelectric color, for the measurement of the color of petroleum oils. Photoelectric color rates petroleum oils in the same order as visual inspection and enables a further characterization by one parameter in terms of relative reddishness or greenishness.

The color definition is independent of the visual response of the operator. The system can be learned easily and requires no previous experience in dealing with oil colors, and the standardization can be checked with aqueous inorganic solu-

tions which are reproducible and easily prepared. From photoelectric color, I. C. I. data can be calculated if desired. Photoelectric color can be correlated with the Union system on a statistical basis.

Acknowledgment

The authors wish to acknowledge the invaluable cooperation of Hellige, Inc., in preparing the experimental equipment required during the course of this work. The instrument developed for the present purpose is known as the Hellige-Diller photoelectric colorimeter, Model 405-A. They also wish to acknowledge the assistance obtained by frequent reference to a private report by V. A. Kalichevsky and B. W. Story.

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Determining the Deterioration of Cellulose Caused by Fungi

Improvements in Methods

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ACCURATE practical methods of determining the factors involved in the deterioration of fabrics and other fiber products are widely needed at the present time. Numerous investigations have shown that cellulose deterioration may be a result of the action of fungi, bacteria, ultraviolet radiation, chemicals, or a combination of these factors. Most of the effort has been directed toward a comparison and appraisal of preventive treatments rather than toward a better understanding of the causes and mechanism of deterioration. Methods of evaluating deterioration have varied so greatly that it is extremely difficult to make accurate comparisons.

The present paper deals largely with (1) experiments on the refinement of technique for testing the amount and rate of cellulosic decomposition that might be caused by various types of microorganisms, and (2) suggestions for the establishment, within certain limitations, of a standardized quantitative technique for testing the effectiveness of mildew-resisting treatments. For the latter purpose certain modifications of the preparatory treatments for the fabric would probably be required.

Literature Review

Several excellent reviews (*5, 6, 7, 12, 13*) on the microflora associated with fabrics and raw fibers composed principally of cellulose have been published. They show that most of the reports on the microbiology of cotton and its manufactured products give only the identification of microorganisms found on these materials. Only a few investigators have attempted to secure quantitative data that may represent the ability of these organisms to destroy cellulose.

For example, Searle (*10*) determined the wet breaking strength of mildewed fabrics to estimate deterioration. He developed a method in which 37.5 × 3.75 cm. (15 × 1.5 inch) strips of cotton fabric were wound on filter candles which had been coated previously with a soil suspension. These strips were then incubated for 3 or 6 weeks by placing each candle in a test tube containing a small quantity of water. A loss of 55 to 93 per cent in strength occurred during 6 weeks' incubation. Searle's method frequently failed to give close agreement between replicates under apparently identical conditions.

Thom, Humfeld, and Holman (*12*) determined the breaking strength of mildewed duck after conditioning in a standard temperature and humidity room for 2 or more days. They abandoned mixed cultures because of the difficulty in obtaining comparable results, and after testing pure cultures of many organisms, selected *Chaetomium globosum* Kunze. A mineral agar was used to support the cellulose sample which was incubated 14 days at 28° to 30° C.

Rogers, Wheeler, and Humfeld (*9*) made quantitative chemical and physical analyses of mildewed duck. They measured the activity of *Ch. globosum* and *Spirochaeta cytophaga* Hutchison

and Clayton, grown on cotton duck on a mineral salts agar medium and incubated for 1 to 18 days at room temperature (about 28° C.). They used square 0.5-liter (16-ounce) screw-cap, closed bottles.

Furry, Robinson, and Humfeld (*4*) studied mildew-resistant treatments on fabrics using the procedure described by Rogers, Wheeler, and Humfeld (*9*).

Other investigators (*2, 3, 11*) have used soil burial tests, or modifications of these, for testing the deterioration of treated and untreated fabrics exposed to field or greenhouse conditions. Their tests usually require long periods of time and provide no check of the casual agencies that are operative in a given test; because of so many unknown or uncontrolled factors, close agreement between results from different tests—if between samples in the same test—would not be expected. Such procedures have, however, resulted in valuable contributions and undoubtedly will continue to be useful for checking and improving laboratory methods.

Materials and Methods

Bleached, 8-ounce Army duck was used throughout these investigations. Before exposure to the attack of the fungi, the fabric was treated to remove sizing finishes, any residual waxes, pectins, and other substances that might serve as added nutrients. For the degreasing procedure the material was extracted twice for 2 hours each in two changes of carbon tetrachloride, followed by a treatment with 0.05 per cent starch and protein-solubilizing enzyme preparation at 60° C. for 1 hour. Finally, the cloth was thoroughly rinsed in distilled water (*1*).

The fabric was divided into large blocks so that each block would furnish one of the ten replicates used for each determination. The blocks were cut into strips measuring 15 cm. (6 inches) in the warp direction by 3.1 cm. (1.25 inches) and by raveling the width was reduced to exactly 2.5 cm. (1 inch) (*1*). The ten replicates used for each determination were selected by a series of random numbers.

Individual incubation chambers were 0.5-liter (16-ounce), square bottles measuring 16.25 cm. (6.5) inches to the neck. Metal screw tops to these bottles were modified to permit a uniform exchange of gases but to exclude any contaminating organisms. Sterile nutrient media in containers with these modified caps remained uncontaminated even after inoculum was pipetted directly on the outer surface of the glass fabric and incubated for several weeks. A circle 4 cm. (1.625 inches) in diameter, was cut out of the center of both the cap and the waxed-paper filler. A round piece of glass filter fabric, the diameter of the filler, was inserted between the cap and the ring of waxed filler (Figure 1).

In order to keep the strip of cotton duck in contact with the liquid medium and yet prevent it from being submerged, a mat of glass fabric was placed in each bottle to support the strip and serve as a wick. These mats, which were approximately 5 × 15 × 0.25 cm. (2 × 6 × 0.1 inches), are made from a material similar to Pyrex and thus are not attacked by organisms. (Detailed description of the glass fabrics that were found to be adapted to these uses can be furnished upon request. An important manufacturer of these products cooperated in furnishing samples and in developing folded-over and sewed strips that would not ravel. Unsewed strips, however, were re-used several times in these experiments with little difficulty from raveling.)

Sufficient liquid medium was pipetted into each bottle to come up to the upper surface of the glass fabric when in the horizontal position. The liquid medium may be conveniently measured into the bottles by means of a measuring or an automatic zero pipet (*8*). The liquid in the culture chamber should not submerge the cotton test strip. The bottles after being capped and stacked horizontally were autoclaved for 20 minutes at 6.8 kg. (15 pounds) pressure.

With only slight modifications this test system could be changed to one of continuous renewal of mineral solutions. This might be useful and advisable in testing treated fabrics when leaching of toxic chemicals is suspected.

Stock cultures of the various fungi used in these investigations were maintained in test tubes on filter paper placed on the surface of an agar slant prepared according to the desired mineral formula. About 10 to 14 days before the spores were required for inoculating the fabric, transfers were made to Petri dishes containing sterile filter paper on mineral salts–agar. The inoculum was prepared by harvesting the fruiting bodies and mycelium on the surface of one piece of 7-cm. filter paper and suspending them in 50 ml. of sterile distilled water in Erlenmeyer flasks containing small glass beads. The flask was shaken until an even suspension was obtained.

(This system of preparing the inoculum has since been simplified. Erlenmeyer flasks with glass beads for supporting the filter paper on the surface of the liquid nutrient are substituted for the agar-containing Petri dishes. Harvest is accomplished by shaking the flask until the inoculum is dispersed by the action of the glass beads, adding sterile distilled water, and pipetting off the suspension. Agar and labor are saved and opportunity for contamination is minimized.)

While it appears that within reasonable limits the amount of inoculum for each strip is not very important, it is recommended that 1 ml. of the resultant suspension be transferred by means of a sterile pipet. The strips of fabric were incubated in a darkened, air-conditioned room which was maintained at 85° to 86° F. (29.4° to 30.0° C.) and 90 to 92 per cent relative humidity.

FIGURE 1. INDIVIDUAL INCUBATION CHAMBER ADAPTED TO THE USE OF LIQUID MEDIUM

Upper right, metal cap and wax paper filler with center removed
Upper left, glass cloth alone and in place in metal cap
Left, center, unsewed glass wick
Right center, sewed glass wick
Bottom, duck strip 6 inches long raveled to 1-inch width
Center, individual inoculation chamber with glass wick and duck strip in place

The liquid nutrient that appeared to be well adapted to the cellulose-decomposing fungi so far tested is represented by Formula A, and is prepared as follows:

	G./l.	
K_2HPO_4	1.3940	(0.008 *M*)
$MgSO_4$	0.7395	(0.003 *M*)
NH_4NO_3	1.0006	(0.0125 *M*)
$CaCO_3$	0.005	
NaCl	0.005	
Fe, Zn, and Mn as SO_4	0.001	

For comparison of formulas and techniques the method described by Rogers, Wheeler, and Humfeld (*9*) also was followed. In each experiment the standard procedure was followed except for the variables under study or when specific mention is made for a certain procedure. The mineral

nutrient agar, designated as Formula B, used by Rogers, Wheeler, and Humfeld (*9*) was prepared as follows:

Tap water, ml.	1000.0
$NaNO_3$, grams	3.0
K_2HPO_4, gram	1.0
$MgSO_4$, gram	0.25
KCl, gram	0.25
Agar, grams	10.0

Both formulas were used with and without agar. After 7 days' incubation the strips were removed from the bottles and carefully rinsed in running water to remove the fungal growth. The air-dried strips were placed in an oven at 80° to 85° C. for 1 hour. These samples were conditioned in constant temperature 21.11° C. (70° F.) and humidity (65 per cent) for at least 4 hours previous to making the breaking strength measurements by means of the motor-driven Scott tester (*1*).

The average loss in breaking strength of the inoculated strips, expressed as departure from the average of the controls, was calculated and recorded as the degree of deterioration.

The pH of the media was determined by the Beckman glass-electrode apparatus before autoclaving and again after harvest. Adjustment of the liquid media to a given pH with hydrochloric acid or sodium hydroxide before autoclaving appears, as judged from several experiments, to be valueless. After autoclaving, the pH of adjusted and non-adjusted media becomes approximately equal.

Experimental Results

It was thought advisable to determine experimentally the length of incubation period and the concentration of *Ch. globosum* inoculum required to yield the most consistent losses in breaking strength. In this experiment the percentage of loss in breaking strength at the 7-, 12-, and 14-day harvest periods was 73, 93, and 97, respectively. Control strips that received identical treatments, except for inoculation, were harvested at 0, 7, and 14 days. The breaking strength values for the corresponding harvests were, 57.6, 53.5, and 52.2 kg. (127, 118, and 115 pounds), respectively. The average for the controls of all other experiments was 59 kg. (130 pounds) after 7 days' incubation. This value has been used in all subsequent calculations.

The inoculum, prepared as described under Materials and Methods, was centrifuged at 2500 r. p. m. for 20 minutes, and 4 ml. of the resultant dense deposit were then suspended in 50 ml. of sterile distilled water. This dilution was designated as concentration K_1. This concentration was further diluted to 1 to 10 and 1 to 50 and these suspensions were designated as K_2 and K_3, respectively. No significant difference in loss of breaking strength was found between inoculum concentrations K_1 and K_2 for the 7-day incubation period. (As used in this paper "significant" refers to statistical odds of 19 to 1 and "highly significant" to odds of 99 to 1. Since the mean values for the results obtained are largely presented in graphical form, the differences required for significance accompany each graph. Significance of main effects may be judged by differences in height of the bar graphs or points on the curve that indicate the means that are to be compared. Significance of interactions may be judged by the degree of failure of the curves to extend in a parallel direction.)

Although the growth resulting from concentration K_3 developed somewhat less rapidly and gave a significantly lower breaking strength for the 7-day harvest, this difference was not detectable for the 12- and 14-day harvest periods. Thus, except for extreme dilutions, it appears that with *Ch. globosum* the amount of inoculum is not of great importance when favorable nutrition and conditions of incubation are provided. These initial experiments also indicated that under favorable conditions active cellulose decomposers, such as *Ch. globosum*, could not be safely incubated much beyond 7 days if reliable breaking strength tests were to be obtained with the Scott tester. In this period of time approximately three-fourths of the original strength is destroyed. Consequently, in succeeding experiments, harvests were made at the end of 7 days, unless otherwise specified.

A subsequent experiment was designed to evaluate the deterioration of cotton duck when no mineral salts were added. Inoculated strips and controls were incubated with distilled water and on water agar, using *Ch. globosum* as the test organism. The procedure, except for mineral nutrients, was standard. The data obtained are presented in Figure 2.

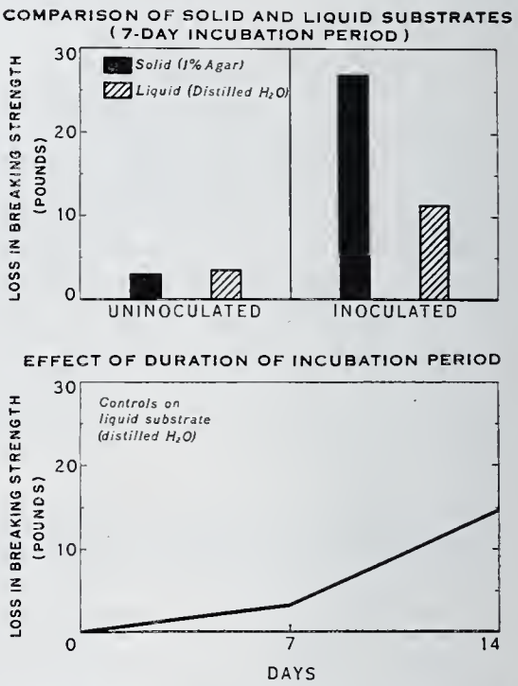

FIGURE 2. LOSS IN BREAKING STRENGTH WITH DISTILLED WATER AND WATER AGAR SUBSTRATES

Difference required for significance at odds of 99 to 1 and 19 to 1: between means for inoculated *vs.* uninoculated and solid *vs.* liquid, 8.80 and 6.56 pounds. Duration of incubation period, 8.60 and 6.38 pounds

When no salts were added, growth of the fungus was very slow on the strips of cotton duck and deterioration was slight. The strips on water agar yielded a highly significant loss in breaking strength when compared with those incubated with distilled water. This is interpreted as indicating that the agar itself is of some nutritive value. Since it was difficult to determine what was being furnished, and since it is general knowledge that agars from different sources vary somewhat in their composition, it seemed desirable to remove this variable. Furthermore, several tests with ether extracts of agar gave no indication of any growth substance that could be detected with the *Chaetomium*. The uninoculated controls gave no evidence that the presence of constituents in agar had any effect on the loss of breaking strength. It does seem important, however, to note that in 7 days about 1.8-kg. (4 pounds) loss in breaking strength was shown for the controls, and that about 6.8 kg. (15 pounds) was registered for the controls that were incubated 14 days. For this reason it seemed important to record the results of the various experiments in terms of the control rather than as actual breaking strength.

Since many investigators of cellulose deterioration, and especially those using *Ch. globosum* as a check on the efficiency of rot-proofing treatments, have used Formula B and closed

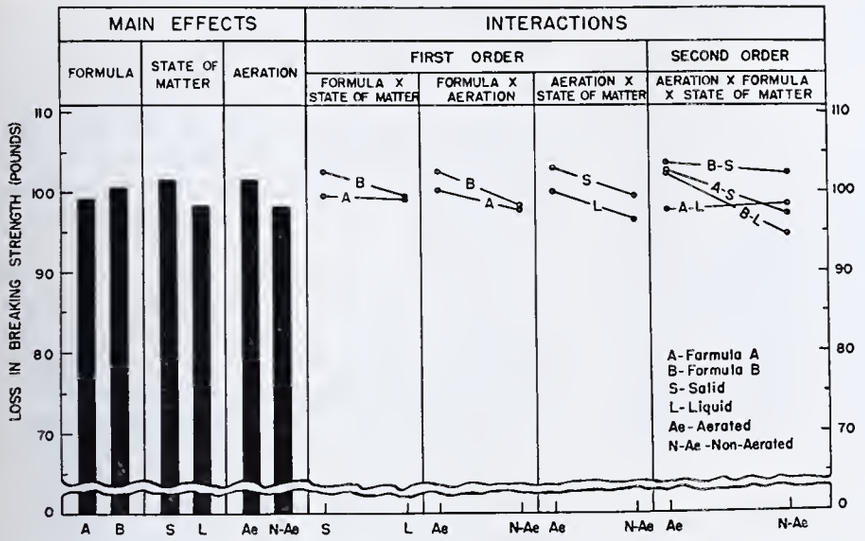

FIGURE 3. EFFECT OF AERATION AND OTHER FACTORS ON BREAKING STRENGTH

Differences required for significance at odds of 99 to 1 and 19 to 1: between means for formulas, state of matter, and aeration, 4.13 and 3.11 pounds

bottles, an experiment was designed to compare Formulas B and A. The effects resulting from the bottles being closed with metal caps and with aerated glass fabric tops were evaluated. By using a $2 \times 2 \times 2$ factorial, this experiment also permitted the further testing of solid *vs.* liquid media.

In Formula A ammonium nitrate was selected as the source of nitrogen, in view of the possibility that all organisms may not be capable of utilizing effectively only the nitrate or only the ammonium radical, and also because the presence of the ammonium and the nitrate radicals increases the buffer capacity of the solution.

A comparison of the data secured with Formulas A and B used in the liquid and solid state, with and without provision for uniform aeration for the culture bottles, is presented in Figure 3.

The nitrogen contents of Formulas A and B were 0.350 and 0.495 gram per liter, respectively. Since statistical analysis of the data from a subsequent experiment showed no significant differences for nitrogen levels ranging from 0.175 to 0.700 gram per liter, it seems logical that the difference in nitrogen equivalent may be largely ignored in evaluating the results for this experiment.

Ch. globosum yielded approximately equal losses in breaking strength, irrespective of the formula or the phase of substrate employed. These results were particularly encouraging since, as is brought out in the discussion, the liquid technique has many advantages over the agar method.

With "closed" bottles the degree of exchange of gases between the container and the outside depends on the tightness with which the cap is screwed. (For convenience, the term "closed" is used here to signify nonperforated lids and does not imply that the bottles were hermetically sealed.) It is almost impossible to repeat this operation with the same degree of tightness for the replicates or different experiments. The glass-fabric top for the culture bottles was employed to aid in removing this variable. Experimentally, a uniform aeration of growth chambers for aerobic organisms is desired. Thus the use of "aerated" and "nonaerated" bottles in these investigations implies uniformly and irregularly ventilated culture chambers, respectively.

A significantly greater loss in breaking strength was obtained from the aerated than from the nonaerated cultures. Although this difference was not great, the trends were so consistent that its failure to attain high significance suggested an examination of the sampling error obtained in the analysis of variance. The results were striking and furnish additional evidence of the need for providing aeration. The graphic presentation of mean-square sampling error or residual variance among replicates for various stratifications of data is given in Figure 4.

The sampling-error variance for Formula A is only about half that for Formula B. The solid state had less within variance than did the liquid state. When sampling error is stratified according to aeration, the mean square for error within aerated bottles is 9.2979 and for closed is 88.1583. One practical implication of the difference is that, to give the same precision in a comparison between means, approximately 9.5 times as many replicates would be required with a closed as with an open system.

Many modifications of Formula A were tested with *Ch. globosum*, but as no promising leads were obtained the data are not presented. However, no differences were found on halving or doubling the amount of nitrogen, using Washington, D. C., tap or distilled water, or in the presence of iron, manganese, zinc, etc. Despite these results the use of distilled water and the addition of the trace elements have been retained in Formula A, since these elements appeared to do no harm for the organisms tested and are known to be required for certain fungi.

Since fungi differ in their nutrient, respiratory, and moisture requirements, an experiment was performed to determine favorable conditions for six fungi that previously had been found to be active cellulose decomposers: *Ch. globosum* Kunze, *Chaetomium elatum* Kunze and Schmidt, *Stachybotrys papyrogena* Sacc., *Metarrhizium* sp., *Alternaria* sp., and *Hormodendrum* sp. (After this manuscript was prepared the attention of the writers was called to the fact that, according to the usage establish by Charles Thom, this fungus should be designated as *Cladosporium* sp.) The numbers under which these specific cultures are maintained in this laboratory are 1042.4, 1043.4, 1331.1, 1334.1, 1022.2, and 1236.1, in the order listed. Herbarium specimens have been deposited with the Division of Mycology and Disease Survey,

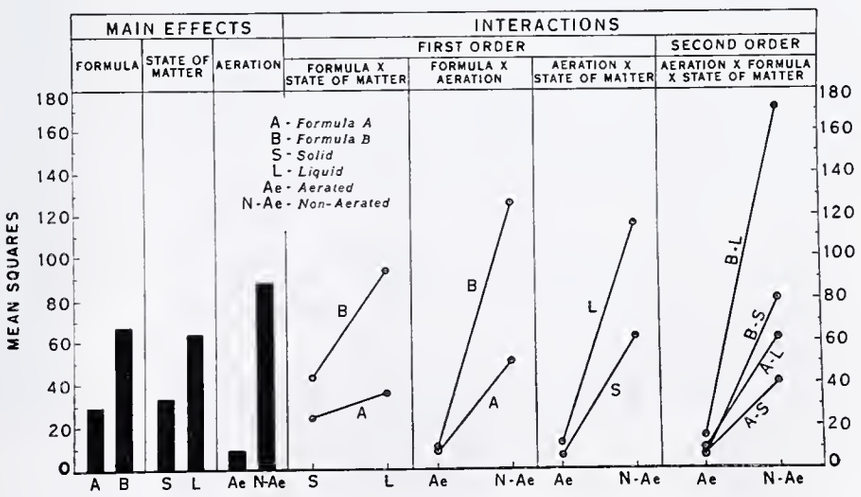

FIGURE 4. COMPARISON OF ERROR VARIANCE

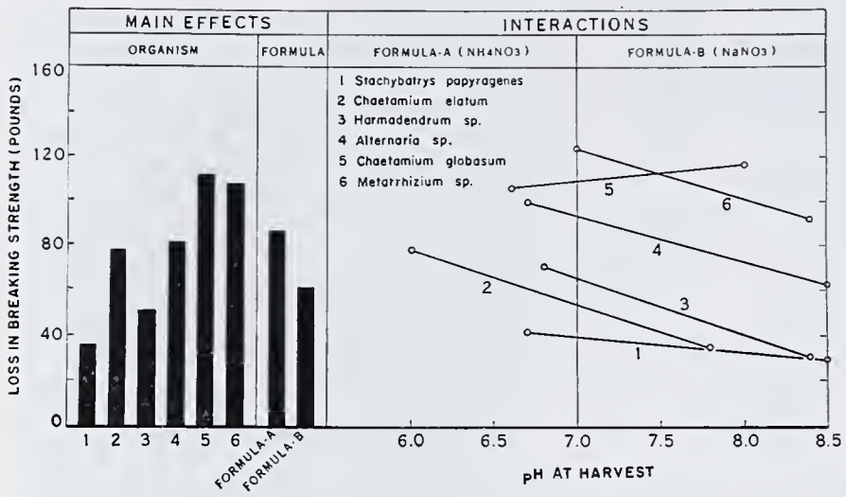

FIGURE 5. EFFECTS ON BREAKING STRENGTH OF SIX ORGANISMS WITH TWO FORMULAS IN LIQUID MEDIA

Differences required for significance at odds of 99 to 1 and 19 to 1: between means for organisms, 17.09 and 12.93 pounds; formulas, 9.78 and 7.47 pounds

Since the results presented in Figure 5 showed a highly significant interaction for organisms with nitrogen sources, it seemed desirable to determine the relation between sources and levels of nitrogen on the two *Chaetomium* species. The influence of three sources and three levels of nitrogen on the deterioration activity of *Ch. globosum* and *Ch. elatum* is presented in Figure 8.

The basic solution was Formula A and equivalent quantities of nitrogen were used for each source. *Ch. globosum* produced a loss in fabric breaking strength of 53.5 kg. (118 pounds) with sodium nitrate, 49 kg. (108 pounds) with ammonium nitrate, and 42.5 kg. (96 pounds) with ammonium dihydrogen phosphate as sources of nitrogen. On the other hand, the growth of *Ch. elatum* under similar conditions resulted in losses of 30.4, 40.4, and 39.9 kg. (67, 89, and 88 pounds), respectively. Levels of nitrogen were of little importance. From the graph it appears that pH, including the effect that levels and sources of nitrogen have on pH, is a very important factor in determining the response of cellulose-decomposing fungi.

U. S. Department of Agriculture, Washington, D. C.

A comparison of the influence on breaking strength of these six fungi when grown on Formulas A and B, modified to 0.350 gram per liter of nitrogen, is presented in Figure 5. In addition to this factorial design, five of the organisms were further tested as to their behavior on liquid and solid media (Figure 6).

The interactions between organisms and formulas show that all organisms except *Ch. globosum* caused a greater loss in breaking strength on Formula A than on Formula B. These differences are highly significant. The loss in breaking strength is associated with change in pH. The pH values at harvest for the fungi grown on Formula B ranged from 7.7 to 8.5. The nitrogen source of the formulas seems to influence the pH of the medium. All fungi studied, except *Ch. globosum*, yielded a greater loss in breaking strength with a pH of 7.0 or less.

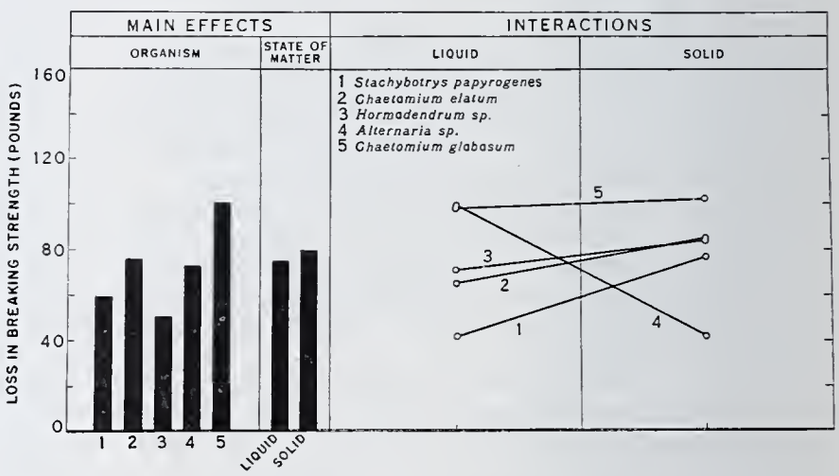

FIGURE 6. EFFECTS ON BREAKING STRENGTH OF FIVE ORGANISMS WITH LIQUID AND SOLID MEDIA

Source of nitrogen, ammonium nitrate (Formula A)
Differences required for significance at odds of 99 to 1 and 19 to 1: between means for organisms, 15.39 and 11.62 pounds; state of matter, 9.74 and 7.35 pounds

Each of these organisms, however, is influenced by other factors, as indicated in the interaction between liquid and solid states. *Alternaria* sp. gave a greater loss in breaking strength on liquid medium than on solid. In contrast, the other organisms, particularly *S. papyrogena*, produced more loss on the solid medium. Subsequent experiments, using double and single wicks with identical amounts of liquid substrate, indicated that this was not a response to the solid agar but was due to a difference in available moisture. Certain of these organisms differ markedly in the rapidity with which they bring about cellulosic deterioration. In Figures 5 and 6 the time of harvest was 7 days for all organisms except *Alternaria* sp. and *Hormodendrum* sp., which were harvested at 14 days. Both these organisms are good cellulose decomposers but develop very slowly. *Metarrhizium* sp., however, develops so rapidly that harvest should be made prior to 7 days.

A photographic comparison of typical response of *Metarrhizium* sp. on the two formulas, after 7 days' incubation, is shown in Figure 7. Under a favorable controlled environment, *Metarrhizium* sp. caused 94.9 and *Ch. globosum* 81.5 per cent loss in breaking strength at the end of 7 days' incubation.

Previous to autoclaving the solutions containing ammonium dihydrogen phosphate, ammonium nitrate, and sodium nitrate the pH values were approximately 5.5, 6.6, and 6.8, respectively. After autoclaving, they were approximately 6.0, 6.7 and 6.9. As the fungus utilized the phosphate salt the pH decreased to 4.7 for *Ch. globosum* and to 5.3 for *Ch. elatum*, whereas with utilization of the sodium salt the pH increased to 8.1 and 7.2, respectively. The ammonium nitrate source appeared to have good buffering capacity, the pH at harvest varying between 5.6 and 6.8 for both organisms grown at the three nitrogen levels.

Discussion

As an approach to a standardized technique applicable to testing mildew resistance of cellulosic materials and comparing the ability of microorganisms to decompose cellulose, the data from these studies indicate clearly that the glass wicks for the support of the test sample in a liquid medium

and the glass fabric for permitting exchange of gases in culture chamber tops have important advantages over the solid-agar, closed-system technique.

1. Less time and work are involved in securing equivalent data. With the solid-medium procedure the mineral salts-agar and the strips of fabric are sterilized separately and the sterile strips are then placed in the incubation bottles on the agar under aseptic conditions. With the glass wick–liquid medium system only one sterilization is required. There is also much less risk of contaminating the sterile strips since they are not transferred from one container to another after they have been sterilized. In addition, the glass wick–liquid medium system could be readily modified to a continuous or periodic medium renewal technique.

2. Experimental results have demonstrated that the aerated system yields reliable results with fewer replicates, and consequently saves test material, time, and work over the nonaerated agar system. The mean-square sampling error between replicates for the nonaerated system with *Ch. globosum* was found to be 88.2 as compared to 9.3 for the aerated system.

3. Agar itself was found to supply sufficient mineral nutrients for slow growth of fungi. Since it is difficult to determine what is being furnished and agars from different sources vary in their usable constituents, it would seem advisable to eliminate this variable.

4. It has been found essential to change the sodium nitrate to some other nitrogen source, because the utilization of the nitrate radical by most fungi resulted in a medium too alkaline for their development. Ammonium nitrate, as nitrogen source, has proved superior to sodium nitrate probably because of its buffering capacity and by supplying both the ammonium and the nitrate radicals for the fungi.

FIGURE 7. RESPONSE OF *Metarrhizium* SP. TO FORMULAS A AND B

Incubated for 7 days. Left, uninoculated control. Center, inoculated sample with Formula A. Right, with Formula B

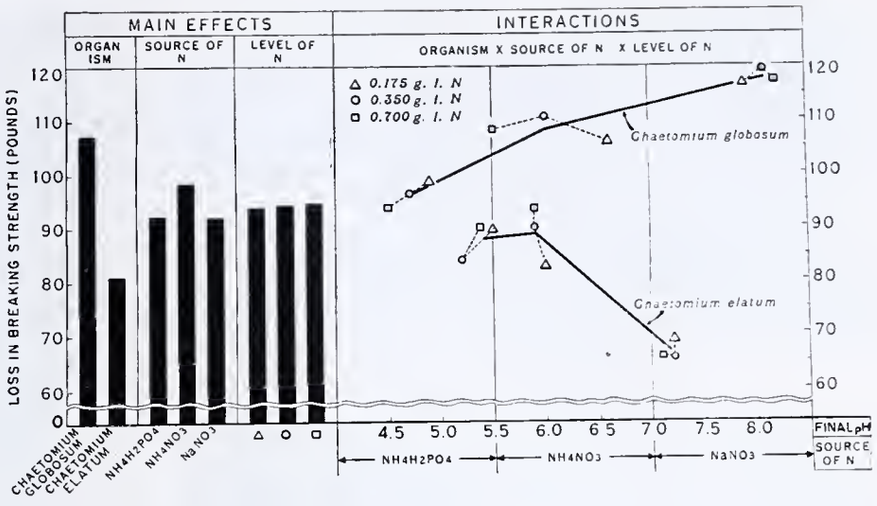

FIGURE 8. EFFECTS OF *Chaetomium globosum* AND *Chaetomium elatum* ON BREAKING STRENGTH

Differences required for significance at odds of 99 to 1 and 19 to 1: between means for organisms, 2.42 and 1.91 pounds; sources of nitrogen and levels of nitrogen, 3.09 and 2.34 pounds

Mixtures of microorganisms have been largely abandoned because of the difficulties involved in getting comparable results with successive samples (*12*). Pure cultures of the individual fungi were used in these investigations. Results with a single organism may not yield the same results as those produced under natural conditions where many organisms are involved, but the same condemnation applies to any controlled method. Thus the choice lies between a carefully standardized test and the actual judgment of exposure to natural conditions, which is costly in time and rarely produces results that are capable of being duplicated.

The selection of the test organism will be influenced by many factors, depending on the problem to be solved and the materials in question. *Ch. globosum* has been recommended by Thom *et al.* (*12*) after an extensive survey of possible test organisms, presumably on account of its ability to decompose cellulose, its frequent occurrence on cotton, and its ease in handling in culture. These studies have shown that *Metarrhizium* sp. is superior to *Ch. globosum* in producing rapid losses in breaking strength, especially on neutral to acid media. This, along with its equal ease of handling, indicates that *Metarrhizium* sp. might be a useful organism for test purposes. It is a common soil-inhabiting organism, and thus it may be an active agent in deteriorating sandbags or the fabrics exposed to the burial test.

In evaluating cellulose deterioration by the activity of fungi, it is desirable to use standardized media in order to facilitate duplication of the work. The media should be buffered so that the original pH is not changed materially by the utilization of any radical or ion. From the results here reported for six fungi it appears that ammonium nitrate fills more of these requirements than does sodium nitrate or ammonium dihydrogen phosphate if but one nitrogen source is to be used.

Summary and Conclusions

A standardized quantitative method for the evaluation of the ability of fungi to decompose cellulose is described. Certain features of this procedure may also prove useful in developing a standardized method for testing rot-proof treatments for fabrics. This technique, employing glass fabric and liquid nutrient instead of solid mineral agar, possesses advantages with respect to accuracy and speed over previously reported methods. Provision for uniform aeration

of culture chambers is highly important, especially in reducing sampling error variability.

Metarrhizium sp. and *Chaetomium globosum* cause very rapid decomposition, are easily handled, and have other features that make them satisfactory test organisms. They are definitely superior to *Chaetomium elatum*, *Alternaria* sp. *Hormodendrum* sp., and *Stachybotrys papyrogena*.

Ammonium nitrate was found to be a better source of nitrogen than sodium nitrate for the development of most of the fungi. The pH of the substrate appears to have an important influence on the activity of cellulose-destroying fungi.

Acknowledgment

Grateful acknowledgment is made to Ruth E. Rogers for suggestions in connection with the textile phase of the investigation, to L. M. Ames for identifying the species of *Chaetomium*, to Vera K. Charles for determining the identity of *Stachybotrys papyrogena*, to Charles Thom for the tentative identification of *Metarrhizium* sp., and to Katharina Bollenbacher and Helen G. Wheeler for valuable laboratory assistance with the transfer of cultures and breaking strength measurements.

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Rapid Volumetric Method for Determination of Sulfate Ion

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BALAKHOVSKI and Ginsburg (*1*) suggested in 1931, but did not investigate, a volumetric method for the determination of sulfate ion, which consisted of adding a known amount of barium chloride to the solution containing sulfate and titrating the excess with sodium pyrophosphate. The following reactions occur:

$$Ba^{++} + SO_4^{--} = BaSO_4(s) \quad (1)$$

$$2Ba^{++} + P_2O_7^{----} = Ba_2P_2O_7(s) \quad (2)$$

An excess of pyrophosphate ion hydrolyzes and the end point is determined by using some such indicator as phenolphthalein. The barium sulfate is found by difference.

Barium pyrophosphate is soluble to the extent of only 0.01 part in 100 parts of water at 20° C. (Hodgman and Lange, *2*), and by using a water and alcohol medium, it was believed this solubility could be further decreased. The sodium salt of the pyrophosphate ion was chosen, since barium pyrophosphate is soluble in ammonium salts.

Since the reaction of barium toward the sulfate ion is known and understood, the problem concerned the reaction of barium ion with pyrophosphate ion. Actually, the method was found to be experimentally unsatisfactory because (1) the alkalinity of the pyrophosphate ion depends upon its hydrolysis in water, and this hydrolysis is too slow to give a satisfactory and reproducible end point. (2) At low alcohol content, the solubility of barium pyrophosphate is great enough to allow some hydrolysis of the pyrophosphate, thus giving a premature end point. The amount of sodium pyrophosphate needed to react with a given amount of barium at various proportions of alcohol is shown in Figure 1. The results are erratic.

As the alcohol content increases, the amount of pyrophosphate ion required to titrate the barium increases rapidly. Since no constant value of barium could be ascertained, the method was abandoned.

Disodium Hydrogen Phosphate Method

Although the sodium pyrophosphate method was unsatisfactory, the authors felt that some other salt might be substituted, and disodium hydrogen phosphate was found satisfactory. The extreme alkalinity of monohydrogen phosphate, HPO_4^{--}, is well known. The presence of alcohol should have little or no effect upon its hydrolysis, and at the same time alcohol serves to cut down the solubility of barium hydrogen phosphate, $BaHPO_4$. The reaction is:

$$Ba^{++} + HPO_4^{--} = BaHPO_4(s) \quad (3)$$

Ammonium compounds must not be present, because of the solubility of barium hydrogen phosphate in the presence of ammonium ions.

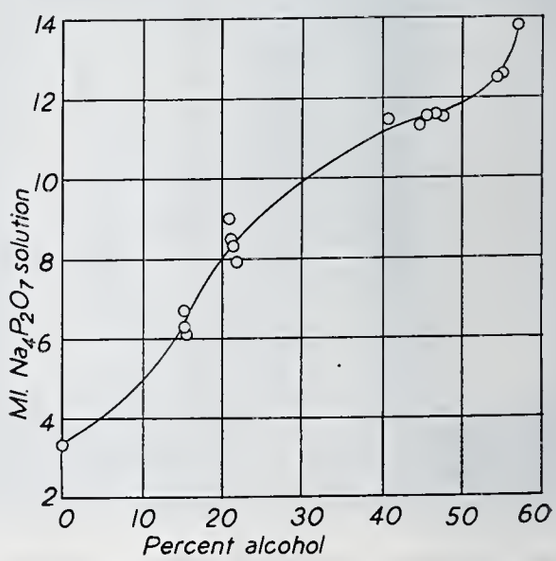

FIGURE 1. STANDARD SODIUM PYROPHOSPHATE SOLUTION REQUIRED TO CHANGE POINT OF METHYL RED IN ALCOHOL-WATER MEDIA

A known amount of barium chloride was added with mechanical stirring, to the acidic solution containing the soluble sulfate. Alcohol was then added to the solution, and the whole was brought to neutrality against methyl red (0.1 per cent in 95 per cent alcohol). The solution with the barium sulfate in suspension was made very slightly acidic by the addition of one drop of hydrochloric acid of concentration equivalent to that of the disodium hydrogen phosphate solution. This latter solution was standardized by the same method against known barium chloride.

Table I. Effect of Alcohol Concentration on Barium Hydrogen Phosphate End Point

Trials	C_2H_5OH	Na_2HPO_4	Alcohol by Volume
	Ml.	*Ml.*	%
1	0.0	0.25	0.0
2	0.5	12.80	2.1
3	1.0	13.90	4.0
4	2.0	14.12	7.7
5	3.0	14.35	11.0
6	4.0	14.58	14.0
7	5.0	14.75	16.8
8	6.0	15.35	19.1
9	7.0	15.51	21.1
10	8.0	15.75	23.7
11	9.0	16.05	25.7
12	10.0	16.30	27.5
13	10.0	16.30	27.5
14	10.0	16.31	27.5
15	10.0	16.30	27.5
16	15.0	16.27	36.3
17	15.0	16.30	37.5
18	15.0	16.33	45.0
19	20.0	16.34	52.1
20	30.0	16.35	62.0

The titration may be done rapidly. It will appear to have gone to the end point when the yellow color is first observed. However, upon standing momentarily, the yellow color fades, probably owing to desorption of barium ion. The true end point will be given when the pink no longer reappears.

Effect of Alcohol Concentration. Constant results for barium content were obtained when the alcohol content was 27.5 per cent or more of the final volume. Ordinarily 20 ml. of unknown was taken for the titration. A white porcelain evaporating dish was found to be ideal for watching the color change of the end point. The effect of the alcohol concentration is given in Table I.

An average value of 16.32 ml. of disodium hydrogen phosphate was taken as having titrated 0.08650 gram of barium chloride. Thus, using methyl red as an indicator, 1 gram of disodium phosphate equals 1.4239 grams of barium chloride.

Table II. Effect of Added Sodium and Potassium Chlorides on Barium Hydrogen Phosphate Method

Salt	Added	$BaCl_2$	C_2H_5OH	Na_2HPO_4	Alcohol by Volume
	Gram	*Gram*	*Ml.*	*Ml.*	%
NaCl	0.05516	0.08650	10	16.25	34.2
	0.11032	0.08650	10	16.30	33.0
	0.16547	0.08650	10	16.30	31.9
	0.22063	0.08650	10	16.32	30.9
KCl	0.06088	0.08650	10	16.28	35.4
	0.12176	0.08650	10	16.31	35.4
	0.24353	0.08650	15	16.31	30.1

Effect of Salts on Barium-Ion Determinations. The effect of the following ions was tested: Fe^{+++}, Fe^{++}, Mn^{++}, Cu^{++}, Cd^{++}, K^+, and Na^+. The heavy metals whose ions form insoluble hydrogen phosphate salts—all the above ions with the exception of sodium and potassium—were found to interfere. This is not of serious consequence, however, since the interfering metal ion is easily removed.

The solutions containing added sodium and potassium chloride were calculated to have required 16.31 ml. of disodium hydrogen phosphate for the amount of barium chloride added. See Table II.

Sulfate Determinations

An excess of approximately 0.05 *M* known barium chloride solution is added with mechanical stirring to an acidified solution of the unknown sulfate at approximately 0.05 *M*. The solution, without filtering, is made neutral to methyl red and one drop of standardized 0.1 *M* hydrochloric acid is added. Sufficient alcohol is now added to ensure a final solution containing more than 27.5 per cent by volume. The excess of barium chloride is titrated with approximately 0.05 *M* disodium hydrogen phosphate to the permanent yellow end point of the methyl red, the disodium hydrogen phosphate is standardized against the barium chloride solution without addition of any sulfate, and the sulfate is then computed by difference.

Experimental Results. Potassium sulfate was used. For standardizing the phosphate solution, 0.00530 gram of barium chloride required 1 ml. of disodium hydrogen phosphate solution. The original addition of barium chloride was 0.14014 gram, being an excess in each case. The results are given in Table III.

Sulfur in Petroleum. The method was checked in one of the Tide Water Associated Oil Company's control laboratories and agreed closely with the gravimetric barium sulfate method. Approximately 0.8-gram samples of petroleum oil containing about 2 per cent of sulfur were weighed, and fired in an oxygen bomb, the resulting acids were washed from the bomb and oxidized completely to sulfate ion, and the method was used as outlined above. The entire procedure required but 35 minutes and agreed with the standard gravimetric method within 1 to 1.5 per cent, being lower in each case.

Table III. Barium Hydrogen Phosphate Method for Sulfate

SO_4 Calculated	Na_2HPO_4	C_2H_5OH by Volume	SO_4 Found	Error
Gram	*Ml.*	%	*Gram*	%
0.03874	10.55	38.10	0.03884	0.28
0.04334	8.65	35.21	0.04347	0.30
0.04334	8.77	35.20	0.04318	−0.38
0.02504	16.50	29.65	0.02476	−1.11
0.04643	7.51	36.50	0.04627	−0.34
0.04643	7.53	36.48	0.04621	−0.47
0.05030	5.95	35.19	0.05007	−0.47
0.05030	5.92	43.70	0.05016	−0.29
0.06060	1.78	46.30	0.06028	−0.53

The last five trials were mixtures of sulfuric and nitric acids in varying proportions. The last three had approximately 0.1 gram of added sodium chloride.

Time. For soluble sulfates, each trial requires about 10 minutes for completion. At no time is filtering necessary. The end point may be approached most rapidly and with the greatest safety when titration is done in a white porcelain casserole. To save time and material, and when the final volume is suspected of being 100 ml. or more, an aliquot should be used.

Summary

The method suggested by Balakhovski and Ginsburg was proved unsatisfactory. Using disodium hydrogen phosphate as the titrating agent, a satisfactory and rapid method for sulfate and barium ions was found. Barium chloride is added in excess to a sulfate-containing solution, and the excess is titrated to the methyl red end point with standard disodium hydrogen phosphate in an alcohol and water medium.

With ordinary precision, and in the hands of a competent analyst, the method may be expected to give results within 0.5 per cent and require 10 to 15 minutes. The method is applicable in control work, especially for rapidly determining the sulfur content of oils, and for rapid barium determinations.

Acknowledgment

The authors wish to express their gratitude to E. T. Lange of the Tide Water Associated Oil Company for his courteous assistance and cooperation in making it possible to test the applicability of this method in the petroleum industry.

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Particle Size Studies

METHODS

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The hydrometer, pipet, and Wagner turbidimeter methods for determining particle size distribution in the subsieve size ranges were studied in detail. The results obtained on a variety of ground materials indicated that the hydrometer and pipet methods give concordant data which are also in agreement with those obtained by air elutriation. On the basis of these results a special pipet for rapid analysis was designed. Using a special technique for size distributions down to 1.25 microns, it was shown that the Wagner turbidimeter gives good results only for ground sand, silica, and certain cements. The data obtained with the Wagner method for other materials were unsatisfactory on a basis of both specific surface and size distribution. The poor results by the turbidimeter on certain materials were shown to be caused by the lack of validity of the empirical conversion of turbidity data to weight concentration; this is of such magnitude that it limits the use of the apparatus to ground silica and certain cements.

A LARGE number of methods have been proposed for evaluating the particle size distribution and the specific surface of pulverulent materials (*35*). Many of these methods have a limited use in industry because they require expensive equipment or complicated techniques, or because the principles upon which their operation is based have not been fully developed. The latter is especially true of methods that have been proposed for a particular purpose or those that measure relative size only. However, a number of methods have widespread industrial use because of their simplicity and speed. The purpose of this investigation was to make a critical study of certain methods in order to determine what theoretical and practical considerations limit their use.

The methods that have the greatest industrial use employ sedimentation or elutriation principles, and, accordingly, base particle size results on the equivalent settling diameter. The principal attributes of such methods are simplicity of operation and relatively good precision, provided certain factors are given consideration.

Temperature Control. All analyses should be run under as nearly isothermal conditions as possible, especially in methods which require 12 to 24 hours to complete the analysis. The important effect of slight variations in room temperature is not the viscosity of the suspending medium but rather on the change of density which produces convection effects.

Dispersion. The necessity for good dispersion cannot be overemphasized, since in order to measure the size of particles they must be separated. This dispersion is best effected by mechanical means and no simple hand stirring can be considered suitable unless the material is larger than 20 to 30 microns. Shaking devices are applicable where long periods of agitation are desired. High-speed stirrers having a rotor enclosed by a stator to give high shearing action without swirling are efficacious (*34*). In most cases agents must be added to aid the dispersion; a number of these have been described (*15, 18, 20, 36*). Consideration must be given to whether the agent merely lowers the surface tension—i. e., a wetting agent—or whether it actually separates the particles and prevents flocculation—i. e., a dispersing agent. For water dispersions a 0.08 per cent sodium metasilicate (Metso granular) has been found satisfactory for many materials. For materials not easily wetted, such as coal, the same solution can be used if the material is first wetted with a small amount of ethanol. For materials that react with or dissolve in water, nonaqueous media (*23*) may be used. The difficulty with such media is to obtain good dispersions. In the case of elutriation the dispersion is obtained by mechanical means, which must avoid appreciable attrition.

Concentration. Consideration must be given to the concentration of the suspension in order to eliminate hindered settling and flocculation as much as possible. In general, sedimentation methods employ concentrations of less than 5 per cent, and hindered settling is not likely to occur (*17*).

Brownian Movement. While Brownian movement is undoubtedly a factor in size determinations by gravity sedimentation of very fine materials, it probably is not important where most of the material is above 1 micron in size.

Physical Properties. The precision of sedimentation measurements is partly dependent upon the accurate determination of the physical properties used in Stokes' law. For this reason the densities of both liquid and solid and the viscosity of the suspending medium should be determined by sound methods (*2, 5*).

Test Procedure. The sedimentation time should be measured accurately, especially for the coarse sizes, since the precision is lowest in that size range. The sedimentation should be carried out in a constant-temperature bath free from appreciable agitation and the sedimentation vessels should be placed in a vertical position to prevent convection currents produced by nonuniform density changes.

Size Limits. The upper limit of sedimentation methods is controlled by the applicability of Stokes' law up to Reynolds numbers equal to 1, and also by the practicability of timing the coarse sizes that the particular method allows. The upper limit

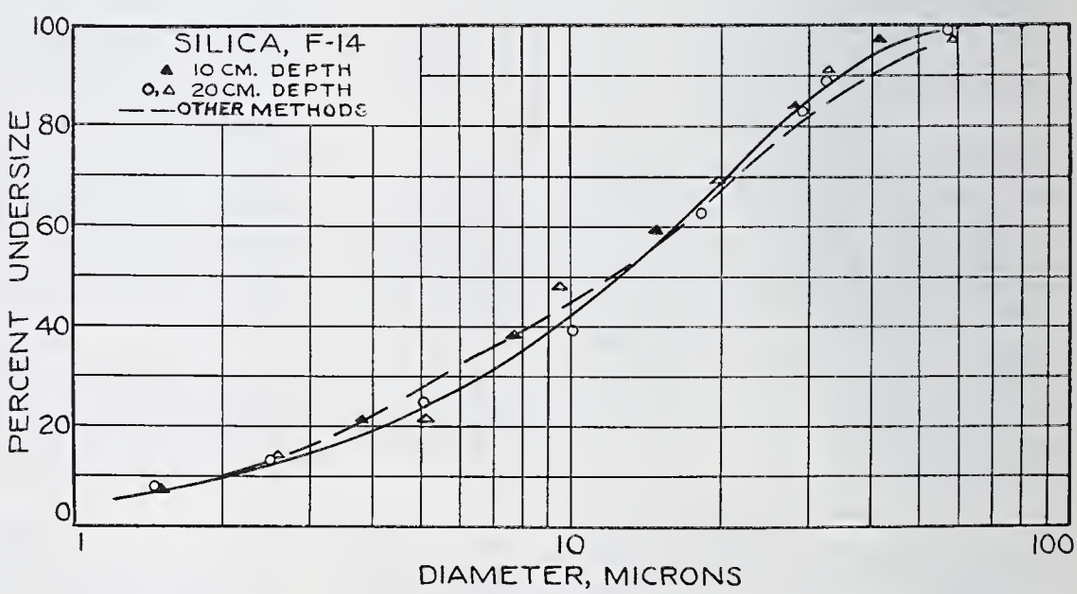

Figure 1. Pipet Analyses

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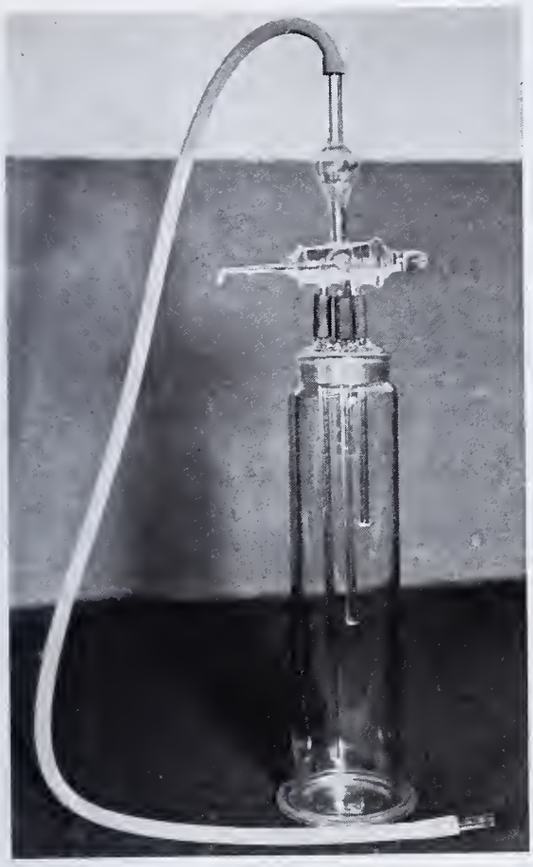

FIGURE 2. SPECIAL PIPET

is, in general, about 75 to 90 microns. The lower limit is controlled by the speed with which the results are desired, but is in general about 0.5 to 1 micron.

CALCULATIONS. The calculations are similar in all methods and are described in detail in another publication (*34*), which also gives the complete procedure employed in the present study.

Study of Individual Methods

All methods studied in this investigation are of the increment type (*35*)—i. e., the size distribution is determined directly in increments rather than indirectly by graphical differentiation of a sedimentation curve as is necessary for cumulative methods (*35*).

PIPET METHOD. The pipet method employing the apparatus designed by Andreasen (*6*) when used under the proper conditions has been found satisfactory for determining particle size distribution. The weight of particles of a given size remaining in suspension is determined directly, the height of fall is determined by direct measurement, the proper technique can be learned quickly, and the apparatus is relatively inexpensive. On the basis of comparative studies, the pipet method is recommended as the best for the determination of particle size distribution based on the sedimentation diameter if complete dispersion of the particles can be obtained.

The results of a number of analyses on ground Ottawa sand, F-14, using the technique described elsewhere (*34*) are shown in Figure 1 along with data obtained by other methods used in the present study. These data and studies of a large number of other materials, show that an average deviation of less than 2 per cent by weight at any given size can be attained by the pipet method.

The principal disadvantage of the Andreasen pipet method is the time required to run the analysis to very small sizes, since in many cases the elapsed time to reach 1.25 microns is 24 hours. As shown in Figure 1, using a cylinder half full, the time can be shortened by reducing the height of fall without greatly affecting the precision. Stokes' law states that the time required to separate a given particle diameter is directly proportional to the height of fall and the viscosity of the medium and inversely proportional to the difference in density of the suspending medium and of the suspended solid. It is, therefore, possible to reduce somewhat the time of test by using liquid media other than water, if good dispersion can be obtained. In an effort to reduce the time of test in the pipet method, a special pipet was designed (Figure 2). Its dimensions and the procedure for use are given elsewhere (*34*). Such a pipet allows an analysis to be made in one fourth the time required for the Andreasen pipet by utilizing shorter distances of fall for the small sizes; the precision in the coarse size range is not affected since a long distance of fall is used for the large sizes. Results shown in Figure 3 indicate that it yields particle size data in good agreement with those from other methods.

Since the pipet method is based on no assumptions other than the validity of Stokes' law, it offers a precise method of particle size analysis to which other methods may be compared and as such serves a calibration method. In the development of other rapid methods the results may be compared with those from the pipet method to prove their validity for the particular problem involved. The pipet method has been used by Hogentogler and Wills (*21*) for soils, by Loomis (*24*) for settling periods of 96 hours on clays, by Andreasen (*7*) in studies on the grinding of iron oxide and barytes, and by Lea and Nurse (*23*) in studies on cement.

The manipulations required in the pipet method are numerous and limit to a certain extent the number of points on the curve that can be determined where a large number of tests are being run by a single person. The use of a cumulative per cent undersize versus log diameter plot of the data where the points are judiciously selected allows interpolation for the intermediate points.

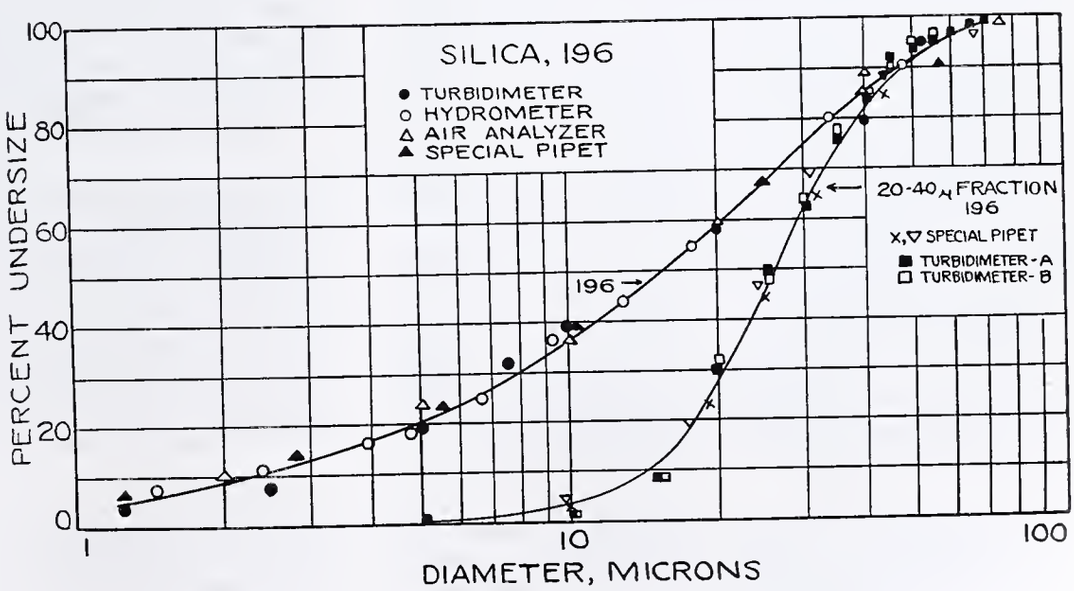

FIGURE 3. SIZE DISTRIBUTION OF SILICAS

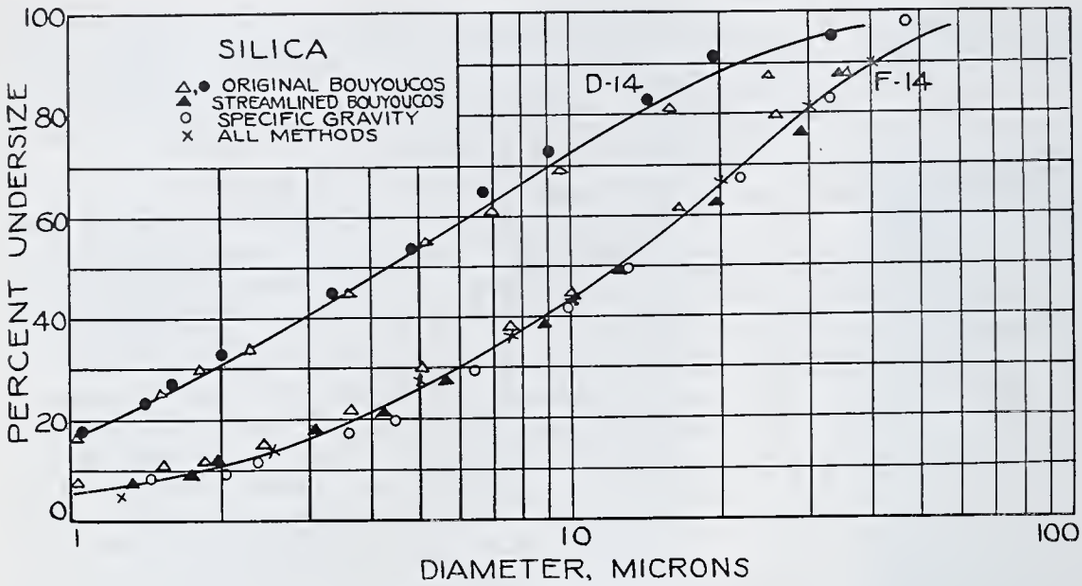

FIGURE 4. SIZE DISTRIBUTION BY HYDROMETER

HYDROMETER METHOD. In the hydrometer method for size analysis, first described by Bouyoucos (*12*), the concentration at some level, at a distance h from the surface, is measured directly, thus eliminating the necessity of taking samples as in the pipet method. h varies with the concentration and has been found to be a function of the type of hydrometer used.

The best discussion of this method was given by Thoreen (*38*), whose work formed the basis for the A. S. T. M. method of test (*3*). Other investigators have discussed this method and described hydrometers, but the streamlined Bouyoucos hydrometer type now manufactured gives results in good agreement with other methods as is shown in Figure 4 for silica, using the author's technique (*34*). For this hydrometer the height of fall, h, is computed as 0.42 times the distance from the surface of the liquid to the bottom of the hydrometer. This factor, which was determined empirically by Thoreen for the old type of Bouyoucos hydrometer, may vary with different hydrometers and for best results should be determined by calibration. The alternative specific gravity type of hydrometer might be used, although there appears to be no necessity for having two types for the same purpose. For this type the height of fall is equal to the distance from the surface to the center of volume. The results obtained with this instrument are shown in Figure 4; because it may give low results, the streamlined Bouyoucos type is preferred.

The hydrometer method has been criticized on the basis of certain theoretical aspects, such as the fact noted by Puri (*28*) that there is a density gradient from the top to the bottom of the suspension. However, these objections have not proved to be critical, since the experimental data indicate that the hydrometer gives results as precise as the pipet method if the possible sources of error are eliminated. Bouyoucos (*13*, *14*) studied the density gradient aspect and showed that in spite of it the hydrometer gives the proper result. Certain investigators have proposed a correction factor for the variation in displacement resulting from the use of containers of different diameters. The experimental data illustrated in Figure 5 show that even a 50 per cent variation in diameter has a relatively small effect on the results and that it is not necessary to make any correction when using ordinary 1000-cc. graduates.

Among the sources of error, which can be eliminated by proper technique, are the lack of maintaining a constant temperature and the indiscriminate use of formulas for correcting the observed readings (which are based on the calibrating temperatures and materials) to per cent remaining in suspension at the temperature of test. Since the hydrometer method for determining concentration depends on the difference in density of the medium and suspension, and since these differences are generally small in numerical value, it is imperative that such densities should be known accurately. The best means of making these corrections is by calibration in a manner which makes one hydrometer universally applicable for any solid-liquid system used (*34*). In this respect a streamlined Bouyoucos hydrometer would be more satisfactory if calibrated in true density than if calibrated for one particular solid-water system as is done at present.

Since the determination of concentration depends on density difference and a small difference may not be recorded on the hydrometer, Bouyoucos (*11*) has suggested using a special hydrometer for the small size ranges when most of the suspended material has settled out (concentrations of less than 10 grams per liter). The use of this hydrometer does not complicate the method unduly if high precision is desired.

The precision with the hydrometer method is equal to that of the pipet, with deviations of less than ±2 per cent. The method has been used extensively for soils on which Thoreen (*38*) has shown that for up to 40 per cent of less than 1 micron the results agree with a pipet method. The precision is best for very fine materials since the larger density differences are evaluated more accurately by the hydrometer. The precision in the coarse ranges depends upon how accurately the particles can be timed. The range of sizes covered is about the same as with the Andreasen pipet, but since the distance of fall decreases with decrease in concentration in the hydrometer and is never as great as in the Andreasen pipet method, the former requires about one half the time for measurements down to 1 micron.

The chief advantages of the hydrometer method over the pipet method are in the more simple operations required since the concentrations are read directly (*16*). In addition, one hydrometer can be used to make a number of analyses simultaneously if they are staggered to allow for the dispersing and 30-minute initial starting period.

APPLICATIONS OF HYDROMETER METHOD. Reimers (*29*) has used the hydrometer method for clays. Bauer (*9*) has used it for evaluating the relative value of deflocculating agents. Biddle and Klein (*10*) have compared the results on cements with those obtained by the Klein turbidimeter. They doubt the use of the hydrometer for cements having surfaces greater than 2000 sq. cm. per gram, because the results did not agree with the turbidimeter. A similar conclusion was reached by Gran (*19*) for results based on the hydrometer and Wagner turbidimeter. In reaching these conclusions all these authors apparently overlooked the fact that the turbidimeters may not be giving the correct result, which is shown below to be the cause of lack of agreement.

The data developed in this study show that the hydrometer has yielded results as precise as the pipet method and may be considered a sound method.

AIR ANALYZER. The air analyzer developed by Roller (*30*, *31*) is one of several types of elutriators that have been

discussed elsewhere (*35*). It has been used successfully by a number of investigators and, as is the case with all elutriators, the size analysis yields fractions of the whole powder that can be studied individually. A number of materials used in this investigation have been analyzed in this apparatus, and the results compared with those from other methods. The apparatus gives good results down to about 2 microns if the material is not subject to attrition effects, but the time per analysis is greater than for other methods and the instrument is considerably more expensive.

WAGNER TURBIDIMETER METHOD. In general, turbidimetric methods for determining particle size distribution have not proved satisfactory, although they have been widely used in various industries and are useful in evaluating relative size. The fact that the fundamental relations for converting turbidity data to weight distribution have not been rigorously established for these methods has resulted in conflicting statements in the literature regarding their accuracy compared to other methods. Turbidimetric methods offer advantages in the small sample required, simplicity of operation, and saving of time; for these reasons a rather comprehensive study was made of one particular method (*4*, *40*), and the results obtained were compared with those from other methods.

The Wagner turbidimeter method is essentially a sedimentation method of the increment (*35*) type in which the change in weight concentration at a given depth and time of sedimentation is computed from the change in turbidity as measured by a photoelectric cell. (The weight concentrations, however, are computed directly to surface in the Wagner equations.) As has been pointed out (*35*), the turbidity relations are very complex, and while the Wagner method was proved valid for one type of material—cements of certain specific surfaces and certain size distributions (*1*)—it appears to be rather limited in its application.

The surface of the particles (assumed as spheres) is computed from the following form of the Beer-Lambert law:

$$-\log T = E = kWl/d \qquad (1)$$

where T = fractional transmission
E = extinction
W = concentration in grams per liter of suspension
l = thickness of the suspension, cm.
d = diameter of the particles, microns
k = optical constant which includes the density of the particle

If the transmission of suspensions of particles exhibiting similar optical effects is obtained, it should be possible to convert the turbidity data to weight and thereby determine the size distribution. However, the general applicability of Equation 1 has not been proved. Wagner (*40*) has stated that the relation is invalid above 60 microns. Since the size at which a No. 325 sieve separates may be considered as 53 microns on a sedimentation basis, this serves as a convenient datum for the application of Equation 1. If Equation 1 is applied to polydisperse powders, W must be the weight smaller than 53 microns and d must be the mean diameter for that material.

In practice Equation 1 is used in the following form by Wagner:

$$E_f = \log(I_{d_2}/I_{d_1}) = S_f/C \qquad (2)$$

where E_f = extinction for the fraction between diameters d_1 and d_2
I_{d_1} = intensity of light just as the last particles of size d_1 are settling out of the light beam
I_{d_2} = intensity of light just as the last particles of the next smaller size, d_2, are settling out of the light beam
S_f = total surface of all particles d_2 and larger, but smaller than d_1
C = optical constant which is determined empirically based on the assumption that C is a constant for all sizes, regardless of the amount and size of other particles that are present

Equation 2 is a special form of Equation 1, since the surface of the particles is proportional to W/d, which permits the calculation of the weight distribution from the values of S_f for the individual fractions. The equation for this computation as derived from Equation 1 is:

$$W_f = E_f d_f / K'' \qquad (3)$$

where W_f = weight (grams per liter of suspension) between size limits d_1 and d_2
d_f = arithmetic mean size of the fraction, microns
K'' = optical constant which also includes the density of the particles and the thickness of the suspension

In order to use Equation 3 it is necessary to know the value of K'', which is obtained by assuming it is constant for all sizes. Thus it may be computed from Equation 5, wherein all unknowns are evaluated from the experimental data.

$$W_F = \Sigma W_f = \Sigma(E_f d_f / K'') \qquad (4)$$

or,

$$K'' = \Sigma(E_f d_f)/W_F \qquad (5)$$

where W_F is the total weight in the size ranges for which Equation 3 is valid—below 53 microns (No. 325 sieve)—and is determined by sieve analysis.

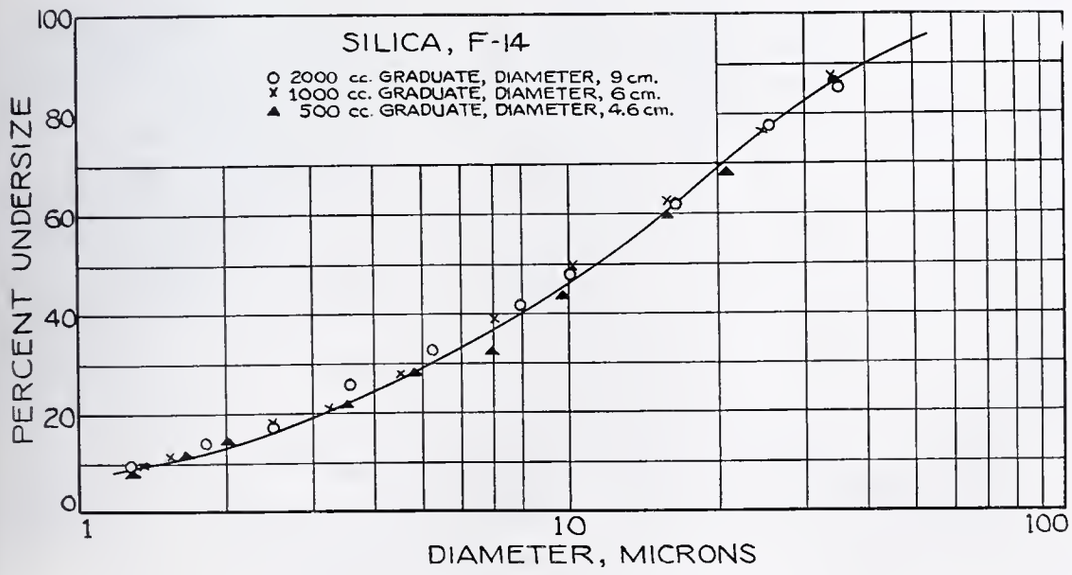

FIGURE 5. HYDROMETER ANALYSES IN GRADUATES OF DIFFERENT DIAMETER

Since the property usually evaluated is surface, the particles in the small size ranges are the most important. This is apparent from a consideration of the equations, since a relatively large error in the coarse size ranges (which affects the weight distribution considerably) does not greatly affect the total specific surface if the surface data in the smaller size ranges are essentially correct.

In order to minimize certain serious criticisms of the apparatus and technique, a number of modifications of the method were necessary.

Table I. Comparison of Data for Amount Passing a No. 325 Sieve

Material	By Nozzle[a]	By Dispersion[b]		Remarks
	%	Min.	%	
Silica 4-168	74.9	2	73.5	Very flaky
	74.9	5	79.1	
Silica D-2	78.8	2	75.2	Normal
Silica W-1	29.7	1	25.3	Very coarse

[a] 1-gram sample at 10 pounds per sq. inch water pressure for 1 minute.
[b] 0.5-gram sample dispersed with mechanical stirrer and washed through sieve with light spray from wash bottle.

Table II. Variation in Properties of Cement 114c Depending upon End Point

Sample Weight	End Point	<7.5 Microns	Specific Surface[a]
Gram	Microns	%	Sq. cm./g.
0.2000	7.5	26.7	1980
0.2000	2.5	19.7	2230
0.1000	7.5	26.1	1925
0.1000	2.5	17.7	2180
0.1000	1.25	15.4	2240

[a] Bureau of Standards value, 1890.

Apparatus Considerations. The slit opening in the apparatus is 1.59 cm. which is not a serious consideration for large sizes, but for fine particles the size at the top of the slit may vary more than 15 per cent from the size at the middle for cement at 7.5 microns and a height of 2.1 cm. Furthermore, the middle of the slit does not give a mean size because of the exponential nature of Stokes' law. The actual variation depends upon the particular solid-liquid system used and the height and time at which measurements are made. By modifying the slit opening and optical system (*34*) the variation in size between the middle of the slit and the top and bottom was reduced to less than 10 per cent for readings of 1.25 microns at 1-cm. height. This modification also produced a light beam that was considerably less divergent than that in the original apparatus. A narrow slit is also necessary in using heights of fall of 1 cm. to prevent the light beam from being reflected from the surface.

The optical system in the Wagner apparatus utilizes white light, which is not satisfactory in the study of materials composed principally of particles less than the wave length of light (about 0.5 micron) but might be adapted for such materials using light sources suggested by Matthews and others (*25*). However, for powders showing less than about 20 per cent below 1 micron, the use of white light is probably satisfactory, based on the work of Stutz (*37*) and the fact that ground minerals show the greatest selective absorption of the shorter wave lengths, which is the region of least sensitivity of the cell. The use of filters to obtain monochromatic light in the Wagner apparatus has practical limitations because of the high intensity source required and the resulting heat effects. An external light source of high intensity is suggested as a means of eliminating the heat effect.

The light energy is dissipated in the cell, which necessitates consideration of temperature effects on convection. In the experiments described below, close control of temperature was maintained by turning the light off between readings for sizes smaller than 7.5 microns. A preliminary 3-minute equilibrium time was adopted for all subsequent readings to allow for photocell fatigue. A similar equilibrium time was used in starting each analysis; this technique was found satisfactory in making readings down to 1.25 microns over a period of 2 hours.

The photocell used in the apparatus is subject to fatigue which can be minimized by proper technique. Because of the response characteristics of this cell, as shown by Müller (*26*), it is desirable to maintain the light intensity at low values in order to obtain a linear relation between current output and transmitted radiation. The use of other cells with amplifying circuits for the apparatus is suggested to overcome the fatigue effect and allow use of lower light intensities to eliminate temperature effects in the sedimentation cell (*41*).

The method described by Wagner for obtaining an initial setting of 100 microamperes, using a meter reading to only 50, is not recommended. If a meter reading to 100 is not available, an initial setting of 50 microamperes can be used with equal precision.

The dispersion method of Wagner was replaced by a stirring apparatus (*34*) similar to that described by Traxler and Baum (*39*). Ponzer and MacPherson (*27*) have studied this phase of the technique indirectly and show the effects of dispersing time on the results for surface. A more direct comparison is shown in Table I. Since the surface computed by the Wagner method is directly proportional to the amount passing a No. 325 sieve, it is essential that a similar amount of particles in the dispersion (used for analysis) also pass a No. 325 sieve. As shown in Table I, this can be attained by modifying the dispersion time according to the nature of the material being analyzed. In the present study, employing the mechanical stirrer (*34*), dispersion times of 1 to 3 minutes were used for the coarse and fine materials, respectively.

Theoretical Considerations. The Wagner treatment for converting turbidity data to surface in absolute units is based on a number of controversial assumptions.

1. The mean size of all particles less than 7.5 microns is assumed to be 3.75 microns. While this might be valid on a weight or number basis, it is not a safe assumption for surface, since a given amount of 1-micron material may possess six times the surface of an equal amount of the same material 6 microns in size. The practical significance of the arbitrary selection of the smallest fraction was pointed out by Roller in the discussion of the paper by Ponzer and MacPherson (*27*), wherein it was shown that the absolute limit of specific surface as measured by the Wagner turbidimeter, using the prescribed technique, was 5080 sq. cm. per gram. Using a final reading of 1.25 microns (as in the present study) and a mean size of 0.63 micron, the limit would be 30,200 sq. cm. per gram (this limit, however, depends upon the density).

The effect of the Wagner assumption is shown by Table II. The variation in weight distribution depending upon the end point used for calculation in the same analysis is large although the surface variation is not. The trend shown for the surface is a very serious point, since it shows the anomaly of coarser material having greater surface. The explanation of these effects is found, in part, in the Wagner assumption of a straight line from the 7.5-micron reading to the origin of the extinction versus size plot, which is correct only for certain size distributions. The discrepancies for cements and ground sand shown by Lea and Nurse (*23*) for the Wagner method can be attributed to this lack of correction for the small sizes which they recognized. Lea (*22*) had shown previously that the Andreasen pipet data gave essentially the same results as the Wagner method if 7.5 microns was assumed the end point for both methods. Although concordant results are obtained, such an end point cannot be considered a solution to the problem if specific surface results are desired.

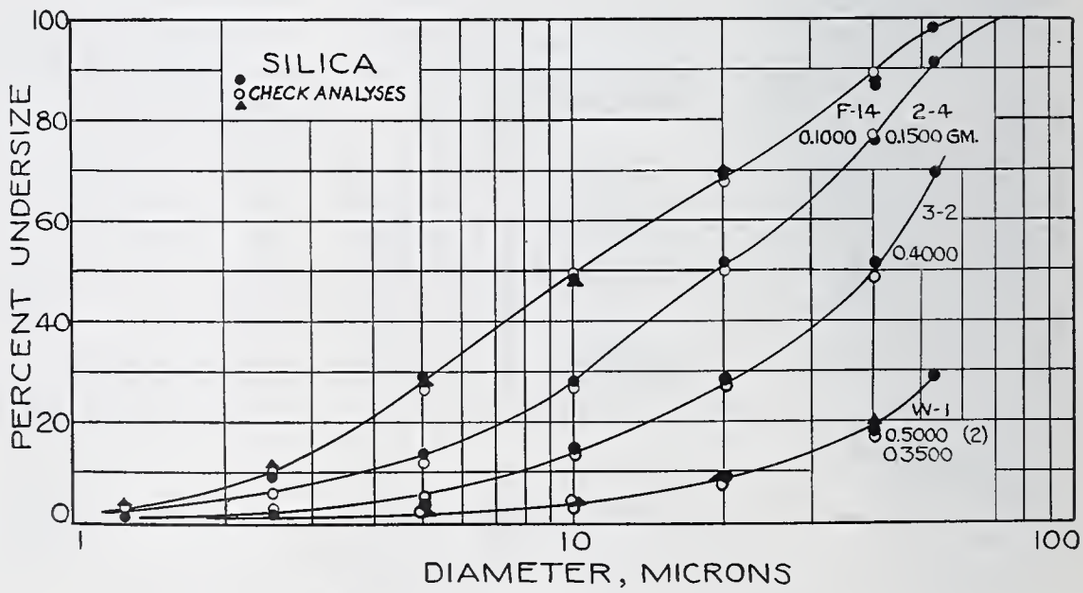

Figure 6. Precision of the Turbidimeter Method

Table III. Precision of Turbidimeter Method

Material	Sample Weight	Passing No. 325 Sieve	Dispersion Time	Initial Transmission	Specific Surface
	Gram	*%*	*Min.*	I_0	*Sq. cm./g.*
Silica F-14	0.1500	98.0	2	100	4160
	0.1500	98.0	3	100	4320
	0.1000	98.0	3	50	4130
	0.1000	98.0	3	100	4210
	0.1000	98.0	3	100	4190
Silica 2-4	0.1500	91.2	2	100	2650
	0.1500	91.2	2	100	2780
Silica 3-2	0.4000	69.4	1	100	1180
	0.4000	69.4	3	100	1380
Silica 4-16	0.1000	97.5	3	100	5860
	0.0500	97.5	3	100	6590
Silica D-2	0.1500	78.8	2	100	2670
	0.1500	78.8	3	100	2380
	1.1500	78.8	2	50	2460
Silica W-1	0.5000	29.7	2	100	533
	0.5000	29.7	2	50	528
	0.3500	29.7	2	100	530

2. The value for C in Equation 2 is assumed to be independent of size. This assumption apparently is the principal cause of the disagreement in results obtained by this and other methods.

3. Of less importance is the concentration effect on the validity of Equation 1. It has been shown (*35*) that for a given polydisperse material (having a mean size, d) Equation 1 is valid for concentrations which yield fractional transmissions greater than 30 per cent; for some materials Equation 1 is valid for even greater concentrations.

Using the modified apparatus and technique (*34*), a large number of analyses were made on a variety of materials to determine the efficacy of the Wagner apparatus. The precision is good, as shown by the results in Table III and Figure 6 for different sizes of materials and two different initial light intensities. These results were obtained in 2 hours, using an end point of 1.25 microns and a height of fall of 1 cm., which is a considerable saving compared to the time required for the hydrometer and pipet methods.

The good results shown for cement (*1*, *8*) and the wide use of the Wagner apparatus would lead to the belief that its success is more than fortuitous. However, a study of the published data shows that the agreement is based on cements all having essentially the same specific surface and similar types of size distribution. When these conditions are changed, as when analyzing a finely ground red slate, the Wagner method does not give good results.

As a matter of interest, an analysis was made on cement 114c, using methanol as the dispersing medium. The results were in fair agreement on the basis of specific surface results, 1950 sq. cm. per gram, although the size distribution results were not very satisfactory. The data indicate the possibility of using some medium other than kerosene in cement analyses, which would be of considerable practical advantage since kerosene possesses properties which change with age.

Comparison of Methods

With the exception of one or two articles there has been available no direct comparison of the particle size distributions obtained by the methods now in general use. Such comparisons have generally utilized but two methods on selected materials and have been prejudicial to one or the other of the

Table IV. Comparison of Data Obtained by Different Methods

Material	Density, D_s	Passing No. 325 Sieve, G_F	Surface Mean Diameter, D_S		Specific Surface, S	
	G./cc.	*%*	*Microns*		*Sq. cm./g.*	
			[a]	[b]	[c]	[d]
Silica 196	2.62	94.8	5.4	5.5	4,250	4,150
20–40 micron fraction, A	2.62	96.0	23	25[e]	1,000	920
A	2.62	96.0	23	31[f]	1,000	740
B	2.62	96.0	23	25[e]	1,000	920
Silica F-14	2.67	98.0	5.1	5.0	4,420	4,500
Silica D-2	2.67	78.8	8.1	7.9	2,780	2,850
Silica D-14	2.67	99.6	2.0	2.0	11,200	11,200
Silica 4-16	2.67	97.5	3.4	3.4	6,600	6,600
Silica W-1	2.67	29.7	30	31	745	720
Silica 1-168	2.67	99.9	4.1	3.9	5,450	5,760
Red slate 642	2.80	96.4	4.0	2.2	5,360	9,740
20–40 micron fraction, A	2.80	85.0	38	18[e]	560	1,190
A	2.80	85.0	38	34[f]	560	630
B	2.80	85.0	29	18[e]	740	1,190
5–10 micron fraction	2.80	99.9	12	7.5[e]	1,710	2,860
	2.80	99.9	12	8.1[f]	1,710	2,640
Red slate T-S	2.80	99.9	1.1	0.87	19,300	24,600
Green slate 470	2.82	96.4	4.2	3.9	5,080	5,450
Green slate 471	2.82	99.9	3.5	3.2	6,100	6,650
Black slate 197	2.91	95.4	8.6	5.1	2,440	4,050
Traprock 113	2.91	83.4	9.8	6.3	2,100	3,280
Cement 114	3.16	87.7	8.9	8.5	2,140	2,230
Limestone 107	2.73	78.2	5.6	6.4	3,940	3,440
Clay C-J	2.67	100	1.0	0.77	21,600	29,200
Tripoli 223	2.60	98.3	9.1	7.8	2,540	2,960
Coal C	1.75	73.4	9.4	9.8	3,650	3,500
Galena G	7.66	65.3	11	15	710	520
Green mica 432	2.92	82.5[g]	6.1	3.1[e]	3,460	6,550
	2.92	82.5[g]	6.1	9.2[h]	3,460	2,230
Talc 573	2.82	99.1	6.1	6.0[e]	3,490	3,540
	2.82	99.1	6.1	11[h]	3,490	1,940
Pumice 571	2.38	99.9	8.8	5.0[e]	2,860	5,060
	2.38	99.9	8.8	5.7[h]	2,860	4,440
Diatoms 939	2.19	96.0	10	4.5[e]	2,680	6,050
	2.19	96.0	10	4.8[h]	2,680	5,700

[a] Computed from turbidity data using Equation 6.
[b] Computed from sedimentation data using Equation 6.
[c] Turbidity surface by Wagner method using 1.25-micron end point and correcting for >53 micron material as in *d*.
[d] Entire surface including that of >53 micron material based on spherical shape, computed by Equation 7.
[e] Sedimentation data only.
[f] Based on microscopic count (*33*).
[g] Value from sedimentation analysis was 95.0 which was used for sedimentation calculations.
[h] Air elutriation data only.

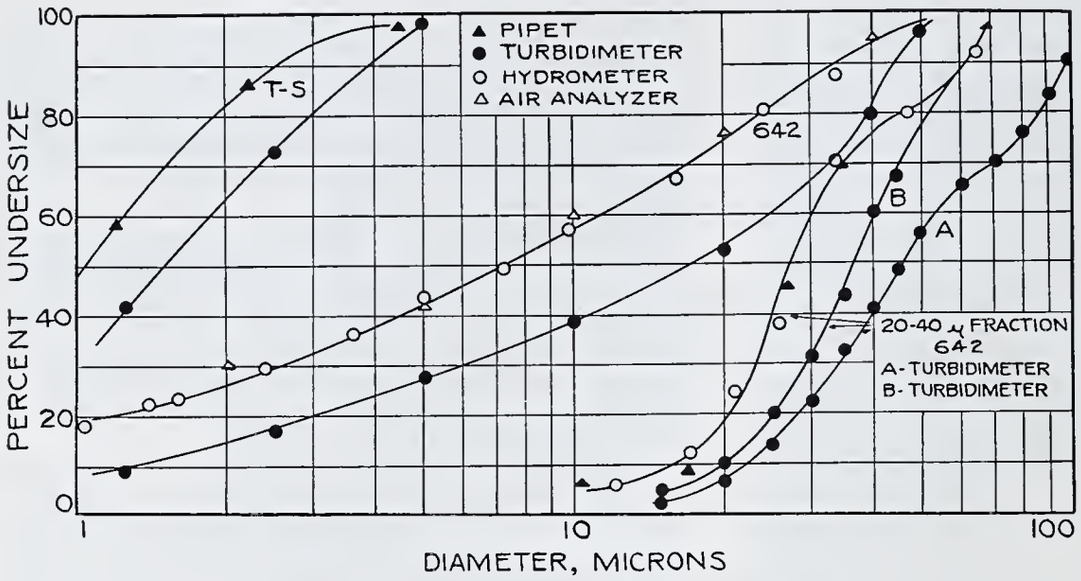

FIGURE 7. SIZE DISTRIBUTION OF RED SLATES

methods used. A rather comprehensive study of the most practical methods was therefore desirable.

The apparatus used in the present study were the streamlined Bouyoucos hydrometer, the Andreasen pipet, the Wagner turbidimeter, and the Roller air analyzer. A variety of materials differing in size distribution and shape were studied in order to cover the types of materials likely to be encountered in practice. In several cases the results were compared with microscopic counts, using the Work (*42*) technique. Since, with the exception of the microscopic counts, all the methods are based solely on the validity of Stokes' law, they should give essentially the same results. Any differences must be attributed to errors inherent in their techniques or theory for computing the distribution. The results of the size distribution analyses are shown in Table IV and illustrated in Figures 1, 3, 7, 8, and 9. The specific surfaces shown in Table IV are based on a shape constant ratio of 6, assuming the particles to be spheres.

The influence of the method of test used and the diameter selected is clearly indicated by the results.

The problem of the point of separation for a No. 325 sieve arises for mica, since the per cent of smaller than 53 microns is considerably less by sieve analysis than by sedimentation. This phase of particle size measurement has been discussed elsewhere (*33*); it is an important consideration in the Wagner turbidimeter calculations. The curves show that air elutriation and the hydrometer and pipet methods give results that are in good agreement except for mica. Figure 9 indicates that the microscope gave good agreement with sedimentation data. However, in Figure 8 the microscope data show a low weight distribution in the intermediate sizes, which may be the result of incomplete fractionation of the portions used for microscopic counting, since the silica D-2 sample was a dry-ground material whereas those in Figure 9 were wet-ground and could be fractionated more readily. Different specific surfaces are obtained for the 20- to 40-micron fractions of silica and red slate, depending upon whether the microscopic or sedimentation diameter is used (Table IV). The data for mica in Figure 8 and other data on talc (not shown) indicate that the platelike shape of these materials produces erratic results. Air elutriation data do not agree with those from pipet and hydrometer analyses, probably because different fluid flow effects are present. The size distribution curves of such materials must be assumed as "statistical" and will depend upon which methods are used. The results for materials like diatomaceous earth are also statistical because of shape considerations.

The most serious disagreement found is in the Wagner turbidimeter.

For silica and cement the results are in good agreement, but other materials yield erroneous data. Furthermore, there is no particular trend; thus the specific surfaces as computed by the Wagner method for coal and limestone are higher than by other methods, but should not be on the basis of the size distribution curves. For slates and traprock the specific surface and size distributions are in proper relation, but they do not agree with the results by other methods. The use of the microscopic diameter for the 20- to 40-micron fraction of red slate shows better agreement with the Wagner turbidimeter than sedimentation results, but in the case of the 20- to 40-micron fraction of silica, the agreement is not as good. The ratio of microscopic diameter to sedimentation diameter for the 20- to 40-micron fraction of red slate is considerably higher than the same ratio for the 5- to 10-micron fraction.

Since the validity of sedimentation methods in general has been proved, the poor results of the Wagner method must be attributed to the empirical treatment of the data.

One aspect of this treatment is shown by the results for 20- to 40-fractions of silica and red slate as illustrated in Figures 3 and 7. The *A* curves were computed by the Wagner method for

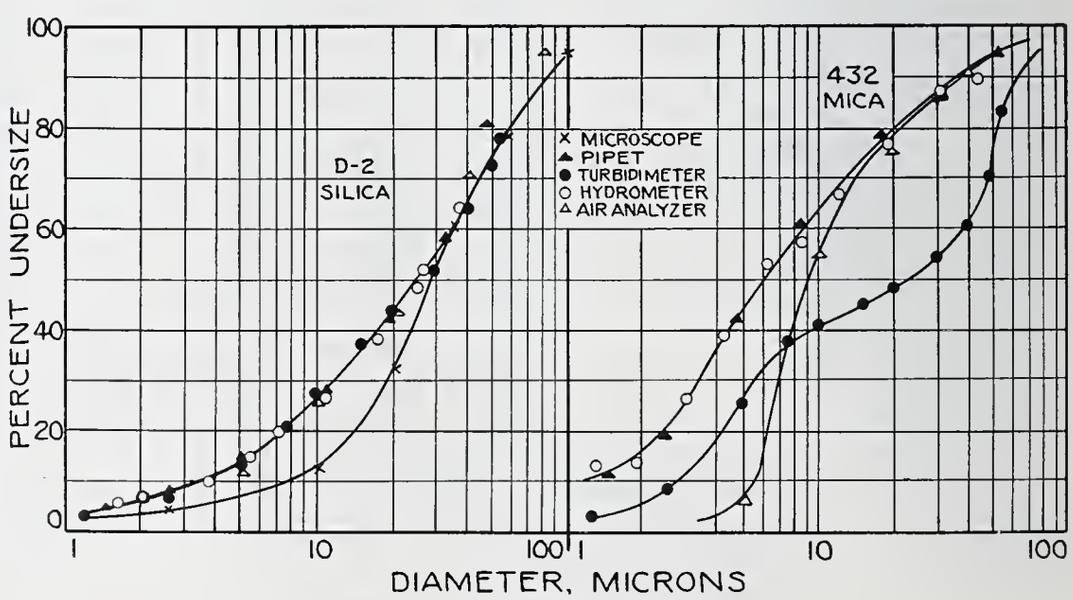

FIGURE 8. ANALYSES OF SILICA AND MICA

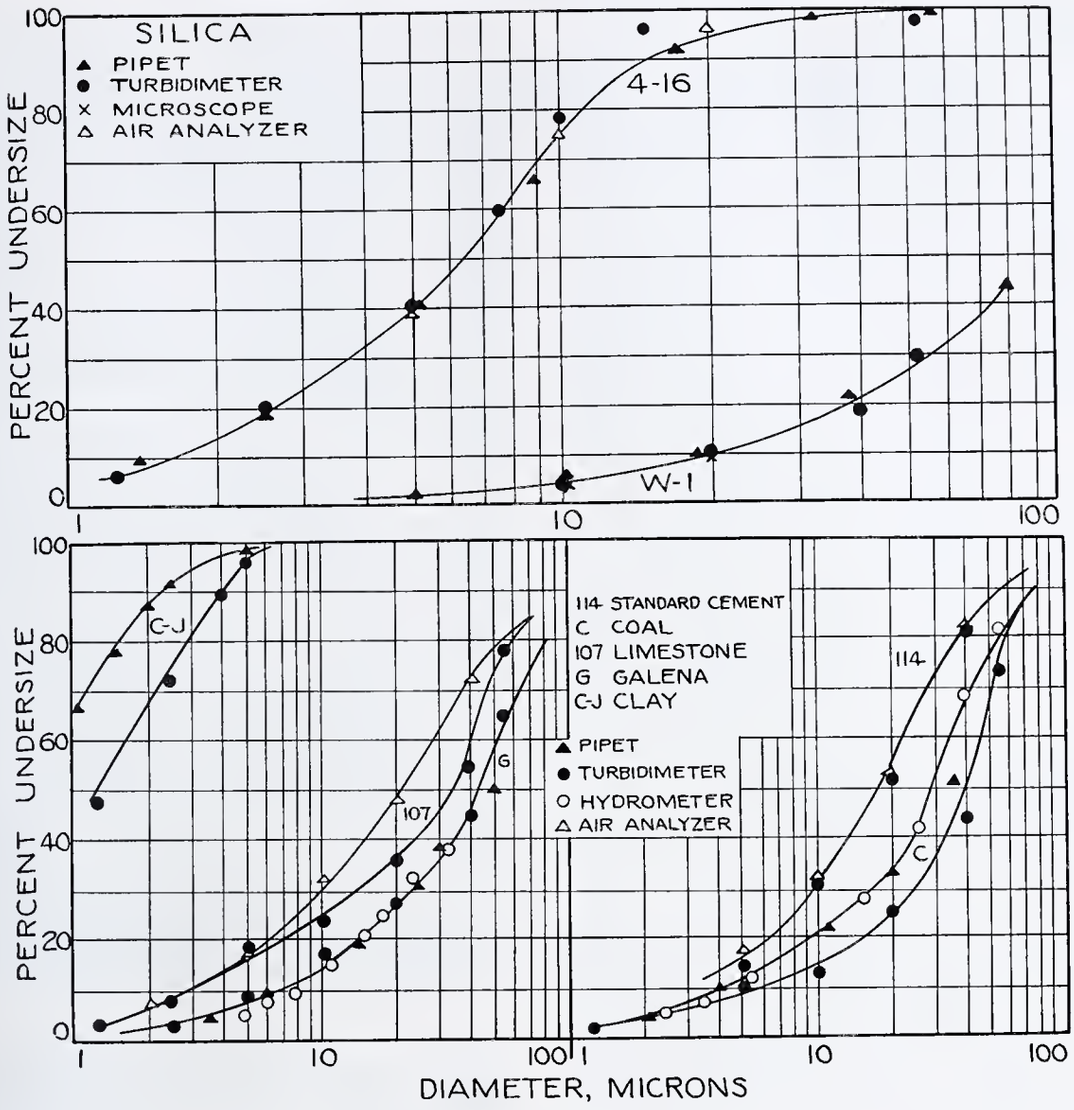

Figure 9. Size Distribution by Various Methods

turbidity data, using readings of 120 microns as the starting point with no correction for sieve analysis. The *B* curves were computed in the manner prescribed by Wagner, using the per cent passing a No. 325 sieve (<53 microns) as the starting point. In the case of the 20- to 40-micron red slate, Figure 7 shows that the per cent of 53 microns by the turbidimeter is 62 (curve *A*) while by other methods it was 85. In the case of the 20- to 40-micron silica, Figure 3 shows that the turbidimeter gives essentially the same value below 53 microns (96 per cent) as does the pipet method (93 per cent). Furthermore, if the starting point for the turbidimeter calculations is taken as 53 microns, using the per cent finer as obtained by other methods, the results show (curve *B*, Figures 3 and 7) that the turbidity data for silica are unaffected, whereas those for the red slate are shifted to the left, resulting in better agreement (compared to the *A* curve) with the analysis by other methods.

These results and those shown in Figures 8 and 9 indicate that the optical properties of silica make it amenable to turbidimeter analysis. Cement probably behaves in a similar manner, which would account for the application of the turbidimeter to this material. However, other materials, of which the red slate is but one example, apparently do not yield satisfactory results because the optical properties of suspensions are not amenable to the Wagner analysis. The results for the fractions of red slate show the same variations as the whole powder. It has been suggested that the high concentrations of red light in the energy from a tungsten filament may be the cause of the disproportionately high transmission for red slate. The available data (*37*) indicate that the wave length of the energy should not greatly affect the light-scattering characteristics of particles of a size greater than the wave length of the incident energy.

Comparison of data by the Roller air analyzer and the Wagner method based on results down to 5 microns was discussed by Traxler and Baum (*39*), who attributed the lack of agreement to optical effects. In this paper a typographical error resulted in a reversal of the concentrations at the recorded transmissions for the red and black slates, which explains why the turbidity curves for these slates have a positive slope whereas the slope for the other materials is negative.

A comparison of results obtained with the Wagner turbidimeter, permeability methods, and air elutriation has been given by Roller and Roundy (*32*). Gran (*19*) has compared data obtained with the Wagner turbidimeter and hydrometer methods and criticized the latter because the results did not agree with the turbidimeter. He states further that the "pumping action probably accounts for the fact that the hydrometer indicates a finer grading by weight than the turbidimeter". However, hydrometer analyses give results in agreement with the pipet method, wherein such pumping effects are not present. Gran also states that errors in the turbidimetric determination caused by optical properties are negligible, in spite of large differences in the results compared with those from the hydrometer method.

The Wagner turbidimeter gives satisfactory results for certain cements and ground silica, but unless it is known that the data are in agreement with those from other methods, the values for *specific surface* are open to question based on the general use of the term. It is suggested that the more relevant term "turbidity surface" be used where turbidimeter analyses are run under prescribed conditions as in cement analysis.

Critical Study of the Wagner Method

The Wagner method has obvious advantages in simplicity of operation and speed which would give it definite superiority

Table V. Wagner Treatment of Turbidity Data

Material	G_F, Sieve Analysis	Turbidimeter Method D_F, Equation 12	Turbidimeter Method Surface[b], Equation 10	Other Methods[a] D_F, Equation 8	Other Methods[a] Surface[b], Equation 11
	%	*Microns*	*Sq. cm.*	*Microns*	*Sq. cm.*
Silica 196	94.8	5.4	4250	5.5	4150
20–40 micron fraction, B	96.0	22	990	24	910
	96.0	22	990	30[c]	730
Silica F-14	98.0	5.1	4420	5.0	4500
Silica D-2	78.8	6.5	2730	6.3	2800
Silica 4-16	97.5	3.4	6600	3.4	6600
Silica, W-1	29.7	11	600	12	550
	29.7	12	550	12	550
Red Slate 642	96.4	4.0	5360	2.2	9740
20–40 micron fraction, B	85.0	26	690	16	1140
	85.0	26	690	30[c]	590
5–10 micron fraction	99.9	12	1710	7.5	2860
	99.9	12	1710	8.1[c]	2640
Green slate 470	96.4	4.2	5080	3.9	5450
Black slate 197	95.4	8.6	2440	5.1	4050
Traprock 113	83.4	8.3	2070	5.3	3250
Limestone 107	78.2	4.4	3900	5.1	3390
Tripoli 223	98.3	9.1	2540	7.8	2960
Coal C	73.4	7.4	3500	7.5	3350
Galena G	65.3	7.4	690	10	510
Green mica 432	82.5[d]	5.1	3320	3.1	6550
	82.5[d]	5.1	3320	9.2[e]	2230
Talc 573	99.1	6.1	3490	6.0	3540
	99.1	6.1	3490	11[e]	1940
Pumice 571	99.9	8.8	2860	5.0	5060
	99.9	8.8	2860	5.7[e]	4440
Diatoms 939	96.0	10	2680	4.5	6050
	96.0	10	2680	4.8[e]	5700

[a] Average of hydrometer, pipet, and air elutriation where they are in agreement, otherwise as noted.
[b] In sq. cm. per gram of whole powder based on spherical shape.
[c] Based on microscopic count.
[d] Percent 53 microns by sedimentation analysis was 95.0 which was used for sedimentation calculations.
[e] Based on air elutriation data only.

if the results could be made to agree with those from other sedimentation methods. For this reason the equations involved were analyzed in detail, in order to determine the magnitude of the errors resulting from the empirical treatment proposed by Wagner. The lack of validity of the empirical treatment is forcibly indicated by the fact that good results are obtained with silica dusts and cements of certain size distributions while results for materials of different colors and size distributions do not agree with the data obtained by other proved sedimentation methods. The materials and data used in the following study were the same as those shown in Table IV. The data obtained by pipet, hydrometer, or air analysis (or averaged data where available) were used for calibration purposes.

Analysis of the Wagner Equations. The surface mean diameter, D_S, for polydisperse powders may be calculated from the size distribution data by means of Equation 6:

$$D_S = 100/\Sigma(G_f/d_f) \text{ microns} \quad (6)$$

where G_f is the weight per cent in the fraction for which d_f is the arithmetic mean diameter of the fraction in microns.

The value for D_S in Equation 6 is for the entire powder and includes consideration of the material above a No. 325 sieve which is normally taken as the lower limit of sieve analysis. If the point of separation of this sieve is taken as 53 microns on a sedimentation diameter basis (*33*), the surface mean diameter of the fraction above 53 microns may be assumed as equal to 100, since it has a negligible effect on the value of D_S except where the material contains but a small amount of <53-micron material. In the latter case the sieve analysis of the >53-micron fractions should also be used in Equation 6.

The specific surface, S, of the entire powder may be computed from D_S, assuming spherical shapes, using Equation 7 where D_s is the density:

$$S = \frac{6 \times 10^4}{D_s \times D_S} \text{ sq. cm. per gram} \quad (7)$$

In the present discussion the spherical shape constant has been employed in order not to complicate the equations unduly. Absolute shape constants are to be preferred, but the same corrections would apply to both the Wagner data and the data from other methods. The use of Equations 6 and 7 assumes the validity of the arithmetic mean diameter for computing the surface in each fraction. This involves an approximation, depending upon the size distributions within the fractions, which is important for the finest fractions and the very fine materials. However, the same approximation applies equally wherever surface is computed from size distribution, regardless of the method used to obtain the size analysis.

To compute the extinction coefficient in Equation 1 for the <53-micron material, the surface mean diameter, D_F, must be based on the weight distribution of the material below 53 microns only, or:

$$D_F = G_F/\Sigma_0^{53}(G_f/d_f) \text{ microns} \quad (8)$$

where G_F is the weight per cent passing a No. 325 sieve (53 microns).

The basis for the Wagner equations is found in Equations 1, 2, 3, and 5 which are combined in a special form involving the "transmittancy constant", C:

$$C = 600wG_F/[D_s\Sigma_0^{53}(E_f d_f)] \quad (9)$$

where w is the weight of sample used in 335 cc. of suspension.

The surface per gram of whole powder in the Wagner method is taken as the surface possessed by those particles of less than 53 microns in 1 gram of whole powder and is computed from:

$$S_F = (C\Sigma_0^{53}E_f)/w = (600G_F\Sigma_0^{53}E_f)/[D_s\Sigma_0^{53}(E_f d_f)] \quad (10)$$

This equation neglects the surface above 53 microns, which is generally not important for most materials as discussed under Equation 6. The equation for other methods of analysis, which is analogous to Equation 10, is from Equations 7 and 8:

$$S_F = (600G_F)/(D_s \times D_F) \quad (11)$$

Comparing Equations 10 and 11 it is shown that if the Wagner method is valid the following must be true: D_F by Equation 12 from Wagner data must be equal to D_F by Equation 8 from data by other methods.

$$D_F = \Sigma_0^{53}(E_f d_f)/\Sigma_0^{53}E_f \quad (12)$$

Furthermore, the relation shown in Equation 12 can be valid, but the size distributions obtained by the two methods might not necessarily agree. The weight distribution is computed from Equation 13.

$$G_f = \frac{G_F E_f d_f}{\Sigma_0^{53}(E_f d_f)} \quad (13)$$

where G_f is the weight per cent in the fraction for which E_f is the extinction and d_f the mean size. The basis for this relation was discussed under Equation 3.

The applicability of the Wagner treatment depends upon the validity of Equations 12 and 13, although neither their derivations nor the theoretical basis for them was given by the author. The principal proof for the Wagner method was the agreement of results obtained with those from other methods on certain cements. In order to determine the validity of Equations 12 and 13, the available data were used in the foregoing equations with the results shown in Table V. (Where the amount of material greater than 53 microns was not sufficient to change the second figure for the value of D_F in Table IV, the same values were used in Table V.) The data in Table V illustrate the errors resulting from the use of Equations 12 and 13 and explain the anomalous results obtained by the Wagner turbidimeter method on certain materials.

In the case of limestone and coal the use of Equation 12 gives a smaller mean size (greater surface) than other methods, while the weight distributions (Figure 9) show these materials to be coarser by the turbidimeter. In the case of galena, the

size distribution agrees (Figure 9), but the surface by the turbidimeter is greater. For slates, the size distributions and specific surfaces are in accord, but the size is greater by the turbidimeter than by other methods. These results and those illustrated in Figure 7 for coarse materials (discussed previously) indicate that the selection and use of Equations 12 and 13 were fortuitous for cement and silica. The discussion given above on the mean size of the 7.5-micron fraction is further evidence of the lack of validity of Equations 12 and 13.

The anomalous results discussed above lie in the fact that an infinite number of size distributions from Equation 13 can yield the same value for D_F in Equation 12. Thus, although the specific surface computed from the Wagner equations (Equation 10 or 12) may be the same as from other methods (computed by Equations 8 and 11), the size distribution computed from Equation 13 for the Wagner data may not be in agreement. This lack of agreement apparently is the result of errors in the conversion of extinction data to weight distribution with compensating errors producing values for D_F that are in agreement with those from other methods. It follows that size distribution is a better basis than specific surface for comparing results from different methods, especially where the computation of specific surface from the size distribution data is based on the same principles.

The importance of the G_F determination is reflected in Equations 12 and 13, and the amount passing a No. 325 sieve after dispersion must be the same as is obtained by sieve analysis, since the latter value is the one used in the Wagner equations. In this respect an evaluation of dispersing action by means of surface results alone, as was done by Ponzer and MacPherson (*27*), is open to question since it merely compares the term $G_F\Sigma_0^{53}E_f/\Sigma_0^{53}(E_f d_f)$ of Equation 10, wherein G_F can vary with the same specific surface result being obtained if variations in the other quantities compensate.

Table VI. Extinction Coefficients[a] on Polydisperse Materials Smaller than 53 Microns

Material	W_F G./l.	E_F	D_F[b] Microns	k
Silica 196	0.424	0.642	5.2	2.06
20–40 micron fraction	1.790	0.565	24	1.99
	1.790	0.565	30[c]	2.47
Silica F-14	0.293	0.496	5.0	2.22
	0.293	0.496	5.0	2.22
	0.293	0.511	5.0	2.28
	0.439	0.717	5.0	2.14
	0.439	0.735	5.0	2.21
Silica W-1	0.310	0.292	12	2.93
	0.444	0.446	12	3.12
	0.444	0.402	12	2.81
Silica D-2	0.351	0.467	6.3	2.19
Silica 4-16	0.145	0.371	3.4	2.29
Silica 1-168	0.149	0.367	3.9	2.52
Red slate, 642	0.144	0.489	2.2	1.96
20–40 micron fraction	1.015	0.444	16	1.85
	1.015	0.444	30[c]	3.44
5–10 micron fraction	0.239	0.240	7.5	1.94
	0.239	0.240	8.1[c]	2.10
Red slate T-S	0.090	0.735	0.87	1.86
Green slate 470	0.202	0.495	3.8	2.44
Green slate 471	0.149	0.471	3.2	2.66
Black slate 197	0.285	0.565	4.9	2.54
Traprock 113	0.374	0.585	5.3	2.17
Limestone 107	0.351	0.686	5.1	2.61
Clay C-J	0.060	0.420	0.77	1.29
Green mica 432	0.299	0.575	3.1	1.58
Talc 573	0.444	0.613	7.0	2.54
Coal C	0.219	0.740	7.5	6.68
Galena G	0.584	0.335	10	1.90
Pumice 571	3.57	1.456	5.0	0.53
Tripoli 223	0.882	0.842	7.8	1.95
Diatoms 939	0.714	0.851	4.5	1.41
Cement 114c	0.267	0.342[d]	7.2	2.42
	0.267	0.442[e]	7.2	3.12

[a] Thickness of suspension, l, was 3.81 cm.
[b] Surface mean diameter of 53 micron material calculated by Equation 8 using sedimentation data only unless otherwise noted.
[c] Based on microscopic count.
[d] In kerosene based on D_F from Equation 12.
[e] In methanol based on D_F from Equation 12.

Table VII. Extinction Coefficients for Fractions of Various Materials

(l = 3.81 cm.)

Material	Coefficients for Fraction Limits in Microns 0–1.25	1.25–2.5	2.5–5	5–10	10–20	20–53
Silica 196	1.67	1.57	2.70	3.08	4.13	...
F-14[a]	1.60	2.10	3.35	2.28	2.51	...
	1.42	2.60	2.88	2.40	2.62	...
D-2	1.88	2.21	2.55	2.22	2.50	2.40
4-16	1.91	2.42	2.24	2.45	2.64	...
Red slate 642	1.41	3.44	3.08	2.78	2.78	
Green slate 470	2.36	1.09	2.44	4.06	3.52	2.79
Black slate 197	1.36	2.12	1.87	4.04	4.30	8.75
Traprock 113	1.39	3.82	2.01	2.26	5.85	4.22
Coal C	9.21	7.75	4.05	1.89	6.65	6.08
Galena G	...	...	1.77	1.47	0.89	1.19

[a] Check runs, using W equal to 0.299 gram.

Table VIII. Extinction Coefficients for Fractions Separated by Air Elutriation

Material	Coefficients for Fraction Limits[a] in Microns 0–2	2–5	5–10	10–20	20–40
Silica 196	0.78	1.78	1.56	2.24	2.61
Green slate 470	1.25	2.29	2.59	3.39	4.92

[a] Mean diameter assumed as mid-point of fraction.

Evaluation of Extinction Coefficients. In view of the lack of agreement in results obtained by different methods, the errors must be attributed to the invalidity of Equation 3 for conversion of the turbidity data to weight concentration. In order to establish this fact the data developed in this study were used for computation of the extinction coefficient, k, by means of Equation 1. The dimensions of k are the extinction per gram per liter per centimeter of thickness of suspension per reciprocal micron—that is, $k = (Ed)/(Wl)$.

Polydisperse Powders. The results for k are given in Table VI for polydisperse powders less than 53 microns in size. The variations in values for k are attributed to differences in optical properties and shape constants. The relatively high values for one silica sample (W-1) are indicative of low transparency for large particles compared to the data for the other silica samples. The high absorbing power of coal is indicated by its high constant, and the low value for galena shows the influence of density. The low value for pumice indicates the high transparency of this material. Certain data in Table VI obtained in the first part of this study are included as a matter of interest although the fractional transmissions are less than 30 per cent.

Fractions. In the Wagner turbidimeter method the measured extinction for any one fraction includes not only those particles in any given fraction but also those particles smaller than the smaller size limit of the fraction. This is true because at any given level in the sedimentation process particles smaller than the lower size limit of the fraction are always being replaced from above as fast as they settle out, thereby maintaining their concentration constant (at the given level) until their sedimentation time has elapsed. The empirical treatment of Wagner implies that the over-all extinction coefficient of any fraction is not influenced by the presence of these smaller particles, even though the extinction coefficients of the individual separated fractions may vary with size.

The over-all extinction coefficients of various fractions of certain materials were evaluated, using Equation 1. The data for the weight of material in each fraction were computed from the weight distribution obtained by other methods of analysis, and the diameter was taken as the mean of the

fraction limits. This method of selecting the mean size of the fractions has been found satisfactory in other studies, provided the fraction limits of the small sizes are close together. The extinction data were taken from the turbidimeter analyses reported above. The results given in Table VII show considerable variation of k with size (even for silica which gives good results by the Wagner method).

In order to determine the individual extinction coefficients (from Equation 1) for closely sized fractions containing a minimum of particles outside the fraction limits, turbidity data were obtained on fractions of two materials that had been separated by air elutriation. The data given in Table VIII show that the extinction coefficients tend to increase as the size increases, with the exception of the 2- to 5-micron fraction of silica. This exception might be the result of incomplete fractionation of the 2- to 5-micron material. Based on the results shown in Tables VII and VIII, it appears that the presence of particles smaller than the lower size limit of the fraction affects the extinction coefficient of the fraction. The variation in amount of these smaller particles probably also influences the extinction coefficient. In the case of silica and cement these variations presumably are not of sufficient magnitude to influence the results. However, for other materials the variations probably account for the anomalous results obtained when using Equations 12 and 13.

The deviations in over-all extinction coefficients with size may be caused by variations in shape constants with size, the lack of validity of Equation 1, the effect of the presence of smaller particles, inadequacy of the apparatus to determine extinction accurately, and experimental error or combinations of these effects. Since the variations are of such magnitude and indicate no trends that can be evaluated separately, it is concluded that turbidimetric sedimentation methods cannot be used with confidence except for certain materials. Silica is amenable to such analysis, probably because the over-all extinction constants for different sizes do not vary sufficiently to invalidate the use of Equation 13 in practice.

It may be argued that it is not possible to obtain true specific surface by any method; but it cannot be disputed that other methods give a weight distribution (based on sedimentation diameter) from which specific surface can be computed if the shape constant ratios are known or assumed. In the case of the Wagner turbidimeter (also a sedimentation method) the accuracy of the results for the weight distribution and calculated specific surface depends upon the type of material being analyzed. However, there is considerable application of the Wagner turbidimeter for rapid control work on a given type of material. If the extinction coefficients for the material are obtained by calibration, it is possible to construct an average curve for k versus size. A size distribution analysis on materials of the same type might then be made by using these values for k at a given size and the observed extinction and employing Equation 1 or Equation 3. In this manner the Wagner turbidimeter might be used advantageously to obtain results on a small sample in a minimum of time.

Conclusions

The pipet method is the best method studied for determining the particle size distribution of subsieve material by sedimentation procedures.

The hydrometer method, when properly calibrated, gives results as precise as the pipet method and may be used as a rapid control procedure. The results are independent of the size of sedimentation cylinder diameters greater than 5 cm.

Air elutriation yields results in good agreement with those from sedimentation procedures, provided there is no attrition and the particles are not platelike in shape and do not flocculate in suspension. This method is particularly applicable for studying the properties of individual fractions.

With the exception of ground silica and certain cements, the specific surface and size distribution of finely ground materials as determined by the Wagner turbidimeter are subject to question.

The use of specific surface alone as a criterion for comparing results in particle size studies may yield anomalous results.

The variation in extinction coefficients of fractions of polydisperse materials invalidates the Wagner empirical treatment of turbidity data except for specific materials such as ground silica and certain cements.

The extinction coefficients (based on Equation 1) of separated fractions (of a given material above the colloidal range) tend to increase with increase in size.

Acknowledgment

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Determination of Beta-Carotene and Neo-Beta-Carotene with the Visual Spectrophotometer

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THE relation of neo-β-carotene to analytical methods for β-carotene has been discussed by Beadle and Zscheile (*1*), who successfully applied a photoelectric spectrophotometric method to the carotene analysis of certain vegetables. They showed that considerable error may be introduced into photometric methods for the analysis of carotene extracts if the content of neo-β-carotene is not considered. The 4500 Å. region is often used for total carotene analysis by visual spectrophotometry. It is clear that if neo-β-carotene is present, analytical results will be low, because neo-β-carotene absorbs considerably less at this wave length than does β-carotene.

In their method of analysis, a double monochromator is employed with the radiation from an incandescent source (T10 bulb, C8 filament, 12-ampere, 6-volt projection lamp) of continuous radiation. Narrow spectral regions are isolated [10.5 Å. at 4360 Å. and 13.8 Å. at 4780 Å.] at wave lengths which are optimum for analysis of mixtures of β-carotene and its neo-isomer. Thus 4360 Å. is employed for determination of total carotene, after which 4780 Å. is used for composition determination.

Since the type of optical equipment employed by Beadle and Zscheile is not generally available, it is desirable that this analytical method be adapted to simpler and less expensive instruments. It is evident (Figure 1, *1*) that certain lines from the mercury arc are favorably situated in the spectrum to permit their use in this analysis. This paper presents the absorption constants required for the use of a mercury arc source with any optical instrument capable of isolating the 4358 and 4916 Å. lines and with a photometer of suitable sensitivity. Comparisons are made between the use of a visual spectrophotometer and of a photoelectric filter photometer.

Among the mercury lines in the region of 3800 to 5200 Å., the 4358 Å. line is most fortunately situated, because at this wave length the absorption curves for the two carotenes are coincident. [In such a region, where the slopes of both standard curves are not extremely steep and absorption coefficients are high, the percentage difference between values at 4360 Å. (*1*) and 4358 Å. is negligible.] The 4339 and 4347 Å. lines are considerably weaker than the 4358 Å. line, are close to the 4358 Å. line, and occur where the absorption coefficients of the two carotenes are nearly identical. The fact that the slopes of the absorption curves of both compounds are steep at 4916 Å. is not objectionable because a line source is used. Another line, of approximately equal intensity, occurs at 4960 Å., the inclusion of which would very seriously affect the composition determination. The problem is thus resolved into an effective isolation of the 4916 Å. line and the group near 4358 Å.

TABLE I. ANALYTICAL CONSTANTS

Wave Length	Specific Absorption Coefficients in Hexane Solution	
	β-Carotene	Neo-β-Carotene
Å.		
4916	142.5	42.5
4358	196	196

Experimental

Table I presents the analytical constants as numerical values from the graphic spectra reported earlier (*1*).

Solutions from fresh spinach extracts were analyzed as standards by the method of Beadle and Zscheile. These solutions were then studied on simpler instruments with a Type A-H4 mercury lamp, with clear standard glass bulb, as the light source.

A Bausch & Lomb Universal visual spectrophotometer was employed, with and without the additional use of Corning filters 038 plus 511. The collimator slit was 0.25 mm. wide. The image of the line was well centered and masked on each side by the jaws of the exit slit.

Results obtained by two operators are compared in Table I with the result obtained on the more accurate photoelectric spectrophotometer. Each visual determination is the average of 10 observations made in rapid succession.

In an effort to obtain greater precision and speed, a modified KWSZ filter photometer (Wilkens-Anderson Co., Chicago, Ill.) was employed with special gas-filled photocells having potassium-coated cathodes to obtain maximum sensitivity in the blue region. The following combinations of filters were tried: for 4358 Å. Corning 038 plus 511, and for 4916 Å. Corning 555 plus GG 11 and Corning 555 plus 556 plus GG 11 (Fish-Schurman glass filter).

Analyses were also made with the visual instrument and incandescent light source (1000-watt projection lamp with T 20 bulb), using wave lengths employed by Beadle and Zscheile (*1*). Slit 1 was 0.20 mm. wide and slit 2 was 0.15 mm. wide at 4360 Å. and 0.7 divisions wide at 4780 and 4850 Å. Results for total carotene were in error by only 2.5 per cent. Analyses for composition had greater errors, although averages for the two wave lengths were in good agreement with correct values. The mercury arc source is a distinct advantage for this type of visual measurement.

TABLE II. ANALYSES OF FRESH SPINACH WITH VISUAL AND PHOTOELECTRIC SPECTROPHOTOMETERS

Wave Length	Filter	Relative Concentration of Carotene	Total Carotene: Visual Operator 1	Total Carotene: Visual Operator 2	Total Carotene: Photoelectric
Å.			*Micrograms/g.*	*Micrograms/g.*	*Micrograms/g.*
4358	None	1.0	52.6	54.9	55.6
	Present	0.5	52.6	56.7	...
	None	0.5	52.6	56.7	...
			Composition (% β-Carotene)		
			%	%	%
4916	None	1.0	93.5	94.5	88.5 (4780 Å.)
		0.5	91.5	96.5	...

Discussion

The 4358 Å. line is very strong and easily used in the visual method with a filter combination of Corning glass filters 038 and 511. This filter is not necessary, however, as shown by the results. The 4916 Å. line is not strong and should not be filtered. Care must be taken to limit the beam to this line only and to exclude the 4960 Å. line.

The results of these two operators disagree appreciably, apparently for subjective reasons, but agreement is better at higher concentrations ($\log \frac{I_0}{I} = 0.5$ to 0.7 for relative concentration of 1.0).

The agreement between the visual and photoelectric spectrophotometric methods is satisfactory for many applications. For composition determination, differences were 3 to 9 per cent, permitting indication of relatively large amounts of isomerization. The experience of both operators with the visual instrument was very limited. It is probable that a more experienced operator would obtain results more concordant with those from the photoelectric spectrophoto-

metric method. Some scattered light was no doubt present in the radiation employed in the above visual measurements. Instrument design and quality of optical parts are important factors.

With the photoelectric filter photometer, absorption values obtained were much too low, indicating that other lines were not sufficiently removed by the filter combinations. It was concluded that the group of lines near 4358 Å. was not completely isolated by the filters available and that such filters alone are inadequate to isolate the 4916 Å. line. These filters are thus inadequate to permit an analysis of this carotenoid mixture with a photoelectric filter photometer.

These analytical constants and this limited experience with the visual method are presented in the hope that they may be useful to workers employing visual equipment. It is noted that Equation 4 (*1*) for calculation of per cent β-carotene is simplified for λ4916 Å. because the denominator becomes 100, the difference between the specific absorption coefficients. It is highly probable that the new types of photoelectric spectrophotometers, which have recently appeared on the market at comparatively low cost and which employ a mercury arc source, would isolate these mercury lines sufficiently well and also provide greater precision and speed than are possible with the visual method.

Summary

β-carotene and neo-β-carotene can be determined in properly purified solutions from spinach extracts by means of a visual spectrophotometer, preferably with a Type A-H4 mercury lamp. A photoelectric filter photometer did not isolate sufficiently narrow spectral regions for accurate analysis of this carotenoid mixture. The total carotene is calculated from the absorption at 4358 Å. and the percentage of either carotene from the absorptions at 4358 and 4916 Å.

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Pipet-Type Capillary Viscometer

For Substances Which Are Solid or Highly Viscous at Room Temperature

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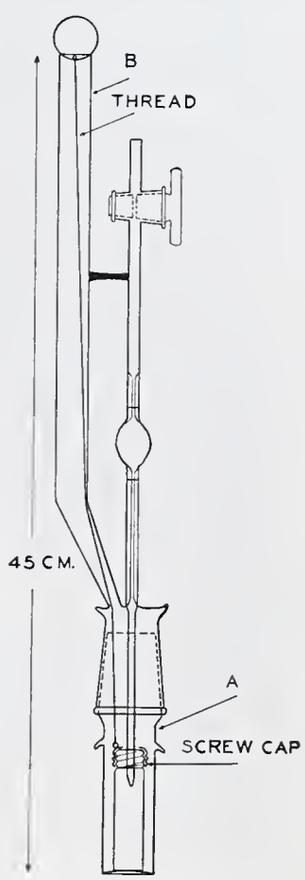
THIS viscometer was developed for routine determinations of the kinematic viscosities of the heavier products resulting from the destructive hydrogenation of coal. These products have a low viscosity index and, although sufficiently fluid at 98.89° C. (210° F.) to permit the use of a capillary viscometer, are either solid or highly viscous at and considerably above room temperature, so that the charging of an Ostwald viscometer is difficult.

The several modifications of the U-tube viscometer having automatic or nonautomatic accurate charging features have recently been briefly discussed (*3*). However, for ease of charging and cleaning, size of sample required, and recovery of the sample, none is as convenient as the pipet-type viscometer such as described by the Bureau of Standards (*2*). A serious disadvantage of this instrument is that it requires a special constant-temperature bath from which it cannot be removed without disturbing the bath. The so-called Bureau of Mines pipet viscometer (*1*) can be handled in the same manner as the Ostwald type, in that several viscometers may be installed in the same bath and can be removed and replaced without disturbing the bath. The pipet, however, must be charged while out of the bath. This limitation not only causes difficulty in charging materials which do not flow readily at room temperature, but also necessitates removal of the pipet for recharging for a check determination. The viscometer described in this communication makes use of a novel method of handling the sample to secure the advantages of both of the above cited modifications of the pipet viscometer.



Method of Use. The sample (5 to 10 cc.) is contained in a sample vial of 15-cc. capacity having a screw cap. With the viscometer removed from the bath, vessel *A* is detached at the ground joint and the sample vial is screwed into the sample vial cap which is suspended by a thread leading up through support arm *B* of the viscometer. The crown of this cap has been cut out, so that the pipet tip can pass through it. *A* is fastened in place with springs and the viscometer is placed in the bath. A small plumb bob, suspended in the support arm, is useful in securing vertical mounting of the viscometer. When the sample has become fluid, the vial is drawn upward until the pipet tip touches or nearly touches the bottom of the vial. A metal disk serves as a counterweight to hold the vial in this position. When the sample and viscometer have attained bath temperature, the sample is sucked up into the pipet to the correct charging level and the vial is then lowered to the bottom of *A*, in which position the pipet tip will clear the top of the liquid in the vial after the contents of the pipet have been discharged. The time required to discharge the pipet can now be determined.

Any number of repeat determinations can be made without dismantling the pipet. After the determinations the sample drains into the vial and is available for other uses with a minimum of loss. Results are reproducible to 0.2 per cent. If the dimensions of the capillary and bulb of the viscometers of this design are made to correspond to those of the Ostwald-Fenske A. S. T. M. viscometers, these viscometers will be about twice as fast, owing to the larger head of liquid.

These viscometers have a record of successful use in the author's own laboratory. Uncalibrated viscometers to accommodate a screw-cap vial 8 cm. in length by 2 cm. in diameter and with capillaries and bulbs of the same dimensions as the Ostwald-Fenske A. S. T. M. viscometers were obtained from the Scientific Glass Apparatus Company.

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Estimation of COOH Groups in Commercial Starches Modified by Oxidation

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RAW starch must be modified by hydrolysis or oxidation before it can be used in certain industrial processes. There are processes in which only oxidized starches can be used.

Hixon (*1*) and co-workers conclude from their work on cornstarch, in which they use bromine as the oxidant, that four types of oxidation may occur:

1. Oxidation of aldehyde groups to carboxyl groups
2. Oxidation of primary alcohol groups to carboxyl groups
3. Oxidation of secondary alcohol to ketone groups
4. Oxidation of glycol to carboxyl groups with concomitant rupture of pyranose ring

Modification both by hydrolysis and by oxidation produces reducing groups, while COOH groups are produced by oxidation only. Therefore, the number of COOH groups is an indication of the degree of oxidation. Modified starches are generally classified according to their viscosities, although viscosity gives no indication of the degree of oxidation. A hydrolyzed starch given a slight oxidation may have the same viscosity as a highly oxidized product.

This laboratory has needed a method whereby the COOH groups in a given starch could be estimated with reasonable accuracy. Three methods have been developed for determining the degree of oxidation of modified starches. The copper method is the most convenient of the titration methods. For routine work the colorimetric method is very satisfactory.

Silver Method

Harris and co-workers (*4*) measured the COOH groups in cellulose by titration with silver *o*-nitrophenolate, using 5 grams of cellulose in equilibrium with 100 cc. of 0.01 molar silver *o*-nitrophenolate. Whereas a value of 6.5 milliequivalents per hundred grams was about the highest they had to measure, in highly oxidized commercial starches 30 milliequivalents per hundred grams are not unusual. If the concentration of silver ions falls below 0.0065 molar, maximum values are not obtained; therefore 1.5 grams of starch per 100 cc. of silver solution was chosen.

PREPARATION OF OXIDIZED STARCHES. Product I. Mix 50 grams of unmodified cornstarch with 100 cc. of water, and into this slurry mix 50 cc. of sodium hypochlorite solution (Chlorox, 5.25 per cent available chlorine). Stir for 3 hours at room temperature, filter, wash, and dry. The oxidant was 5.25 per cent on the weight of the starch.

Product II is prepared in the same way as Product I, except that 2.62 per cent of oxidant on the weight of the starch is used.

Product III is the unmodified starch.

PREPARATION OF SAMPLE. Treat about 35 grams of starch with 150 cc. of sulfuric acid (2.5 per cent by volume) for 2 hours at room temperature with occasional stirring. Transfer to a Büchner funnel and wash with distilled water until the wash water gives no precipitate with silver nitrate solution. Dry below 60° C., transfer to an air-tight container, and determine moisture.

SOLUTIONS. Prepare silver *o*-nitrophenolate by melting 14 grams of C. P. *o*-nitrophenol and adding 14 grams of C. P. silver oxide. This forms a cake which is good for about 10 liters of solution. A saturated solution is almost 0.01 molar.

Prepare and standardize 0.01 molar ammonium thiocyanate (*2*).

Prepare ferric alum indicator (*2*).

Prepare dilute nitric acid by boiling concentrated C. P. nitric acid to free it of lower oxides and diluting 1 to 5 with distilled water.

PROCEDURE. Weigh a 0.75 gram sample and transfer to a 125-cc. stoppered flask. Pipet 50 cc. of silver solution into the flask, stopper, shake well, and set aside overnight. Pipet 25 cc. of silver solution into a 250-cc. flask as a blank.

After about 18 hours, pipet carefully, taking care not to disturb the starch, 25 cc. of the clear supernatant liquid into a 250-cc. flask. Add 5 cc. of dilute nitric acid and 5 cc. of indicator, and titrate with standard ammonium thiocyanate from a 25-cc. buret. Report as milliequivalents per 100 grams of dry starch.

TABLE I. CALCULATIONS FOR PRODUCTS I, II, AND III

Product	1 Dry Wt. of Sample Represented by 25 Cc. of Silver Solution	2 0.00991 *N* NH_4CNS for Blank	3 0.00991 *N* NH_4CNS for Sample	4 Blank minus Sample	5 1.0 *N* Silver Solution	6
	Gram	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Milliequivalents/100 grams*
I	0.353	20.10	11.8	8.3	0.0824	23.3
II	0.360	20.32	16.25	4.07	0.0403	11.2
III	0.362	21.80	20.42	1.38	0.0137	3.8

1. Dry starch represented by 25 cc. of silver solution.
2. Cc. of standard ammonium thiocyanate required for blank.
3. Cc. of standard ammonium thiocyanate required for sample.
4. Column 2 minus Column 3.
5. Column 4 multiplied by normality of standard ammonium thiocyanate solution.
6. Column 5 divided by Column 1 and multiplied by 100.

TABLE II. SILVER METHOD

Product	1 No Acid	2 Treated with Acid
	Milliequivalents per 100 grams	
X_1	4.6	5.1
X_2	4.7	5.2
X_3	4.9	5.7
X_4	4.7	5.1
X_5	5.4	6.0

In commercial starches most of the COOH groups have been neutralized, and the purpose of the dilute sulfuric acid treatment is to convert them back to COOH groups. One may suspect that COOH groups are lost in this purification process. The COOH groups were determined by the silver method on five different batches of the same commercial product (Table II).

The slightly lower values in Column 1 may be due to a small number of metallic ions that are as tightly bound as is silver ion.

PURIFICATION. Wash with distilled water until wash water gives no precipitate with silver nitrate.

Treat with dilute sulfuric acid and wash with distilled water until wash water gives no precipitate with silver nitrate.

Copper Method

SOLUTIONS. To prepare 0.01 molar copper acetate, dissolve 2 grams of C. P. neutral cupric acetate in distilled water and dilute to the mark in a 1-liter flask.

Prepare 0.01 molar sodium thiosulfate and standardize by conventional methods (*3*).

Starch solution. Add 1 gram of pure soluble starch to 100 cc. of boiling water with stirring.

PROCEDURE. Pipet 25 cc. of 0.01 molar copper acetate into a 250-cc. flask and stopper for a blank. Weigh 0.75 gram of sample purified as in the silver method into a 125-cc. flask. Pipet 50 cc. of 0.01 molar copper acetate into a flask, shake, stopper, and set aside overnight. After about 18 hours pipet carefully 25 cc. of the clear supernatant liquid into a 250-cc. flask. Add 5 cc. of 2 *N* acetic acid and enough C. P. potassium iodide to

Table III. Copper Method

Product	1 Dry Wt. of 25 Cc.	2 Blank	3 Sample	4 Blank minus Sample	5 1.0 *M* Copper Solution	6 Valence of Copper X Cc. of 1.0 *M* Solution	7
	Gram	*Cc.*	*Cc.*	*Cc.*			*Milliequivalents per 100 grams*
I	0.353	25.00	20.45	4.55	0.0428	0.0856	24.2
II	0.360	25.15	22.73	2.42	0.0228	0.0456	12.6
III	0.362	25.15	24.70	0.45	0.00428	0.00856	2.4

1. Dry weight of starch represented by 25 cc. of copper solution.
2. Cc. of standard thiosulfate required for 25 cc. of copper solution.
3. Cc. of standard thiosulfate required for 25 cc. of copper solution, from sample.
4. Column 2 minus Column 3.
5. Column 4 times normality of thiosulfate.
6. Column 5 times valence of copper.
7. Column 6 divided by Column 1 and multiplied by 100.

Table IV. Comparison of Values by Copper and Silver Methods

Product	Copper	Silver
	Milliequivalents/100 grams	
I	24.2	23.3
II	12.6	11.2
III	2.4	3.8
A	5.9	6.0
B	9.2	9.3
C	11.6	10.4
D	19.6	18.7
E	21.0	17.6
F	27.8	23.8
G	30.4	28.9
H	2.4	3.5
J	2.4	3.7

dissolve the cuprous iodide formed. Add about 1 cc. of starch solution and titrate with standard thiosulfate. Express as milliequivalents per 100 grams of starch (Table III).

The values obtained by the copper method are slightly higher wherever oxidation is moderate or high (Table IV). This may be due to the higher concentration of copper ions at equilibrium—e. g., twice as many monovalent silver ions as divalent copper ions are taken out of solution for a given number of COOH groups.

Products A, B, C, D, E, F, and G are commercial oxidized cornstarches; H is an unmodified cornstarch; J is a hydrolyzed, thin-boiling cornstarch.

Colorimetric Determination of COOH Groups in Modified Starch

Solutions. Copper acetate, 1 per cent, and potassium ferrocyanide, 1 per cent.

Procedure. Transfer about 1 gram of unknown to a 50-cc. beaker, add 25 cc. of copper solution, stir well, and let stand for 10 minutes. Filter at the pump on a small Büchner funnel. Wash with distilled water until wash water gives no test for copper with potassium ferrocyanide solution. Transfer to a 50-cc. beaker, add 25 cc. of potassium ferrocyanide solution, stir well, and let stand 2 minutes. Filter and wash until wash water is colorless. Dry and compare with standards.

Standards may be prepared by treating known oxidized products as described above.

Acknowledgment

The author is very grateful to Dr. Frieden, chief of the Stein-Hall Technical Department, New York, N. Y., for his helpful suggestions and also for permission to publish this article.

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Colorimetric Method for Phosphates

Modification of A. O. A. C. Molybdenum Blue Method

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A GOOD review of colorimetric methods for phosphates and a discussion of their relative values are given by Woods and Mellon (*3*). All the methods depend on formation of the complex phosphomolybdic acid and a selective reduction of this acid to form a blue compound of unknown composition, generally referred to as molybdenum blue. The color developed is not stable and the results are so variable as to require the use of standards run parallel with the unknown. In spite of the vast amount of work reported, there seems to be no basic study of the reactions involved.

The procedure of the Association of Official Agricultural Chemists (*1*) with slight modification was used for study because of the extreme simplicity of the reagents and method. An excess of an acidic solution of ammonium molybdate is added to the sample to form phosphomolybdic acid, which is then reduced with 0.5 per cent solution of hydroquinone according to the method of Bell and Doisy (*2*). A 20 per cent solution of sodium sulfite is next added before diluting to volume. The modifications were a proportionate increase in the volumes of sample and reagents used and color measurements by means of a Coleman Universal spectrophotometer.

In carrying through this procedure it was observed that the color first developed was green, which became blue on addition of the sodium sulfite. Since the sodium sulfite would alter the pH of the solution, the effect of pH on the color was studied. The percentage transmission–wave length curve of the colored solution was found to be smooth, having but one maximum in the visible range. The maximum point was used to identify the color, as it would correspond to visual identification. The readings for stability of color density were made at the point of maximum transmission at intervals of 3, 10, and 15 minutes after the addition of the reducing agent. A solution of C. P. potassium dihydrogen phosphate was used as the source of phosphate for all tests.

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A preliminary experiment was run using varying amounts of sodium hydroxide in place of the sodium sulfite (Table I). There was a decided change in the position of the transmission maximum, but the density of both colors was found to be variable. The relation of the two colors and their maxima are shown in Figure 1. A gradual shift in the pH was also observed. The decomposition noted was a decided fading in the intensity of the color and the formation of a grayish turbidity.

To cover the pH range in which the color change was observed, the acidity of the ammonium molybdate reagent was changed from 3 *N* to 1 *N* sulfuric acid and varying amounts of 20 per cent sodium succinate were used instead of sodium sulfite, in order to rule out any effect of the sulfite ion. The results are shown in Table II.

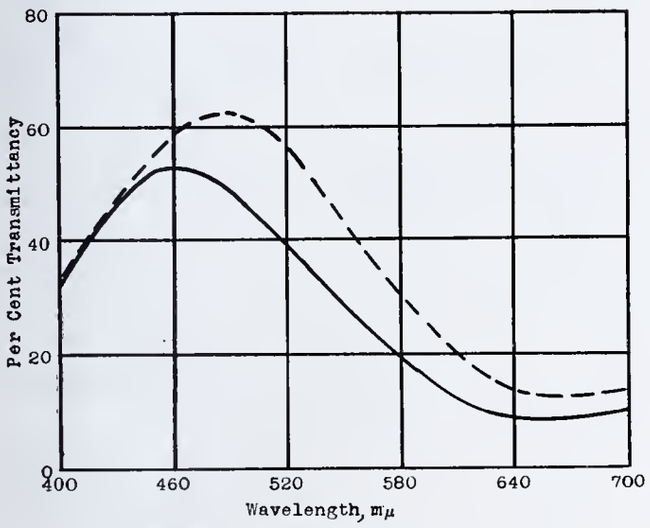

FIGURE 1. PER CENT TRANSMITTANCY–WAVE LENGTH CURVES OF MOLYBDENUM BLUE

At pH 1.3 (broken line) and 4.7 (solid line). Concentration same for both

These findings show that there is a range of color density stability corresponding to a color having a maximum transmission at 460 mμ. The decrease in color density at the highest pH may be explained by the "decomposition" observed at higher pH values. The recorded decrease in color density would therefore be due to a slow decomposition. As the pH is increased, the decrease in color density becomes even more rapid until a point is reached where there is total decomposition, as recorded in Table I.

TABLE I. PRELIMINARY EXPERIMENT

1.0 *N* NaOH *Ml.*	pH	Transmission Maximum *Mμ*
0	1.3	485
1.0	1.6	485
2.0	4.4	460
3.0	Decomposition	

TABLE II. EFFECT OF pH

Sodium Succinate *Ml.*	pH	Transmission Maximum *Mμ*	Change in Color Density
0	1.3	485	Increase
0.20	1.9	485	Increase
0.50	2.5	475	Increase
0.75	3.2	465	Increase
1.00	4.0	460	None
1.50	4.3	460	None
2.00	4.7	460	None
2.25	4.8	460	None
2.75	4.9	460	Decrease

TABLE III. EFFECT OF pH ON BLANKS

Sodium Succinate *Ml.*	Transmission Origina %	Transmission after 4 Hours %
0	98.3	88.5
0.25	98.3	92.9
0.50	98.3	94.2
1.00	98.3	97.8
1.50	98.3	98.0
2.00	98.3	98.3
2.50	98.3	98.3

To determine the reason for the increase in color density at the lower pH values, blanks were run in the same manner as the preceding experiment (Table III). Per cent transmission was referred to distilled water.

Since the only source of molybdenum blue in the experiment is the molybdate, it may be concluded that there is a slow reduction of molybdate in acid solution and that the rate of reaction is reduced to zero when the color having a transmission maximum at 460 mμ is formed.

Modified Procedure

These findings led to the development of a modified procedure.

REAGENTS. Ammonium molybdate solution, 5 grams of ammonium molybdate per 100 ml. of 1 *N* sulfuric acid.

Hydroquinone solution, 0.5 gram of hydroquinone per 100 ml. of distilled water made slightly acid with one drop of concentrated sulfuric acid per 100 ml.

Sodium succinate solution, 20 grams (anhydrous basis) of sodium succinate per 100 ml. of distilled water.

SAMPLE. All the phosphorus should be in the ortho form of the acid and in clear, colorless solution made neutral or slightly acid to litmus (pH 5.0).

PROCEDURE. To a 25-ml. volumetric flask add an aliquot of sample up to 15 ml. containing not more than 0.3 mg. of phosphorus. If less than 10 ml. is used, make the volume up to 10 ml. with distilled water to prevent precipitation of phosphomolybdic acid. Then add the following in order, mixing well after each addition (Mohr pipets may be used): 2 ml. of ammonium molybdate solution, 2 ml. of hydroquinone solution, and 2.5 ml. of sodium succinate solution. Make up to 25 ml. with distilled water and measure the color at 460 mμ within 4 hours, as succinate may crystallize out since the pH is near its isoelectric point.

Each reaction seems to be instantaneous and complete, since delays as long as 15 minutes in the addition of any of the reagents have no effect on the final result, outside of the slow reduction of molybdate before the buffer is added. There is no appreciable change due to this cause for periods up to 5 minutes.

Buffers other than sodium succinate may be used. If a 20 per cent sodium sulfite solution is used, the amount needed in the above procedure is 3 ml. The author selected the succinate buffer since it is more stable than sulfite over long periods of storage.

The effective range of this method is 0 to 0.35 mg. of phosphorus, in which the system follows Beer's law very closely.

Discussion

The author suggests that the reason for the observed color stability is that it occurs in the isoelectric range of both the phosphomolybdate and molybdate where reactivity is at a minimum. If the hydroquinone is added after the buffer, there is no reaction.

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Analysis of Indium Alloys

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IN THE course of investigations conducted in the Metals Research Laboratory on binary indium alloys, it became necessary to do considerable quantitative analyses in order to determine the percentages of the constituents present. As this work required the greatest accuracy consistent with a reasonable time factor per analysis, a thorough investigation of the methods available was conducted.

A careful literature research revealed that very few accurate methods for analysis of indium alloys had been reported. Laboratory tests soon showed which of these could be depended upon to give usable results. As it was desired, in many cases, to have a fairly rapid means of determining the purified indium solution, a combination of several methods was worked out. In general, the methods of alloy analysis reported by Moser and Siegmann (*9*) were found entirely satisfactory and could be utilized to prepare the indium solutions for the potentiometric titration method of Bray and Kirschman (*1*), by means of which a rapid and reasonably accurate determination of indium could be obtained.

Analyses of indium-lead, indium-zinc (*2*, *9*), and indium-aluminum (*9*) alloys were accurate to 0.1 to 0.2 per cent, this degree of accuracy being highly reproducible. In the case of indium-tin alloys, however, no usable method was found which could reproduce results to better than 1 per cent. All available procedures were investigated and several original ones tested by the author, but as yet a high degree of accuracy for indium-tin determinations has not been found. The suggested separations of Nizhnik (*10*) and Erämetsä (*4*), however, seem to offer the best methods for laboratory use.

One of the greatest sources of error encountered in indium alloy analyses was that due to iron, particularly so where titration with potassium ferrocyanide was used (*1*, *6*). Moser's (*9*) method for the removal of iron from indium solutions has proved entirely adequate and rapid, though the equally efficient procedures of Mathers (*7*) and Prichard (*8*) may be employed.

Because of the increased interest in indium and indium alloys, it is hoped that the methods outlined here will be of value to workers in this field.

Procedures

INDIUM-LEAD ALLOYS. Dissolve a 0.1-gram sample of alloy in 20 cc. of aqua regia, and salt out the major portion of the lead with absolute alcohol in an ice-salt bath. Filter off lead chloride, wash with 1 to 1 hydrochloric acid plus alcohol solution, and save for subsequent lead determination. Now collect filtrate and washings, evaporate to dryness, and dissolve residue in 10 cc. of 0.5 *N* hydrochloric acid. Dilute solution to 100 cc., run in hydrogen sulfide, and filter off lead sulfide. Wash with ammonium sulfide solution and add precipitate to lead chloride residue. Adjust acidity to 0.03 *N* with hydrochloric acid, heat to boiling, and run in hydrogen sulfide to precipitate iron sulfide. Filter and wash, collect filtrate and washings, and evaporate solution until all hydrogen sulfide is removed. Dissolve any precipitate with hydrochloric acid, then heat again. Precipitate indium hydroxide with concentrated ammonium hydroxide. To determine gravimetrically merely ignite indium hydroxide and weigh as indium trioxide. If the potentiometric titration with potassium ferrocyanide is used (*1*), dissolve the indium hydroxide in 20 cc. of 0.1 *N* hydrochloric acid and dilute the solution to 250 cc. for titration.

To determine lead in the alloy, convert lead sulfide to lead chloride, add to the first precipitate of lead chloride, and dissolve the entire amount in hot water. Add saturated potassium chromate solution to precipitate lead chromate which is dried and weighed as such.

Table I lists results of some typical analyses.

INDIUM-ZINC ALLOYS. Dissolve a 0.1-gram sample of alloy in 25 cc. of concentrated hydrochloric acid, dilute to 50 cc., and neutralize with concentrated ammonium hydroxide. Add 10 cc. of dioxygen solution and after a few minutes run in sufficient 10 per cent potassium cyanate to make it alkaline to methyl orange (yellow). Heat to boiling, filter, and wash coagulated indium hydroxide immediately. Save filtrate and washings for zinc determination. Dissolve indium hydroxide in 15 cc. of 0.5 *N* hydrochloric acid and dilute to approximately 150 cc. For the best iron separation adjust to 0.03 *N* in hydrochloric acid, and then run in hydrogen sulfide while the solution is hot (90° C.). Filter off ferrous sulfide, and wash precipitate several times with ammonium sulfide solution. Collect filtrate and washings, heat to remove excess hydrogen sulfide, and then evaporate down to a small volume. Add hydrochloric acid to dissolve indium sulfide precipitate, and dilute to 150 cc. Heat to about 60° C. and add 1 to 1 ammonium hydroxide to precipitate indium hydroxide. To determine gravimetrically, ignite indium hydroxide to indium oxide and weigh. For potentiometric titration with potassium ferrocyanide (*1*) dissolve indium hydroxide in 20 cc. of 0.1 *N* hydrochloric acid and dilute to 250 cc.

To determine the zinc, collect filtrate and washings from the indium-zinc separation and add 10 cc. of hydrochloric acid. Place the solution on a hot plate and allow to boil until evaporated down approximately 50 per cent. Now remove it, dilute to 100 cc. with distilled water and precipitate zinc hydroxide with 1 to 1 ammonium hydroxide. Ignite zinc hydroxide and weigh as zinc oxide.

INDIUM-ALUMINUM ALLOYS. Dissolve 0.1-gram sample in 1 to 1 hydrochloric acid, dilute to about 50 cc., and neutralize with ammonium carbonate after adding 2 grams of sulfosalicylic acid. Make the solution neutral to methyl orange and acidify with acetic acid (just slightly acid). The volume of the solution at this point should be between 125 and 150 cc. Run in hydrogen sulfide, filter off indium sulfide precipitate, and wash it, being careful to save all the filtrate and washings for the aluminum determination. Now dissolve indium sulfide precipitate in dilute hydrochloric acid, and dilute so as to adjust acidity to 0.03 *N*. Run hydrogen sulfide into hot solution (90° C.) to precipitate ferrous sulfide, which is filtered off and washed with ammonium sulfide solution. Evaporate filtrate and washings containing indium down nearly to dryness on a hot plate and then add 10 cc. of 1 *N* hydrochloric acid to dissolve the indium sulfide. After solution is complete, add 50 cc. of distilled water and precipitate indium hydroxide with concentrated ammonium hydroxide. Filter off this precipitate, wash,

TABLE I. TYPICAL ANALYSES

Analysis	Sample	Indium Present	Lead Present	Indium Found	Lead Found
	Gram	*Gram*	*Gram*	*Gram*	*Gram*
A	0.1000	0.0960	0.0040	0.09595	0.0041
B	0.1000	0.0960	0.0040	0.09597	0.0039
C	0.1000	0.0960	0.0040	0.09593	0.0038
D	0.1000	0.0798	0.0202	0.07975	0.0203
E	0.1000	0.0798	0.0202	0.07977	0.0200
F	0.1000	0.0798	0.0202	0.07977	0.0201
			Zinc Present		Zinc Found
G	0.1000	0.0972	0.0028	0.09694	0.0027
H	0.1000	0.0972	0.0028	0.09716	0.0024
I	0.1000	0.0972	0.0028	0.09720	0.0026
J	0.1000	0.0754	0.0246	0.07537	0.0244
K	0.1000	0.0754	0.0246	0.07540	0.0246
L	0.1000	0.0754	0.0246	0.07538	0.0242
			Aluminum Present		Aluminum Found
M	0.1000	0.0500	0.0500	0.0498	0.0498
N	0.1000	0.0500	0.0500	0.0498	0.0499
O	0.1000	0.0500	0.0500	0.0499	0.0498
P	0.1000	0.0600	0.0400	0.0599	0.0398
Q	0.1000	0.0600	0.0400	0.0596	0.0396
R	0.1000	0.0600	0.0400	0.0598	0.0399

and ignite to indium oxide for gravimetric determination. For potentiometric titration, dissolve indium hydroxide in dilute hydrochloric acid and dilute the solution so that it is not more than 0.04 *N* in hydrochloric acid.

Determine the aluminum in the usual way by precipitating aluminum hydroxide and igniting to aluminum oxide for weighing gravimetrically.

Indium-Tin Alloys. Treat 0.1 to 0.2 gram of the alloy sample with concentrated nitric acid, and remove the major portion of the tin as metastannic acid. Dissolve this metastannic acid and reprecipitate to assist removal of any occluded indium solution. Now add filtrates and washings from both precipitations, precipitate indium as the hydroxide with 2 *N* sodium hydroxide (*4*), and then redissolve by addition of an excess. Boiling the solution, or addition of ammonium chloride causes reprecipitation of indium hydroxide; tin is not precipitated under these conditions. As this separation is not complete, neutralize the filtrate with hydrochloric acid and boil, causing 0.1 per cent of dissolved material to precipitate. Repeat this procedure two or three times for best separations. Repeated solution and reprecipitation of indium precipitate, however, still left more than 1 per cent of tin which could not be removed from the indium. Determine indium and tin as the oxides.

In attempting to locate a suitable means of separation of indium and tin for accurate quantitative analyses, many types of separation were studied and tested. The volumetric determination of tin (*11*) with iodine in the presence of indium gave poor results, as did the determination of indium potentiometrically with potassium after distillation of stannic chloride from indium solution. The precipitation of indium by means of 8-hydroxyquinoline (*5*) and also by 8-oxychinolate (*3*) likewise proved unsatisfactory.

Summary

Available methods for indium alloy analysis were investigated and the most accurate and efficient chosen by means of analytical tests. For rapid determinations of indium, the potentiometric titration of Bray and Kirschman using potassium ferrocyanide was used.

Satisfactory procedures for indium-lead, indium-zinc, and indium-aluminum were determined, but no precise method for indium-tin alloys could be found which permitted a determination of greater than 1 per cent accuracy. In the case of indium-lead, indium-zinc, and indium-aluminum alloys, however, an accuracy of 0.1 to 0.2 per cent was attainable.

Acknowledgment

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Viscosity Determination of Polymer Solutions

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A new set of bubble tubes extends the Gardner-Holdt series to viscosities of 1000 poises, and thus adapts this rapid method to the measurement of viscosities of high polymers in volatile solvents. The eleven tubes in the set are numbered from $^1/_2$ to 10, and cover the range from 50 to 1000 poises in steps of 100. To minimize effect of temperature, each tube is filled with a mixture of a 105 viscosity-index lubricating oil and a polybutene of 12,000 molecular weight. Diameter of tubes is 21.3 mm., double that of the regular Gardner-Holdt series. Length is the same. These dimensions make for ease in filling, and save 80 per cent of the measuring time.

IN CONNECTION with recent work (*2*) requiring the rapid and precise determination of polymer solution viscosities, the necessary accuracy was found obtainable with the Gardner-Holdt rising air bubble viscometer (*1*). Results are duplicable, since no solvent is lost, as in using the majority of other viscometers. Further, by inverting the standard tubes at their designated temperature (25° C.) alongside a water bath maintained at a different temperature, and containing the sample tubes arranged on a hinged mounting, it is possible to take measurements at a series of temperatures with speed and with an accuracy sufficient for most purposes.

The highest viscosity available in a closely related Gardner-Holdt series is the Z6 tube of the "heavy bodied series" (148 poises), and hence the choice of polymers and their solution concentrations was delimited at that value. It was also found difficult to fill sample tubes of 10.65-mm. inside diameter with highly viscous solutions of polymers in volatile solvents, without losing appreciable solvent. In addition, were it attempted to extend the viscosity range with the small diameter tubes, the time of bubble ascent would become excessive (Figure 1).

A series of four experimental tubes, of the same length (11.4 cm.) but of progressively larger diameter, was accordingly constructed from regular Pyrex tubing. Carefully made flat bottoms permitted standing perfectly upright on a horizontal surface, without the aid of a holder. Times of bubble

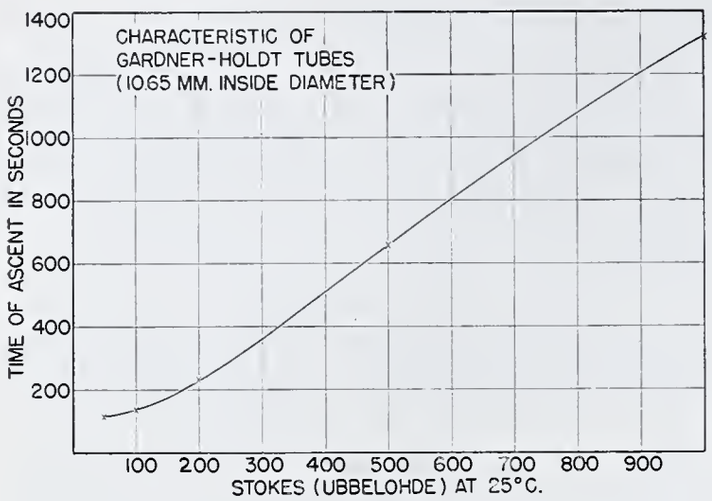

FIGURE 1

ascent, from the moment of inversion to the incipient flattening of the bubble against the cork, are shown in Figure 2.

Tube Size and Contents

It was found that the tube of largest inside diameter—21.3 mm. (double the Gardner-Holdt size)—averaged approximately one fifth the time for bubble ascent throughout the range (cf. Figures 1 and 3), the 1000-poise tube requiring 4 minutes and 20 seconds as compared with Z6 (148 poises) 3 minutes and 8 seconds.

Eleven tubes were chosen to make up the set. Ten cover viscosities, in steps of 100 stokes, from 100 to 1000, and are numbered 1 to 10. Tube 1/2 has 50 stokes' viscosity. Table I shows the values as determined in a No. 4 Ubbelohde tube at 25° C.

TABLE I. BUBBLE ASCENT VALUES

Tube No.	Ubbelohde Stokes at 25° C.	Bubble Ascent, Seconds	Tube No.	Ubbelohde Stokes at 25° C.	Bubble Ascent, Seconds
1/2	50.1	20	6	600.1	153
1	100.1	30	7	700.5	188
2	200.0	44	8	800.8	210
3	299.4	67	9	901.1	239
4	400.8	98	10	1002.5	260
5	499.7	129			

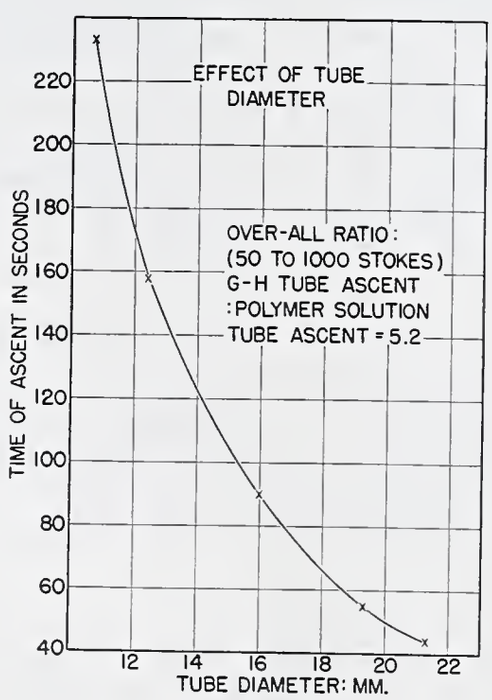

FIGURE 2

Since the two ingredients used to make the blends have respective densities at 25° C. of 0.93 for the 12,000 molecular weight polybutene, and 0.87 for the 105 viscosity-index lubricating oil, stokes and poises are herein used interchangeably. (Gardner-Holdt "poises" refer to the petroleum oils contained in the standards tubes. Since, however, most varnishes and resin cuts possess similar densities at 25° C., no great error is involved.)

Relative bubble dimensions, in the present eleven-tube set, correspond to those in the Gardner-Holdt "varnish series". Bubble height was chosen at 35 mm., providing a length approximately twice the diameter. When three tubes were "filled" with the 200-stoke blend, times of ascent for bubbles respectively 42, 35, and 28 mm. long were 41, 43, and 44 seconds. Hence, as stated in the directions for using the Gardner-Holdt viscometer, "slight variations of the bubble size do not affect the determination, provided the length of the bubble is greater than its width".

To test the relative accuracy of the set, four solutions of 80,000 molecular weight polybutene were made in ordinary rubber solvent, with concentrations of 140, 160, 180, and 200 grams per liter. Results are plotted in Figure 3.

It appears that 20 to 40 per cent solutions of 12,000 molecular weight polybutene in light petroleum lubricating oils behave substantially as Newtonian liquids at such low rates of shear as occur in a rising air bubble viscometer, since no suggestion of "tail" is visible at the bottom of the rising bubble in any tube in the set. Instead, the bottom is as rounded as the top, exactly as in the bubble in a straight petroleum bright stock.

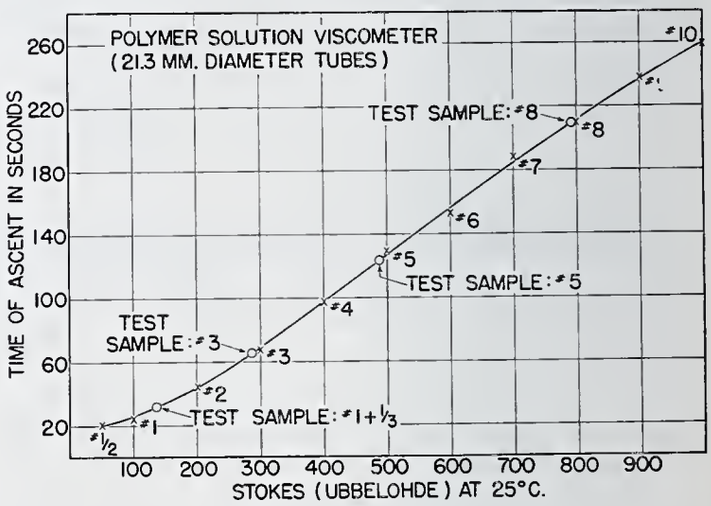

FIGURE 3

It is believed that choosing blends of 12,000 molecular weight polybutene and a high viscosity-index straight petroleum oil provides for minimum temperature effects when measuring viscosities of polymer solutions.

Acknowledgment

Opportunity is taken to thank W. A. Schaefer, of the Esso Laboratories, who constructed the tubes.

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Measuring the Concentration of Dissolved Oxygen in Dairy Products

A Voltammetric Method

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A STUDY of the oxidative reactions in dairy products has created a demand for an accurate and rapid method of measuring the concentration of dissolved oxygen. Recently Sharp *et al.* (*9*) devised a method in which the amount of ascorbic acid oxidized in milk under the influence of ascorbic acid oxidase is correlated to the amount of dissolved oxygen present. This produces excellent results but is a "spot" method and does not give a continuous record of the oxygen concentration over a period of time. The latter feature is desirable in studying rates of oxidation reactions and perhaps in controlling the concentration of oxygen in manufactured or processed products.

The use of the dropping mercury electrode in analytical chemistry was developed first by Heyrovský (*2*). Vitek (*10*), the first worker to apply the method to oxygen analysis, reported an accuracy of ±2 per cent when the concentration of oxygen in water varied between 0.04 p. p. m. and saturation. The method is based essentially on the measurement of the current produced by the reduction of oxygen when a potential is imposed on the dropping mercury electrode at a voltage above the oxygen decomposition potential. The amount of current produced depends on the rate of oxygen diffusion to the mercury surface and this, in turn, depends on the concentration of oxygen. The dropping mercury electrode method has been applied to the determination of dissolved oxygen in biological fluids, water, and sewage (*1*, *4*, *7*, *8*). The usual complete current-voltage curve as obtained from a polarograph is not necessary for the quantitative determination of dissolved oxygen, as has been shown by successful measurements of oxygen concentration by the deflections of a galvanomcter at a single potential (*1*, *4*, *7*). Petering and Daniels (*8*) made the measurements by determining the difference in galvanometer deflections at two different potentials.

Apparatus

The apparatus used in this study is described by Ingols (*3*) in his paper dealing with dissolved oxygen in sewage. The light reflected from a sensitive mirror galvanometer is focused on a photoelectric cell and the current thus produced is amplified and recorded by a Type B Cambridge thread recorder. A saturated calomel half-cell (Leeds & Northrup No. 7724) was used as the reference electrode. Since the measurements of dissolved oxygen were made at temperatures near 0° C., the saturated potassium chloride solution used in the electrode was held at the same temperatures for several days to ensure solution equilibrium. When not in use, the calomel half-cell was immersed in saturated potassium chloride solution and stored at the low temperatures. These precautions prevent equilibrium disturbances due to temperature changes.

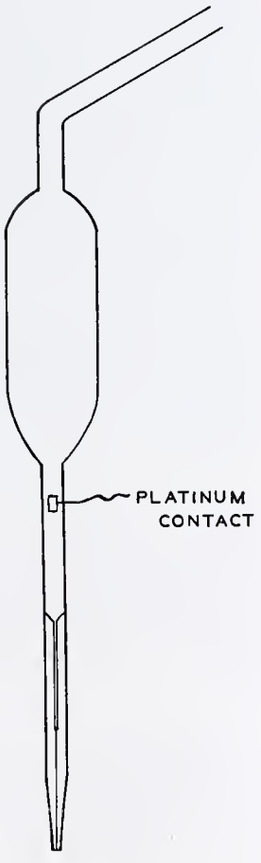

FIGURE 1. MERCURY RESERVOIR AND MICROPIPET FOR DROPPING MERCURY ELECTRODE

Success in the use of the method is largely dependent on the preparation and maintenance of the dropping mercury electrode. Uniform and regular mercury drops are essential for accurate and reproducible results. The mercury must be clean, since dirt or oil readily clogs the small capillary through which the mercury flows. If rubber connections are used between the mercury reservoir and the pipet, they must be scrupulously clean and should be short. Rubber connections may be eliminated by sealing the microcapillary tip directly to the glass mercury reservoir.

The mercury reservoir and the microcapillary tip used in these studies are shown in Figure 1. The reservoir consists of a 50-ml. volumetric pipet, to which is sealed the microcapillary tip, drawn to the proper dimension. Electrical contact to the mercury is made by sealing a platinum wire into the stem of the reservoir. The bore of the microcapillary tip should not be smaller than 50 microns and preferably near 60 to 70 microns. The microcapillary should be 2 to 3 cm. long.

The mercury should form a drop every 1.5 to 2.5 seconds. The drop rate may be controlled by means of a mercury-leveling bulb and a manometer (*6*). Kolthoff and Laitinen (*5*) have described a dropping electrode, with a constant head of mercury, which can be used successfully.

Long use of the mercury cathode in milk may be assured by proper construction of the pipet, by using thoroughly cleaned mercury, and by observing the following precautions in the manipulation of the electrode:

Start the flow of mercury drops before the electrode is inserted into the fluid to be tested.

While the drops of mercury are still falling, remove the electrode from the fluid being tested and swab the capillary tip with water and finally with ether.

Stop the flow of mercury only after the capillary rests in a pool of mercury or in distilled water.

Application of the Method to Milk

The milk samples should be collected in such a manner that no further absorption of oxygen occurs. This can be done satisfactorily by allowing the milk to overflow a wide-mouthed sample bottle which is subsequently closed with a rubber stopper fitted with a small glass tube and a short length of rubber tubing. A pinchcock is used to close the rubber tube as soon as the stopper is inserted into the sample bottle. This procedure prevents the trapping of air in the bottle. Brown glass bottles are desirable for eliminating the influence of direct or diffused light on oxidation reactions that may occur in milk. The sample is immersed in ice water, so as to minimize consumption of oxygen by bacterial growth or chemical reaction. The eventual oxygen determination is made at this temperature, which is easily kept constant, an essential precaution during the determination. Determinations at higher temperatures may be made, but the instrument must be standardized for any given temperature.

The source of current for the instrument used in these studies is the ordinary 110 to 120-volt alternating current. The current should be turned on and the instrument allowed to reach equilibrium before determinations are made. The recorder is then adjusted to zero by the zero point adjustor, the flow of mercury from the pipet is started by raising the mercury-leveling

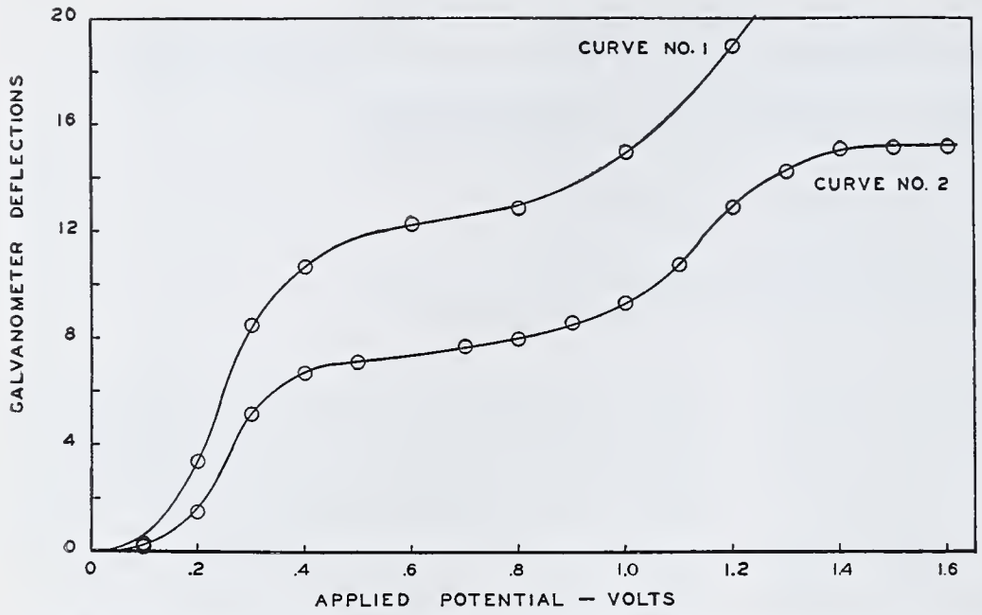

FIGURE 2. CURRENT-VOLTAGE CURVES IN MILK SATURATED WITH ATMOSPHERIC OXYGEN AT 1° C.

Curve 1 determined at full galvanometer sensitivity. Curve 2 determined at 2/5 galvanometer sensitivity.

TABLE I. RESIDUAL CURRENT OF MILK WHEN OXYGEN IS REMOVED BY YEAST

Voltage	Galvanometer Deflections 1	2	Av.
0.8	0.40	0.30	0.35
1.0	0.45	0.45	0.45
1.2	0.50	0.55	0.53
1.4	0.80	1.00	0.90

bulb, and the calomel half-cell and the dropping electrode are inserted in the milk. The tip of the dropping electrode should extend at least 3 or 4 cm. below the surface, so that diffusion of oxygen from the air will not interfere. The drop rate of the mercury is adjusted by lowering the leveling bulb, so that a drop falls every 1.5 seconds. The slight deflection of the galvanometer with the formation of each drop provides a means of measuring the rate with a stop watch. Current equilibrium is reached in about 30 seconds. The galvanometer deflection may be noted or a continuous record may be imposed on the drum recorder. The recorder marks the galvanometer deflection at minute intervals.

The galvanometer deflection due to the residual current for milk is small but, nevertheless, for highly accurate results, it must be determined and subtracted from the total deflection. The amount of the residual current may be determined by removing all the oxygen from milk by physical means, by chemical reactions, or by microbiological methods. In these studies both bacterial cultures and yeast were used satisfactorily and with identical results. The results obtained with yeast at different voltages are shown in Table I.

Repeated determinations of the residual current on different samples of milk showed that the current is small and does not vary significantly with different milks. For the routine analysis of oxygen in milk, therefore, it is not necessary to determine the residual current on each sample. It is essential, however, that the value be determined anew if there is a change in the galvanometer or in the electrical circuit.

The presence of metallic ions, such as copper, iron, etc., affects the magnitude of the residual current. Copper and iron, the two most likely contaminants, are exceedingly low in normal milk but may be dissolved into processed milk from the equipment used in processing. If they are known to be present in quantities above the normal or if there is doubt about their presence in abnormal amounts, the residual current should be checked, especially when making studies of their influence on oxidation reactions.

Vitek (*10*) and others (*6*) reported that the relationship between the deflections of a galvanometer and the oxygen concentration is linear. Petering and Daniels (*8*) and Ingols (*4*) found a linear relationship only at a potential of 1 volt. At other potentials maxima in the current-voltage curve alter this relationship. These maxima are due to the electrostatic adsorption of reducible molecules on the surface of the mercury which cause a current to flow that is greater than the limiting current set by the diffusion rate.

Two current-voltage curves were determined in milk which had been saturated with atmospheric oxygen (Figure 2). Curve 1 was determined at full galvanometer sensitivity and curve 2 at 2/5 sensitivity. The sensitivity of the galvanometer was reduced for the second curve, so that the deflections at higher potentials could be measured, since the maximum deflection which could be recorded on this instrument was 20.

It is apparent that the current-voltage curve of milk does not show maxima; the relation between galvanometer deflections and oxygen concentration at any given voltage, therefore, should be linear. The curve obtained at 2/5 galvanometer sensitivity is almost identical with the one reported by Kolthoff and Laitinen (*5*) for oxygen in 0.1 *N* potassium chloride solution which contained a trace of methyl red. The curve has two waves, the first corresponding to the reduction of oxygen to hydrogen peroxide and the second to the reduction of oxygen to the hydroxyl ion. Since twice as many electrons are involved, the height of the second wave is twice the height of the first wave.

Since the method of analysis is entirely empirical, the instrument must be standardized with milks containing known concentrations of oxygen. Proof that the galvanometer deflections are proportional to the oxygen concentration was obtained by determining the deflections for known oxygen concentrations at several different potentials. This was done by mixing definite proportions of oxygen-free milk and milk saturated with air according to the following technique:

One quart of milk was de-oxygenated with yeast and cooled to 1° C. in an ice bath to retard further growth. A second quart of milk, held at 1° C., was saturated with atmospheric oxygen by forcing air through a gas diffuser bulb into the milk for 15 minutes. The two milks were mixed in various definite proportions by a procedure which avoided further appreciable absorption of oxygen from the air. The galvanometer deflections were determined immediately for each mixture at three different voltages and with the galvanometer at full sensitivity.

The graphs in Figure 3 show that the relationship between the concentrations of dissolved oxygen and magnitude of the galvanometer deflections is linear. All three series of points extrapolate to zero. The corrected galvanometer deflections, therefore, are directly proportional to the oxygen concentration. Since this is true, the concentration of oxygen in milk may be determined at a potential ranging from 0.8 to 1.2 volts. A voltage of 1.2 was chosen for making

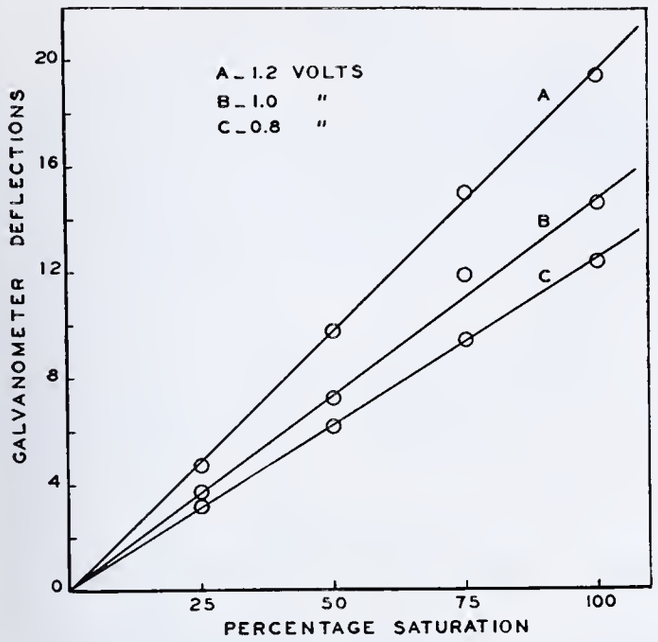

FIGURE 3. RELATIONSHIP BETWEEN THE CONCENTRATION OF DISSOLVED OXYGEN IN MILK AND GALVANOMETER DEFLECTIONS

TABLE II. CONCENTRATION OF OXYGEN AND CORRESPONDING GALVANOMETER DEFLECTIONS IN MILK SATURATED WITH AIR AT 1° C.

No.	Oxygen as P. p. m., C_1	Galvanometer Deflection,[a] D_a
1	11.28[b]	19.4[c]
2	11.27[b]	19.6[c]
3	10.97[b]	19.3[c]
Av.	11.14	19.4

[a] Not corrected for residual current.
[b] Average of four determinations.
[c] Average of seven determinations.

measurements with the instrument used in these studies because it permitted the use of the full scale on the drum recorder.

In order to convert galvanometer deflections to concentration of oxygen it was necessary to ascertain the oxygen content of milk at a definite point. This was done most conveniently by saturating milk with air at some given temperature, measuring the galvanometer deflection, and determining the concentration of oxygen by some suitable method. Sharp's (*9*) method was used for this purpose. Table II shows the concentration of oxygen and the corresponding galvanometer deflections in milk saturated with air at 1° C. Once the corresponding values for a given temperature are established, it is not necessary to repeat the chemical determination.

The concentration of oxygen in milk corresponding to any determined galvanometer deflection may be calculated by applying the formula

$$\frac{D_u - D_0}{D_a - D_0} \times C_1 = C_2$$

in which D_a is the galvanometer deflection corresponding to the known oxygen concentration, C_1

D_u is the galvanometer deflection of the unknown sample of milk

D_0 is the galvanometer deflection due to the residual current

C_2 is the concentration of oxygen as p. p. m. in the unknown sample of milk

The following is an example of the calculations involved in the determination of oxygen in a sample of milk in which $D_a = 19.4$, $D_u = 10.5$, $D_0 = 0.5$, and $C_1 = 11.14$.

$$\frac{10.5 - 0.5}{19.4 - 0.5} \times 11.4 = 5.88$$

Thus, the milk contains 5.88 p. p. m. of dissolved oxygen.

D_a has been determined on a large number of air-saturated samples of whole milk, skim milk, cream, and sour milk, and has been found the same in all instances. This indicates that the method determines the concentration of dissolved oxygen only in the aqueous phase, which apparently is not influenced by the amount of fat or serum solids. Consequently, the concentration of solids must be considered in calculating the dissolved oxygen in any given dairy product. Since the total solids content of normal mixed dairy herd milk does not vary a great deal, an average value can be used for all milks without introducing a significant error in the determination. In other dairy products that vary considerably, the total solids must be determined in order to obtain the correct concentration of oxygen in the whole product.

The method has been used for determining the dissolved oxygen in a number of commercial milk samples. The samples were collected, cooled in an ice bath to 1° C., and the determinations made immediately thereafter. Each sample was then saturated with air at 1° C. and oxygen determinations were made again. The results are shown in Table III.

Referring to the data in Table III, the mean galvanometer deflection in the milk samples saturated with atmospheric oxygen is 19.46 with a probable error of ±0.05. The mean concentration of dissolved oxygen is 11.17 p. p. m. with a probable error of ±0.03. The standard deviations are, respectively, 0.30 and 0.18. These mean values agree well with those shown in Table II.

Conclusions

The dropping mercury electrode method of measuring the concentration of dissolved oxygen has been applied successfully to milk.

The relationship between the concentration of dissolved

TABLE III. DISSOLVED OXYGEN CONTENT OF COMMERCIAL MILK SAMPLES

Description of Sample	Oxygen Content Before saturation A	Before saturation B	After saturation A	After saturation B
	P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*
Certified, raw	11.0	6.18	19.5	11.20
Certified, pasteurized	10.7	6.00	19.3	11.08
Certified, pasteurized, homogenized, vitamin D	13.2	7.48	19.9	11.43
Guernsey, grade A, raw	10.3	7.21	19.2	11.02
Guernsey, grade A, pasteurized	12.8	7.24	19.3	11.08
Guernsey, grade A, pasteurized	11.0	6.18	19.1	10.96
Grade B, pasteurized	13.8	7.83	19.8	11.38
Grade B, pasteurized, paper bottle	15.9	9.07	20.0	11.49
Pasteurized, homogenized, vitamin D	12.5	7.07	19.9	11.44
Grade A, pasteurized, homogenized, vitamin D	12.4	7.01	19.3	11.08
Grade A, pasteurized	13.3	7.54	19.8	11.38
Grade B, pasteurized	11.8	6.65	19.0	10.90
Grade B, pasteurized	13.1	7.42	19.7	11.32
Guernsey, pasteurized	12.8	7.24	19.2	11.02
Raw	12.2	6.89	19.2	11.02
Grade A, pasteurized	14.0	7.95	19.3	11.08
Jersey, pasteurized	13.9	7.89	19.3	11.08
Raw	12.0	6.77	19.1	10.96
Guernsey, pasteurized	10.8	6.06	19.3	11.08
Grade A, pasteurized	10.3	5.77	19.8	11.38
Grade B, pasteurized	13.2	7.48	19.6	11.26

(A = galvanometer deflection, B = concentration of oxygen in p. p. m. Residual current was 0.53 galvanometer deflection.)

oxygen in milk and the magnitude of the galvanometer deflections is linear.

The concentration of oxygen in milk may be determined at a potential ranging from 0.8 to 1.2 volts.

The slight variations in the solids content of normal milks do not introduce significant errors.

The statistical analyses of the data on the air-saturated milks show that the method is highly reliable.

Acknowledgment

The authors wish to express their appreciation to the Cambridge Instrument Co., New York, N. Y., for the loan of the instruments used in this study, and to the Federal Work Projects Administration (O. P. 65-1-22-477) for laboratory assistance.

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Determination of Calcium in the Presence of Magnesium by Standard Soap Solution

A Rapid Titration Method

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Calcium in water can be determined in the presence of magnesium by direct titration with Clark's soap solution with the addition of ammonium chloride and pH adjustment. Ions common to industrial waters do not interfere. The accuracy and rapidity of this method are equivalent to those of the usual soap titration for total hardness.

INDUSTRY has needed a rapid method for the determination of calcium in the presence of magnesium, but a review of the literature failed to yield a simple, convenient, and yet satisfactory method that would give results comparable to those encountered in the total hardness determination recommended by the American Public Health Association (*1*).

Froboese (*5*) stated that a separate determination of magnesium could be made in the presence of calcium by precipitation of the calcium as calcium oxalate and titration of the magnesium by potassium palmitate in the presence of the calcium oxalate precipitate. Durovdier (*4*) separated the calcium oxalate by filtration before titration of the magnesium and claimed a sensitivity of 1.4 mg. of calcium oxide or 1 mg. of magnesium oxide per liter. Using either procedure the calcium content can be obtained indirectly by subtracting the magnesium content from the total hardness. Breazeale (*2*) titrated calcium in the presence of magnesium by employing alkaline tartrate which converts the magnesium into a complex that does not react with soap. In Langelier's method (*7*) the sample is first neutralized to methyl orange and then the carbon dioxide is removed either by boiling or in his mechanical apparatus. The magnesium is precipitated by the addition of carbonate-free sodium hydroxide, and the calcium is titrated with standard potassium oleate solution in the motorized unit.

Of the above methods, none had both the accuracy and speed desired for the authors' work.

Ammonium chloride has long been known in the gravimetric determination of calcium and magnesium as a reagent for eliminating the interference of magnesium during the calcium precipitation with ammonium oxalate.

The procedure described in this paper makes use of the fact that magnesium in the presence of ammonium chloride and within prescribed pH ranges will not react with a standard soap solution, which can therefore be used to titrate calcium directly.

Reagents. Clark's soap solution (*1*, *3*), 1 ml. = 1 mg. $CaCO_3$ equivalent. Ammonium chloride (reagent grade), 10% solution. Phenolphthalein, 1% solution [50% alcoholic, neutralized]. Sodium hydroxide, 0.77 *N* (carbonate free, *6*). Calcium chloride, 1 ml. = 1.084 mg. $CaCO_3$ equivalent. Magnesium chloride, 1 ml. = 2.239 mg. $CaCO_3$ equivalent.

Experimental

Standard solutions of calcium chloride and magnesium chloride were prepared gravimetrically, and from them, seven stock solutions of varying calcium and magnesium content were made. Six 50-ml. samples were taken from each stock solution and placed in 0.24-liter (8 ounces) narrow-necked glass-stoppered shaker bottles and 1 ml. of the ammonium chloride was added to each one. To the samples sufficient sodium hydroxide was added to bring the pH values to 8.0, 9.0, 10.0, 11.0, 11.7, and 12.0, respectively, using a Beckman pH-meter. Correcting for the sodium ion, the values of the last three are actually 11.1, 12.1, and 12.7. The quantity of sodium hydroxide necessary to adjust to these pH values was determined on separate samples.

Standard soap solution was then added from a buret in small portions, approximately 0.2 ml. at a time, and after each addition the bottle was shaken vigorously and placed in a horizontal position. The end point was considered to have been reached when a lather was formed which covered the entire surface of the liquid and lasted for one minute. The number of milliliters of soap solution required in each titration was recorded in Table I.

Table I reveals that a pH of 11.7 is optimum for this procedure. When magnesium is present in the absence of calcium, a minimum pH value of 11.7 is necessary for the titration value to agree with the blank of 0.20 ml. required for distilled water. This pH value also agrees with the fact that in the other calcium and magnesium concentrations a pH value of 11.7 was necessary to obtain a constant relationship between the milligrams of calcium added and the milliliters of soap solution required minus the 0.2-ml. blank, as shown in the last column of Table I.

TABLE I. EFFECT OF pH

(Each sample contains 1 ml. of 10% NH_4Cl)

Ca Added as $CaCO_3$	Mg Added as $CaCO_3$	0.77 *N* NaOH Added	pH	Soap	Mg. Ca Added as $CaCO_3$ (Equivalent) Ml. of Soap minus Blank Under pH 11.7	At pH 11.7 and over
Mg.	*Mg.*	*Ml.*		*Ml.*		
0	0	0.10	8.0	0.20	..	..
0	0	0.80	9.0	0.20	..	..
0	0	2.10	10.0	0.20	..	..
0	0	2.50	11.0	0.20	..	..
0	0	3.30	11.7	0.20	..	..
0	0	5.15	12.0	0.20	..	..
0	5.42	0.10	8.0	6.70	..	..
0	5.42	0.75	9.0	6.15	..	..
0	5.42	2.10	10.0	5.50	..	..
0	5.42	2.60	11.0	1.30	..	..
0	5.42	3.35	11.7	0.20	..	..
0	5.42	5.10	12.0	0.20	..	..
1.08	4.33	0.10	8.0	3.60	0.32	..
1.08	4.33	0.90	9.0	4.90	0.23	..
1.08	4.33	2.15	10.0	4.95	0.23	..
1.08	4.33	2.60	11.0	1.35	0.94	..
1.08	4.33	3.30	11.7	1.20	..	1.09
1.08	4.33	5.10	12.0	1.15	..	1.14
1.62	3.79	0.10	8.0	4.70	0.36	..
1.62	3.79	0.80	9.0	2.00	0.90	..
1.62	3.79	2.05	10.0	1.90	0.96	..
1.62	3.79	2.50	11.0	2.40	0.74	..
1.62	3.79	3.35	11.7	1.65	..	1.12
1.62	3.79	5.15	12.0	1.55	..	1.20
2.71	2.71	0.10	8.0	5.15	0.55	..
2.71	2.71	0.90	9.0	2.80	1.04	..
2.71	2.71	2.20	10.0	2.75	1.06	..
2.71	2.71	2.70	11.0	2.80	1.04	..
2.71	2.71	3.40	11.7	2.60	..	1.13
2.71	2.71	5.10	12.0	2.50	..	1.17
3.79	1.62	0.10	8.0	3.95	1.01	..
3.79	1.62	0.90	9.0	3.85	1.04	..
3.79	1.62	2.25	10.0	3.75	1.07	..
3.79	1.62	2.70	11.0	3.70	1.09	..
3.79	1.62	3.30	11.7	3.50	..	1.15
3.79	1.62	5.20	12.0	3.50	..	1.15
4.33	1.08	0.10	8.0	4.50	1.01	..
4.33	1.08	0.80	9.0	4.15	1.10	..
4.33	1.08	2.10	10.0	4.10	1.11	..
4.33	1.08	2.50	11.0	4.15	1.10	..
4.33	1.08	3.20	11.7	4.10	..	1.11
4.33	1.08	5.10	12.0	3.90	..	1.17
5.42	0	0.10	8.0	5.20	1.09	..
5.42	0	0.85	9.0	5.00	1.13	..
5.42	0	2.10	10.0	4.90	1.15	..
5.42	0	2.55	11.0	4.95	1.14	..
5.42	0	3.50	11.7	4.90	..	1.15
5.42	0	5.20	12.0	5.05	..	1.12

This being true, a curve was plotted (Figure 1) using the values obtained from the samples titrated at a pH value of 11.7, the total milliliters of soap solution required being plotted against the milligrams of calcium present. This gave a linear function whose equation is

$$x = k(y - 0.2 \text{ ml.})$$

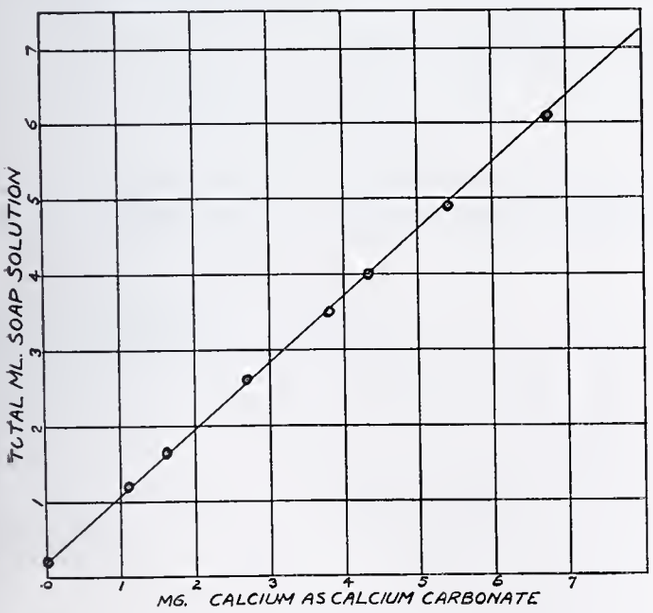

FIGURE 1

where x = mg. of calcium present
y = ml. of soap solution titrated
k = constant
0.2 ml. = blank required for distilled water

The constant, k, for the above curve was found to be 1.14.

A variation of the amount of ammonium chloride added to the sample was found to have little effect on the results, as shown in Table II. However, as the concentrations of the ammonium chloride were increased the resulting lather of the end point was not so definite as with lower concentrations. In the cases where no ammonium chloride was added to the samples, difficulty was encountered in obtaining consistent end points.

Procedure

A 50-ml. sample of water is measured into a 0.24-liter (8-ounce) glass-stoppered bottle and neutralized to the phenolphthalein end point, and 1.0 ml. of the ammonium chloride reagent and 2.7 ml. of 1 *N* sodium hydroxide are added, bringing the pH of the resulting solution to approximately 11.7. A variation in the pH value of the solution before titration, from 11.7 to 12.0, is allowable without affecting the stability of the end point or the accuracy of the determination.

The soap solution is then added in small portions from a buret. The bottle is shaken vigorously after each addition, and placed

TABLE II. EFFECT OF VARYING AMMONIUM CHLORIDE CONTENT

[Each sample adjusted to pH 11.7 (before titration) with sodium hydroxide]

Present Ca as $CaCO_3$	Present Mg as $CaCO_3$	10% NH_4Cl Added	Ca as $CaCO_3$ Found from Figure 1
Mg.	*Mg.*	*Ml.*	*Mg.*
5.42	0	0	6.00
5.42	0	1	5.35
5.42	0	2	5.45
5.42	0	3	5.40
5.42	0	5	5.35
4.33	1.08	0	4.55
4.33	1.08	1	4.25
4.33	1.08	2	4.30
4.33	1.08	3	4.25
4.33	1.08	5	4.30
3.79	1.62	0	4.00
3.79	1.62	1	3.75
3.79	1.62	2	3.80
3.79	1.62	3	3.85
3.79	1.62	5	3.85
2.71	2.71	0	3.00
2.71	2.71	1	2.75
2.71	2.71	2	2.75
2.71	2.71	3	2.75
2.71	2.71	5	2.80
1.62	3.79	0	2.05
1.62	3.79	1	1.70
1.62	3.79	2	1.70
1.62	3.79	3	1.70
1.62	3.79	5	1.75
1.08	4.33	0	1.40
1.08	4.33	1	1.20
1.08	4.33	2	1.25
1.08	4.33	3	1.25
1.08	4.33	5	1.20
0	5.42	0	0.45
0	5.42	1	0
0	5.42	2	0
0	5.42	3	0
0	5.42	5	0

TABLE III. EFFECT OF VARIOUS IONS

Present Ca as $CaCO_3$	Present Mg as $CaCO_3$	Ions Present SO_4^{--}	Ions Present SiO_2	Ions Present HCO_3^-	Ions Present Fe^{+++}	Ca as $CaCO_3$ Found
P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
71	31	50	...	...	..	71
71	31	100	...	...	..	71
71	31	200	...	...	..	73
71	31	500	...	...	..	72
71	31	1000	...	...	..	70
71	31	..	5	...	..	73
71	31	..	25	...	..	73
71	31	..	50	...	..	73
71	31	..	100	...	..	71
71	31	..	...	50	..	73
71	31	..	...	100	..	71
71	31	..	...	200	..	71
71	31	..	...	500	..	70
71	31	..	...	...	5	72
71	31	..	...	...	10	71
71	31	..	...	...	20	70
71	31	..	...	...	50	70

on its side, and the formation of a lather is observed. This procedure is continued until a lather is formed which covers the entire surface of the liquid and lasts for 1 minute. If the test requires more than 7 ml. of soap solution, an aliquot portion is diluted to 50 ml. with distilled water so that the final end point will be less than 7 ml.

Calculation of Results

The calcium content of the sample as p. p. m. of calcium carbonate is obtained from the formula

$$[\text{Ml. of soap (titrated)} - 0.20 \text{ ml.}] \times \frac{1000 \times 1.14}{\text{No. of ml. of sample}} = \text{p. p. m. of Ca as } CaCO_3$$

Effect of Ions

In order to determine whether other ions usually found in industrial water interfere with the test, various amounts of silicate, sulfate, bicarbonate, and iron were added to different samples; they did not noticeably affect the accuracy of the method, as shown in Table III. Since ammonium chloride is added in the determination, chloride can also be regarded as noninterfering.

Industrial and Synthetic Waters

A series of synthetic samples was analyzed by several different operators (Table IV). The maximum error was small and on a par with the standard soap method.

A list of industrial waters that have been analyzed is given in Table V, showing that good accuracy can be expected from this method.

Conclusions

This method gives accurate results for calcium in the presence of magnesium, and is as easily executed as the standard soap method. It is not affected by normal concentrations of ions found in industrial waters. The difference between a total hardness determination by the usual Clark method and by the method described reveals the approximate hardness due to magnesium. It is therefore possible to determine distribution of hardness in terms of calcium and magnesium by this new modified method. Its application to boiler waters will be the subject of a later paper.

Table IV. Operator's Tolerance

Present as $CaCO_3$ Ca	Mg	Ca as $CaCO_3$ Found by Operator A	B	C	D
P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
21.7	86.7	21	24	20	23
54.2	54.2	51	54	53	50
108.4	0	106	105	106	106

Table V. Analysis of Industrial Waters

Test No.	Source	Gravimetric Ca as $CaCO_3$	Gravimetric Mg as $CaCO_3$	Dilution Factor	Ca Found as $CaCO_3$	Total Hardness by Soap
		P. p. m.	*P. p. m.*			
1	Feed	19.7	...	1	18	29
2	Raw	35.8	...	1	35	50
3	Raw	46.9	...	1	46	73
4	Raw	56.7	...	1	53	64
5	Well	171.4	29.4	2	168	196
6	City	268	202	5	256	480
7	Test well	156.5	76	2	158	220
8	Well	26.1	17.2	1	26	46
9	Test well	372.2	195	5	360	570
10	Well	370	176.4	5	380	555
11	City	81.7	41.7	1	80	122
12	Well	472	242.4	5	465	730
13	Well	183.6	83.3	2	184	270
14	Well	322.6	141	5	320	460
15	Well	497	245	5	485	730

Acknowledgment

The authors are indebted to C. A. Noll, chief chemist, for his suggestions and to W. H. & L. D. Betz for making this investigation possible.

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Colorimetric Determination of Phenothiazine with Palladous Chloride

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CONSIDERABLE interest has been manifested in the use of phenothiazine as an insecticide and in the determination of small amounts found in spray residues. Eddy and De Eds (*2*) determined small amounts of phenothiazine colorimetrically by oxidizing an alcoholic solution to a highly colored red compound with bromine water. Recently, Cupples (*1*) reported additional information concerning this reaction.

The authors have observed that phenothiazine reacts with a number of inorganic ions. Most of these ions are oxidizing agents and the red or green colored solutions formed are due to an oxidation of phenothiazine. Palladous chloride reacts with phenothiazine, yielding a dark blue colored solution or precipitate which is not an oxidation product but a complex having the formula $Pd(C_{12}H_9NS)_2Cl_2$.

A procedure for the colorimetric determination of small amounts of phenothiazine based on this reaction, methods for the preparation of the complex, and a description of its properties are presented in this paper.

Reagents

Phenothiazine. A compound obtained from the Eastman Kodak Co. (P1456) was purified by recrystallization from ethyl alcohol. The purified compound melted at 182° C. An acetone solution containing 0.05 mg. per ml. was used. (An ethyl alcohol solution of the same concentration may be used.) The solution slowly acquires a pink color; for this reason a fresh solution should be prepared at least once a week.

Sodium Acetate–Hydrochloric Acid Buffer. Fifty milliliters of 1 *M* sodium acetate were added to 50 ml. of 1 *M* hydrochloric acid and the solution was diluted to 250 ml. with water. This buffer has a pH of 2.6 which increases to 2.9 upon dilution of 5 ml. to 25 ml. with water.

Palladous Chloride. A standard palladous chloride solution containing 1 mg. of palladium per ml. in 1 *M* hydrochloric

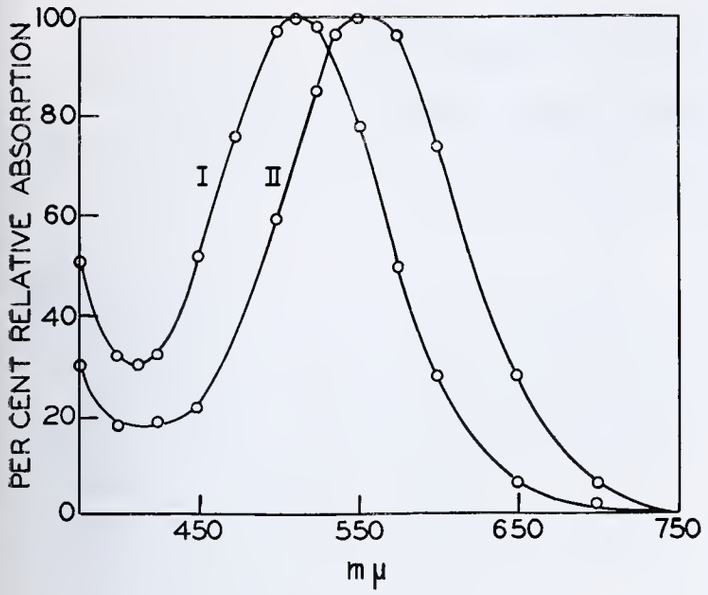

Figure 1. Relative Absorption of Palladous Chloride–Phenothiazine Compound
I. Ethyl acetate medium
II. Buffer medium

acid was diluted with water to give a solution containing 0.03 mg. of palladium per ml.

Procedure

Transfer 5.0 ml. of the sodium acetate–hydrochloric acid buffer to a 25-ml. volumetric flask and, after adding 1.0 ml. of the palladous chloride solution, add the amount of acetone required to give a constant amount in the standards and unknowns. Limit the acetone concentration to 20 per cent and preferably less. Add sufficient water to bring the solution to within several milliliters of the mark, add the desired volume of phenothiazine solution, make up to the mark with water, and mix well. The unknowns are prepared in the same manner, adding the desired volume of an acetone solution containing the sample to be determined. Color comparison should be made immediately and may be performed with 50-ml. Nessler tubes or other suitable cylinders. The increments of phenothiazine used in preparing the standards are as follows: 0 to 40 micrograms, 2.5 micrograms; 45 to 100 micrograms, 5 micrograms. These values also correspond to the sensitivity at the respective concentrations. Best results are obtained using 40 micrograms or less. Amounts of phenothiazine in excess of 100 micrograms may be determined, but difficulty in the color matching frequently arises due to decreased stability of the colored solutions at these higher concentrations.

Discussion

A blue to purple solution is formed, depending upon the amount of phenothiazine added to the buffered solution containing palladous chloride. The colored complex is completely removed if the solution is extracted with ethyl acetate. In the process the color of the complex changes, resulting in a red ethyl acetate solution. This suggests the possibility of performing the color comparison in this medium. The red color observed in the ethyl acetate, however, is less stable than the blue color formed in the buffered solution and consequently less suitable for colorimetric purposes. It might, however, have some application for the qualitative detection of phenothiazine where interfering colors are present.

The stability of the blue solutions, while more satisfactory than that in ethyl acetate, is not so great as might be desired for colorimetry, but by preparing the standards and unknowns at as nearly the same time as possible and comparing the color immediately, satisfactory comparisons are obtainable. This is especially true when determining small amounts of phenothiazine where the stability of the colored solution is greatest. Attempts to stabilize the colored solutions by the addition of gelatin, gum ghatti, and gum damar were unsuccessful.

The acetone concentration should be controlled closely because the intensity of the color formed is dependent upon it. A constant amount should be used as determined by the volume of the unknown that is to be employed. Where possible, a constant volume of 1 or 2 ml. of acetone is recommended and in no instance should it exceed 5 ml. A marked decrease in the sensitivity results at higher acetone concentrations.

When the sample containing phenothiazine must be prepared for analysis with ethyl alcohol as a solvent, the method is applicable if an alcoholic solution of phenothiazine is used for the preparation of the standards. The methods using acetone or ethyl alcohol are similar. A constant amount of ethyl alcohol must be used in the standards and unknowns and should not exceed 20 per cent.

The use of buffered solutions is recommended because small amounts of acids or salts alter the color intensity considerably in unbuffered solutions. Using the designated buffer concentration, the limits of acids or salts that may be present without error are: 0.05 ml. of 1 *M* hydrochloric acid and 0.2 ml. of 1 *M* sodium chloride. The stability of the colored solutions formed is increased by the presence of the buffer.

The reverse procedure—i. e., addition of palladous chloride to phenothiazine—may be employed but the colored solutions formed are less stable and less reproducible. Phenothiazine may also be used for the detection of palladium but is less satisfactory than other reagents previously employed (*3*).

Preparation and Properties of Complex

The complex must be precipitated from acetone-water or alcohol-water mixtures in order to obtain a pure product. Otherwise, a compound contaminated with phenothiazine results, owing to the low solubility of the latter in aqueous medium.

Transfer 5 ml. of the palladous chloride solution (5 mg. of palladium) to a beaker, dilute with water to 100 ml., and add 80 ml. of acetone (or alcohol). Add 10 ml. of a filtered acetone solution containing 40 mg. of phenothiazine. After the suspension has stood for several hours, filter through a sintered-glass crucible and dry at 120° for 2 hours.

The product obtained is a bluish-black solid, insoluble in water, sodium acetate–hydrochloric acid buffer, and carbon tetrachloride; sparingly soluble in ethyl alcohol; moderately soluble in ether and ethyl acetate; and readily soluble in acetone and dioxane (deep purple colored solutions). The complex is destroyed by concentrated acids and bases.

The complex has the composition $Pd(C_{12}H_9NS)_2Cl_2$ as found by analysis. Calculated for $C_{24}H_{18}N_2S_2PdCl_2$: C, 50.07; H, 3.14; Pd, 18.52; Cl, 12.31. Found: C, 50.40; H, 3.47; Pd, 18.30; Cl, 12.2.

Relative absorption data for the colored solution formed in the presence of the sodium acetate–hydrochloric acid buffer and the red solutions obtained by extracting with ethyl acetate are plotted in Figure 1. These measurements were made with a Beckman spectrophotometer, Model D. Although the solutions are not sufficiently stable to permit extensive use of transmittancy measurements, a limited application such as this is possible. Curve II was obtained for a solution prepared according to the procedure given for the colorimetric determination using 1 ml. of phenothiazine. Curve I represents the values obtained after extraction with ethyl acetate. The general character of the two curves is similar but the maximum shifts to a shorter wave length upon extraction.

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Identification of Alcohols

By Means of the Optical Properties of the Esters of Carbanilic Acid

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THE optical properties of the esters of carbanilic acid were suggested by the authors in a previous paper (*1*) as a means for identifying alcohols. They studied the esters of fifteen normal primary alcohols and recorded the optical data, and have since prepared and studied twenty-three more esters.

The procedures followed were similar to those employed in the previous work. The esters were prepared by the action of phenylisocyanate on the alcohols (obtained from the Eastman Kodak Company, Rochester, N. Y.). The esters were recrystallized from petroleum ether, a portion was then recrystallized from ethanol, and the crystal system, optic sign, sign of elongation, dispersion, and extinction angle were determined (Table I).

All the compounds so far investigated belong to the monoclinic system. Their optical character is biaxial. It was impossible to obtain values for some of the indices because of the difficulty of obtaining an orientation which would permit the observation of those values. For nine of the compounds, the values of gamma could not be determined because these values were greater than the maximum refractive index obtainable with the potassium mercuric iodide solutions. For these compounds the lower limiting values given in Table I were determined experimentally or calculated from the birefringence.

The optical properties of the compounds recrystallized from ethanol checked with those observed when the compounds were recrystallized from petroleum ether. The crystals from ethanol are larger and better formed, and give clearer interference figures. The additional recrystallization is especially valuable for those esters which have low solubility in petroleum ether. The crystals of glyceryl carbanilate were so poorly formed even after repeated recrystallizations that determination of the optical properties was impossible.

In confirming the identity of terpineol, ethylene bromohydrin, ethylene chlorohydrin, and menthol, the crystals of the esters should be oriented with the long direction more nearly parallel to the 3 o'clock–9 o'clock direction in the polarizing microscope while the value for beta is observed. Alpha or gamma is found when the long direction of these crystals is more nearly parallel to the 6 o'clock–12 o'clock direction. For all others these orientations are the reverse. The optical orientation should be established by means of an interference figure before observing the values for alpha or gamma.

The identification of some alcohols by means of certain optical properties of their carbanilates has been found possible, even when the refractive indices are identical or nearly so. It is possible to distinguish bornyl carbanilate from isoamyl carbanilate by their dispersion. The refractive indices of crystals of the urethan prepared from ethylene chlorohydrin and those of diphenyl urea, which is formed when water is present, lie very close together. However, crystals of diphenyl urea show an interference color of a gray of a high order. Beveled edges show many bands of color. Thin crystals do not show intense polarization colors. When a melt under a cover glass is prepared, the polarization colors are not intense, and interference figures usually show only the dark isogyres. Crystals of the ethylene chlorohydrin carbanilate recrystallized from petroleum ether or rapidly precipitated from hot ethanol solutions show slightly more vivid polarization colors. The crystals obtained by slow evaporation of the solvent from ethanol solutions or from fusion of the material under a cover glass exhibit intense polarization colors. The interference figures show colored bands in addition to the dark isogyres.

The components of mixtures of alcohols may be identified by the procedure suggested in the previous paper. Twelve binary mixtures of alcohols in equal proportions were used in preparing carbanilates. The resulting crystalline mixtures were composed of two kinds of crystals, each of which exhibited the same optical properties which it possessed when prepared from the corresponding pure alcohol. The only exception was that of the ethyl and methyl mixture previously reported. Frequently some characteristic serves to indicate the identity of the individual crystal. In a mixture of 3-methylcyclohexanol carbanilate and 4-methylcyclohexanol carbanilate, the former can be quickly distinguished, for its crystals are optically negative while those of the latter are optically positive. Confirmation is obtained by determination of the refractive indices. In the preparation and recrystallization of carbanilates from a mixture of alcohols, care must be used to prevent the loss of one of the components; otherwise the result would indicate a pure alcohol instead of a mixture.

Table I. Optical Properties of Esters of Carbanilic Acid

Ester	Melting Point, ° C.	Extinction Angle	Optic Sign	Refractive Indices Alpha	Beta	Gamma	Elongation	Dispersion
Allyl	67	25	−	1.507	1.586	1.659	±	$\gamma > \rho$
Isoamyl	55	10	−	1.482	1.560	1.626	±	$\gamma > \rho$
Borneol	138	30	−	...	1.561	1.621	±	None
Isobutyl	82	18	−	1.481	1.572	1.656	±	$\gamma > \rho$
Cetyl	76	5	−	...	1.541	1.610	±	$\gamma > \rho$
Cinnamyl	89	10	+	1.530	1.589	>1.718	±	$\rho > \gamma$
Cyclohexanol	83	32	+	1.487	1.632	>1.775	±	$\rho > \gamma$
Diethylcarbinol	48	45	−	1.499	1.568	1.616	±	$\gamma > \rho$
Ethylene bromohydrin	76	7	+	1.595	1.655	>1.718	−	$\rho > \gamma$
Ethylene chlorohydrin	51	27	+	1.583	1.628	>1.718	−	$\rho > \gamma$
Ethylene glycol	148	7	−	...	1.625	>1.718	±	$\gamma > \rho$
Furfuryl	44	15	+	1.519	1.599	>1.718	±	$\gamma > \rho$
Menthol	111	5	+	1.558	1.573	1.597	−	$\rho > \gamma$
p-Methoxybenzyl	90	9	+	1.550	1.602	>1.718	±	$\rho > \gamma$
Methylbenzylcarbinol	86	19	+	1.532	1.584	1.651	±	$\gamma > \rho$
2-Methylcyclohexanol	103	20	−	...	1.575	1.698	±	$\rho > \gamma$
3-Methylcyclohexanol	92	42	−	...	1.562	1.667	±	$\gamma > \rho$
4-Methylcyclohexanol	123	43	+	1.488	1.613	>1.738	±	$\rho > \gamma$
Methylisopropylcarbinol	69	14	−	1.492	1.558	1.617	±	$\gamma > \rho$
Myristyl	71	25	−	1.542	1.573	1.577	±	None
Isopropyl	88	10	−	1.510	1.573	1.616	±	$\gamma > \rho$
Terpineol	112	34	−	1.511	1.607	>1.718	−	$\rho > \gamma$
Tetrahydrofurfuryl	58	43	+	1.570	1.608	...	±	$\rho > \gamma$

Summary

The optical crystallographic data for twenty-three esters of carbanilic acid have been determined and compared with data for fifteen esters and for diphenyl urea reported in a previous paper. The crystallographic and optical properties of the esters of carbanilic acid are suitable constants for identifying the alcohol either pure or in mixtures.

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Design of Still Heads for Batch Fractionation in Laboratory Columns

The Vapor Take-Off System

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Low-holdup reflux regulators for batch distillation are described in which the product is removed in the vapor phase. Possible designs are discussed with reference to glass models, and detailed designs are given for still heads of general application. Details of heads applied to 25-mm. Stedman packed columns show how these designs may be modified to suit special requirements.

DURING the past few years experimental and theoretical work in connection with batch fractionation has served to emphasize the importance of the effect of holdup. Considerable ingenuity has been demonstrated in the design of still heads to eliminate reflux holdup in order that full advantage may be taken of the fine fractionation achieved by modern low-holdup column packings. This paper is concerned with further refinements in the design of still heads suitable for the control of batch fractionation on laboratory columns.

A column fitted with an overhead condenser of the type shown in Figure 1, may be operated on total reflux with a minimum of reflux holdup. If the condenser is so designed that only the latent heat is removed from the condensing vapors and the reflux is returned to the column at its boiling point, the liquid film will be of minimum thickness and none of the packing will be used as a heat exchanger to warm up cooled reflux. When the system is extended to modes of operation other than total reflux the product is usually collected from the liquid reflux system, a technique which increases the reflux holdup. This may be avoided by using a vapor-phase product drawoff (*1*, *2*, *3*), and the auxiliary condenser and control systems described below make use of this principle.

Types of Auxiliary Condenser and Control Systems

In the closed system shown in Figure 1, 2, a second condenser has been added and so arranged that condensate collected in this second condenser cannot return to the column. In such a system the rate of condensation on the cooling surface of the auxiliary condenser determines the rate of product removal. The rate of heat transfer depends on the supply of product to the condenser, the difference in temperature between the vapor and condensing surface (Δt), and the material and design of the condenser.

Figure 1, 3 to 11, indicates in diagrammatic form the various methods of controlling vapor take-off which are described below. The merits and demerits of these systems and their operating characteristics are summarized in Table I. Since the reflux holdup is the same for all these systems, the term "holdup" in the table refers to run-down line holdup.

Performance tests on models of these condensers (designs 3 to 11) were undertaken in order to study their behavior with reference to the following fundamental factors:

The holdup must be a minimum.
The response to the control must be rapid.

Table I. Characteristics of Condenser and Control Systems

System	Control	Response, Product Rate: Decreasing	Response, Product Rate: Increasing	Stability of Product Rate	Variables Which May Possibly Affect Stability	Run-down Holdup	Behavior on Total Reflux	Possible Contamination	Suitability for Laboratory Use
3	Vapor rate, stopcock in vapor line	Quick	Quick	Excellent	Small pressure changes in reflux system	Nil	Excellent	Grease from stopcock	Unsuitable owing to stopcock in vapor line; see, however Bruun (*2*) or Bush and Schwartz (*4*)
4	Water rate, stopcock in condenser water inlet	Fair	Poor	Poor	Top temperature, cooling water pressure and temperature	Nil	Response slow and condenser must be lagged	None	Unsuitable, poor response, needs continuous adjustment
5	Water temperature, mixing of hot and cold water to condenser inlet	Fair	Fair	Poor	Hot and cold water temperature and pressures, top temperature	Nil	Response fair and condenser must be lagged	None	Unsuitable, too many auxiliary controls for water temperature, poor response
6 7	Water level, cocks in condenser inlet and outlet or constant-level device	Poor	Fair	Poor	Water temperature and rate, top temperature	Nil	Response fair and condenser must be lagged	None	Unsuitable, poor response
8 9	Condensate level, constant-level device or stopcock on product line	Quick, lag in response of column condition	Quick	Excellent	Water rate and temperature, top temperature in 8 and stopcock grease in 9	High with low rate and vice versa	Tends to incorporate rundown holdup in column holdup	Stopcock grease in 9	Ease of control makes 9 attractive in spite of contamination and holdup
10	Removable condensing surface	Quick	Quick	Good	Water rate and top temperature	Nil	Requires mercury seal	From mercury seal on total reflux	Has limited application; see, however, Fenske's design (*5*)
11	Gas blanket	Quick	Quick	Good	Water temperature and pressure, partially self-stabilizing towards top temperature changes	Nil	Excellent	None	Most satisfactory

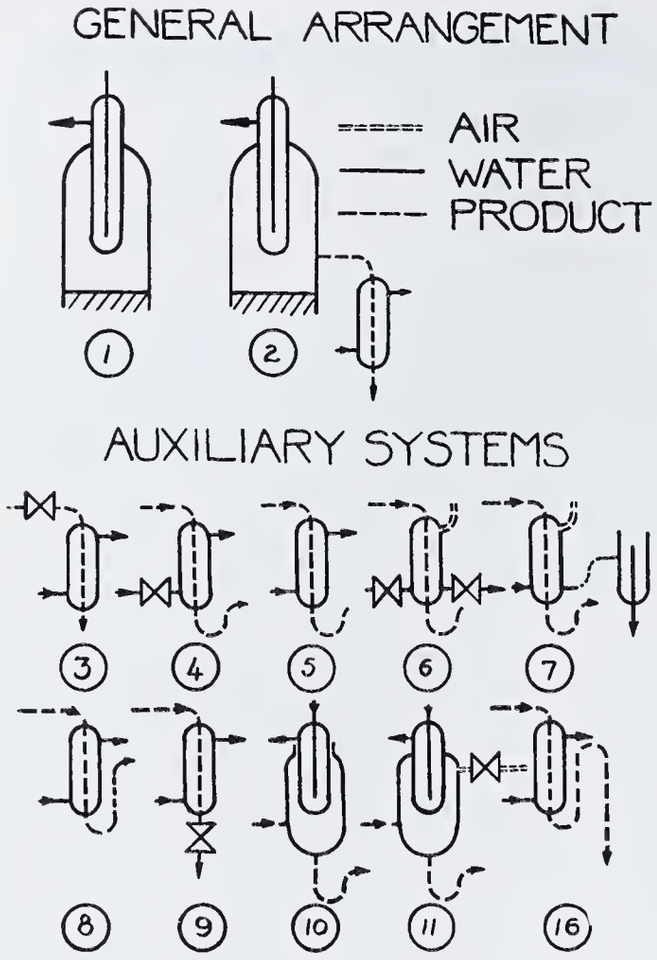

FIGURE 1

The control must be positive and stable.

The system must permit of operation on total reflux.

The use of stopcocks or mercury seals should be avoided when possible, particularly where they may come into contact with gasoline vapors.

Design Details

The performance tests indicate that designs 9 and 11 are the most suitable for laboratory control, but before the construction of this equipment is described other factors which affect the design details must be considered. In the first place it is an advantage if the auxiliary equipment can be made small and compact enough to be supported only by the column, so as to avoid the necessity for expansion joints which may either cause holdup or give rise to leaks. Such heads can be changed very easily if broken or if the operation of the still is to be changed, but in order to do this it is necessary to remove the product through the head and not from the side of the column.

The use of vapor-tight connections between the head and the column is unnecessary providing the distillation takes place at atmospheric pressure. Condensers of the type shown in Figure 2, 12, are in constant use for total-reflux work and for certain organic reactions such as nitrations, since a stirrer and thermometer can be easily accommodated. However, in the latest designs, it has been found possible to use ground joints which can be water-cooled and can be protected from product vapors, which might attack the lubricant, by the use of an air blanket.

If the reflux is overcooled and returned to the column substantially below its boiling point, the top few plates are used as a heat exchanger and there is a loss of efficiency. As a consequence devices other than "drippers" for measuring reflux rates have been avoided. Reflux rates and ratios can most accurately be determined by preliminary experiments with pure compounds in which the power required by the still pot and column heaters can be compared with the pressure drop across the column when the top and bottom rates are the same. In such experiments holdup is of no consequence and several devices may be used which otherwise would not be permissible.

Finally, the location of the thermometer is of some importance. Top temperature is not, in the writer's experience, a satisfactory criterion of purity when close fractionations are being dealt with and as a consequence temperature records to within 0.2° C. are usually sufficient. Unless a reboiler of the Cottrell type is used batchwise or in conjunction with a large column, the holdup (about 10 ml.) associated with it seriously affects the sharpness with which a cut may be made, and very often it will be found that the Cottrell temperature lags below the top temperature. It is possible to fabricate reboilers with a holdup of less than 1 ml., provided they are located within the column itself and utilize the main condenser for cooling and the column vapor for heating, but as they are of limited use it is not considered necessary to describe them here. Moreover, in a column with fifty or more plates very little difference in temperature can be detected in the top few plates (unless the reflux is overcooled) and little improvement in the vapor liquid equilibrium can be obtained by running the product through a reboiler. Therefore in order to obtain a true top temperature it is better to suspend the thermometer or thermocouple in the vapor at the top of the column and arrange a suitable guard so that the liquid reflux does not flow over the thermometer bulb or thermocouple tip.

Constructional Details

The simplest head based on design 9 is shown in Figure 2, 13. An eight-turn coil of 3-mm. inside diameter Pyrex tubing forms the main reflux condenser. The vapor line, *A*, and the water

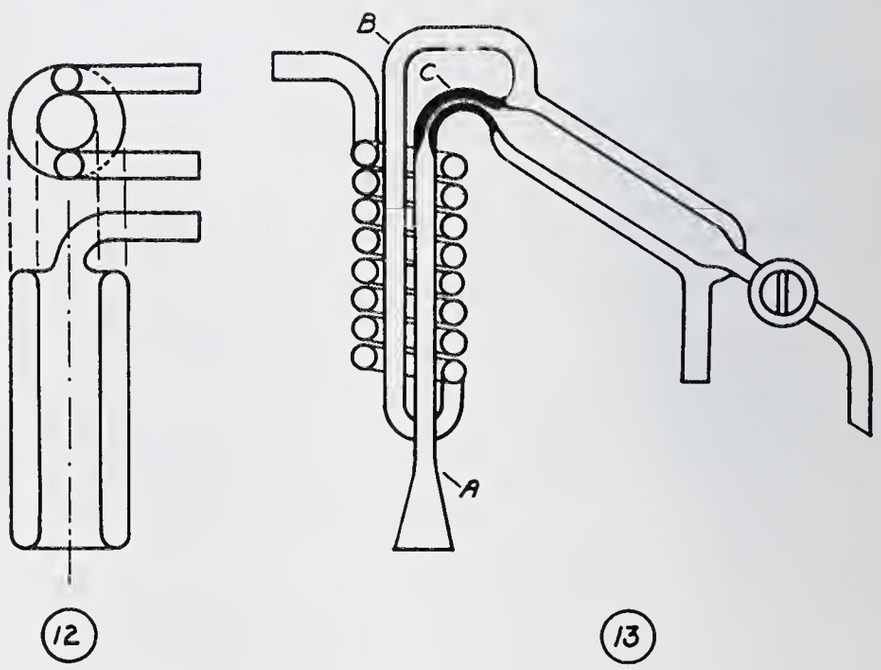

FIGURE 2

inlet line, B, are accommodated axially as well as a thermometer which is not shown in the figure. It is difficult as a consequence to make the outside diameter of the condenser coil sufficiently small to enter a column 2.5 cm. (1 inch) in diameter. However, if such heads are required for columns smaller than 2.5-cm. (1 inch) diameter, they may be fabricated from tinned copper tubing. The product condenser consists of a 5-cm. (2 inch) length of tube flattened in order to reduce the holdup. It is better to draw this flat tube from previously flattened thick-walled tubing than to flatten tubing of the correct size and wall thickness. This tube is enclosed in an ordinary water jacket, the water outlet of which is fused to the water inlet, B, of the main condenser. This water tube serves as a support which maintains the relative positions of the main and auxiliary condensers. It is important that the thermometer and vapor tube should not touch the main condenser.

The head is rested in the top of the column with the stopcock closed, and water circulated through the condensers. The column is brought into operation and total reflux conditions are established by condensation on the main coil condenser.

In order to remove product from the still a small amount of air is then withdrawn through the stopcock. Product vapor is withdrawn from below the main condenser via the vapor tube into the auxiliary condenser where it condenses. If the stopcock is closed the auxiliary condenser first fills rapidly and then the liquid level will continue to climb slowly to the top of tube A until total reflux is established. The stopcock may now be opened and the level of the product in the auxiliary condenser will fall until the exposed condenser surface is just sufficient to condense a volume of vapor which corresponds to the product demand. This condition may be maintained throughout the distillation, the product rate being controlled by the stopcock the setting of which is facilitated by filing a short scratch around the key barrel starting from the bore hole.

If a small amount of air remains in the auxiliary condenser when the head is primed it may slow down the action of the auxiliary condenser by blanketing the surface or even seal off the condenser completely by remaining trapped in the head at the top of tube A. This can be obviated and priming facilitated by sealing a side tube and stopcock to the bend, C, in the vapor tube. The bell on the collecting end of the vapor tube must have a terminal diameter sufficient to reduce the velocity of the entrant vapor appreciably below the vapor velocity in the column; otherwise vortex rings travel down the outside of the vapor tube and draw air from above the main condenser, which can then pass up the vapor tube and blanket the auxiliary condenser. With a narrow-mouthed vapor tube this phenomenon persists even if the vapor tube projects as much as 20 cm. (8 inches) below the main condenser or if the top of the condenser system and the space between the condenser and column are plugged. Glass wool packed below the main condenser but above the mouth of tube A will keep the air from the auxiliary system but the bell mouth is the most satisfactory solution of the problem.

A development of this head is shown in Figure 3, 14, which has been successfully used for the delivery of constant volume (10-ml.) samples in the fractionation analysis of gasolines. The main condenser, A, 14, is of a design similar to that shown in 12. The heat transfer through the cooling water is partly by convection and partly due to forced circulation, and although it would appear that such a design would not be so satisfactory as one in which complete circulation obtains it has been found suitable in practice. In slightly larger models it would be preferable to run the water inlet line, B, down inside the water space to the bottom of the condenser. Both surfaces of this main condenser may be in contact with the vapor as far as the hole, C, which pierces the water annulus, but above this hole air is trapped between the condenser and column wall and this serves to protect the lubricant of the ground joint from product vapors. The inner surface of the condenser is, of course, available above the equalizing hole. The product vapor is removed through a bell-mouthed tube and condensed in a small auxiliary condenser, so that the operation of priming the head is the same as for the design shown in 13. A thermometer is suspended from the closed end of the extension tube, D, so that the bulb lies beside the bell and the stem is not in contact with the condenser. Under these conditions the bulb records the vapor temperature, and not the temperature of the cooled reflux. The pressure inside the still is controlled via the tube, E, which is in connection with a manostat assembly. After passing the product-rate control cock, F, the liquid product is collected in the jacketed receiver G, which is protected against loss by the flattened condenser, H. The product siphons intermittently from this receiver at intervals determined by the control-cock setting. If the still pressure is widely different from atmospheric this form of delivery is not suitable since although an auxiliary receiver below the siphon

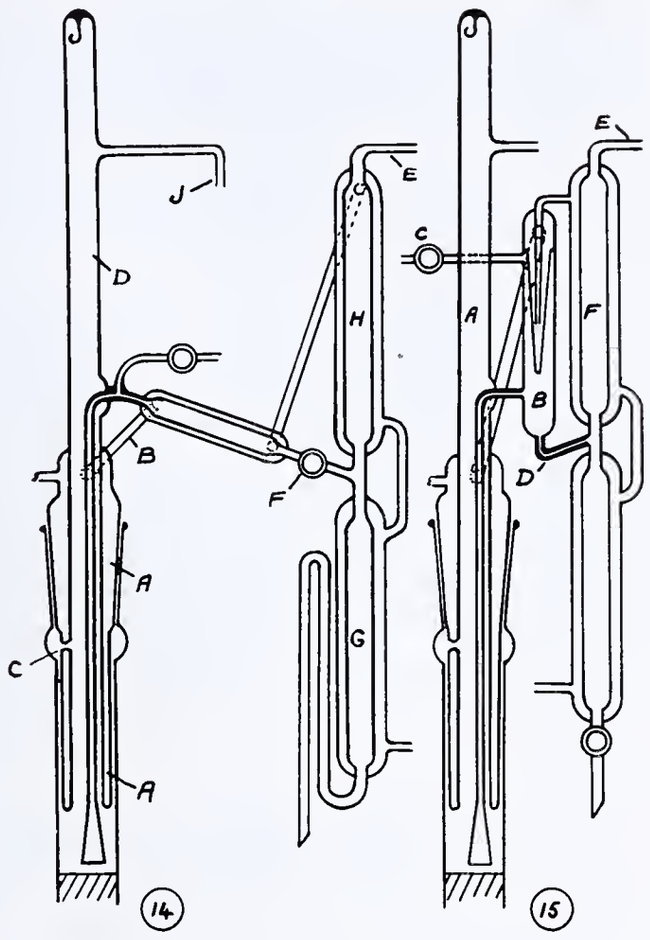

FIGURE 3

connected by an equalizing line to tube E would enable the siphon to operate, the difficulty attendant on changing such receivers renders the head unsuitable. For pressures around atmospheric a rubber connection is made from the side tube, J, to one side of a manometer which is used to indicate the pressure drop across the packing. In order to simplify the drawing both the thermometer and equalizing tube from D to the top of G are omitted.

Figure 3, 15, shows a general-purpose head based on design 11. The bayonet-type main condenser, thermometer extension, vapor tube, and pressure-control outlet are similar to those shown in 14. (Aniline point thermometers are suitable for these types of heads since the main condenser assembly only obscures the unetched part of the stem and a few of the lower graduations.) The vapor outlet leaves the thermometer extension, A, 15, and communicates via a short capillary tube with the side of the small chamber, B. The head of this chamber carries a very small bayonet-type condenser for condensing product, while the lower end is sealed to a short capillary liquid-seal tube, D. Sealed into the chamber is the permanent gas inlet, C. The auxiliary bayonet condenser should not reach to the bottom of B, since for very high reflux ratios it is better to use the walls of the lower part of B as the condensing surface. The liquid seal, D, communicates with a jacketed receiver through a tube wide enough to prevent siphoning, and with the pressure control inlet, E, through a flattened protecting condenser, F. The equalizing line from A is connected to the bottom of F opposite the entrance of the seal, D, but to simplify the diagram both it and the thermometer are again omitted. On total reflux the head is full of permanent gas (air) except for the lower end of the main condenser. The head is primed by removing a small amount of air from B through tube C. The product vapor rises up inside the vapor tube with a slight "pumping" action for 1 or 2 seconds until the tube is warmed up, and, if sufficient air has been removed from B, the product vapor condenses on the lower walls of B and the condensed liquid fills the seal. The bayonet condenser is drawn to a point so that when the air blanket is slowly raised there is no marked discontinuity in the increase in condensation. When Δt is large the lower part of tube B may be lagged with felt or cotton wool in order to give a more sensitive control of low product rates. It has been found possible to keep a constant product rate of 1

ml. per hour for 48 hours, since the rate is not controlled by narrow openings which may become blocked with grease or, as may happen in gasoline fractionation, by traces of moisture. The auxiliary systems of both designs 14 and 15 may of course be used satisfactorily with simple main condensers as in Figure 1, 2.

The flexibility of these systems of vapor take-off may be demonstrated by two other modifications which have proved useful for special applications.

A closed tube may be inserted axially through the condenser in design 15 and the upper end of this tube brought out through the thermometer support tube above the vapor line and sealed into the permanent gas inlet line. The tip of this closed tube is located close to the thermometer bulb in the product vapor so that an increase in "top" temperature will cause the air in the closed compensating tube to expand and, as a consequence, drive down the blanket in chamber *B*. If the volume of air in the compensator is adjusted (by the addition of a few drops of oil) it is possible to oppose the increase in Δt due to a rise in top temperature by a reduction in available condensing surface, so that the product rate remains substantially constant.

It is sometimes an advantage in precise fractionation to increase the reflux ratio as the cut point is approached. Design 9 may be modified to do this automatically, as shown in 16 in diagrammatic form. The vapor line communicates directly to a vertical condenser which is emptied intermittently by a siphon. As product condenses in the lower part of this condenser-receiver, the product level rises and cuts off part of the available condenser surface. As a result there is a progressive decrease in product rate as the distillation proceeds until the siphon is primed and the cycle repeated. The volume of the cut depends on the volume of the receiver up to the bend in the siphon, the final product rate on the residual condenser surface above the top of the siphon, and the initial product rate on the total condenser surface. It is possible therefore to design such a head to meet any specified conditions of distillation. If the top temperature is increasing throughout the run there will be a slow increase in product rate unless the water temperature is raised or the area of condenser remaining above the siphon progressively reduced by an air blanket in the water jacket.

Acknowledgment

Thanks are due to Trinidad Leaseholds Limited for permission to publish this laboratory application of still-head design.

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An Oil Manometer-Manostat to Control Column Throughput

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THE importance of control of distillation rate (throughput) for optimum column performance is well recognized. Control of the heat input to the still pot by rheostat or Variac alone, except over short periods of time, has been found undependable because of the marked effect, especially at low throughput, of even small variations in line voltage on the total heat supply to the assembly. This is particularly true where tall columns are used and where the column insulation is dependent upon heat compensation. [For short columns (not over 90 cm., 3 feet) in which column and still pot are vacuum-jacketed, such as Podbielniak type (*4*), Variac control is probably much more dependable.] Any serious departure from the optimum throughput of a given column (especially a packed column) by increase or decrease of rate may seriously impair the column efficiency (HETP); if the rate is too fast, it may cause flooding; if too slow, it may fail to supply adequate reflux. The latter condition is especially likely to result where "product" or "take-off" is maintained at a constant value by a stopcock setting, or some similar means is used for reflux ratio control.

Manostatic control of heat input to the still pot, by taking advantage of the relationship of column pressure drop to distillation rate, has been used by Othmer (*2*), Rossini and Willingham (*5*), and Selker, Burk, and Lankelma (*6*). The manostatic liquid (mercury or other conductor) provides the switch mechanism for opening and closing the control circuit to the still-pot heater. Mercury, as the manostatic liquid, can be used advantageously only with columns having appreciable pressure drop. With recent developments in columns having very low pressure drop, mercury is too heavy and lighter liquids, such as are used in the control described by Selker *et al.* (*6*), are necessary. When a two-fluid manostatic system is used and the conducting manostatic liquid is an aqueous salt solution, use of the control device is restricted to column operation at atmospheric pressure because of the excessive volatility at reduced pressure of the water in the manostat.

The manostatic control described in this paper utilizes oil alone (mineral oil of very low vapor pressure) as the manostatic liquid. The movement of the meniscus, as a result of

Table I. Throughput

Test No.	Throughput Min.	Throughput Max.	Throughput Av.	Δp No.[a] Min.	Δp No.[a] Max.	Total Time Elapsed	No. of Throughput Determinations[b]	Difference between Minimum and Maximum Throughput	Deviation from Average Throughput	
	Cc./hour			*Mm. of oil*		*Hours*		*Cc./hour*	*Cc./hour*	*%*
1	26	35	30½	28	30	16	29	9	±4.5	±14.8
2	32	42	37	29½	32	2	34	10	±5	±13.5
3	92	100	96	40	43	72	46	8	±4	± 4.2
4	132	146	139	49	52	17	22	14	±7	± 5.0

[a] Pressure drop as measured by distance between upper and lower levels of manometric fluid.
[b] Obtained at still head by counting drops at varying intervals during test.

pressure variations corresponding to variations in column throughput, actuates a photoelectric relay which controls the heat input to the still pot. The results of some tests on throughput constancy when this device was used are shown in Table I.

The manometer-manostat assembly is shown in Figure 1. The mineral oil used (Nujol), previously degassed under a high vacuum, fills the apparatus to level C. It has a density, at room temperature, of 0.862; therefore, when vertical distance AC is 15.7 mm. of oil it represents a pressure drop (Δp) of only 1 mm. of mercury. The oil as a manometric fluid is thus almost sixteen times more sensitive than mercury.

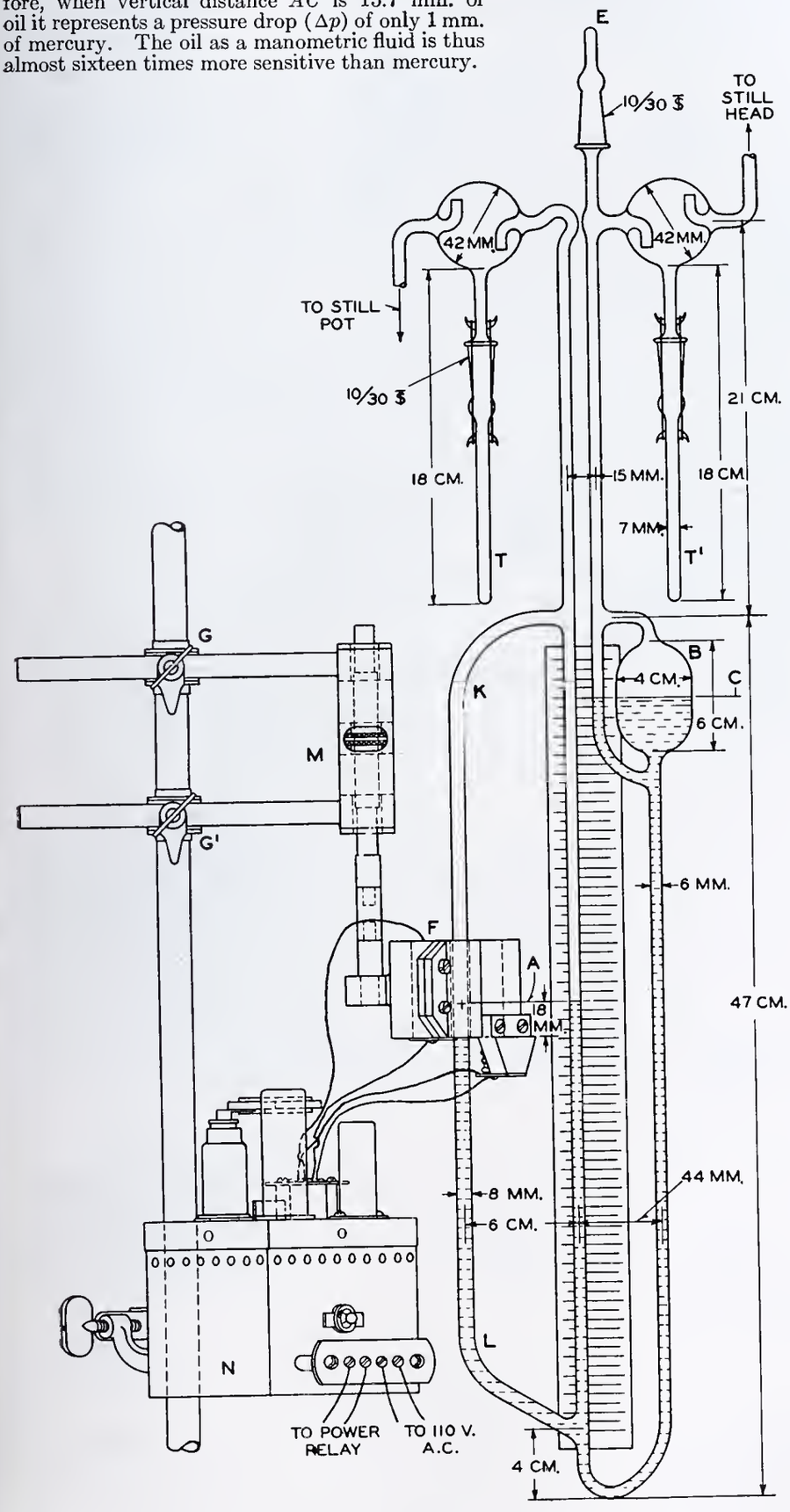

FIGURE 1. MANOMETER-MANOSTAT ASSEMBLY

Since the volume of oil in bulb B is relatively large, the level of oil in it is practically stationary for all probable values of Δp, or distances between the upper and lower levels of oil in the two sides of the manometer. The photocell and the light source are contained in a brass housing, F, which can be moved by means of clamps G and G^1 to any desired setting along the manometer by-pass tube, KL, which acts as the manostat. The micrometer screw, M, permits a slow raising or lowering of the mechanism for a precise setting of the reflux rate. T and T^1 are trap tubes. Directly below the photocell mechanism is a small metal box, N, containing a two-stage alternating current-operated photoamplifier relay circuit connected to the phototube in housing F. The amplifier circuit operating a small sensitive relay mounted inside the box was described by Shepard and Schrader (*7*). The two thermionic tubes and electrolytic condenser are mounted on top of the box. The other circuit parts, together with the sensitive relay, are mounted inside.

Figure 2 is a detailed drawing of the metal housing for the phototube and 6-watt, 115-volt lamp constituting the movable phototube mechanism. Figure 3 shows the electrical hookup between the amplifier circuit and the still-pot heater.

Operation

The heat input to the still pot is set roughly by means of a Variac (Figure 3) until a reflux at the top of the column, slightly above the rate desired, is attained—as measured by a drop counter or other suitable means. The phototube mechanism is then moved down along arm KL (Figure 1) until the sensitive relay is actuated to close the circuit. This signifies that the transparent oil, acting as a cylindrical lens to give a line image of the light source on the phototube cathode, W, has been interposed between the light source, S, and the aperture, O (Figure 2). [Thus its operating principle is different from the "opaque meniscus" principle described by Josten (*1*).] The mechanism is then moved slowly upward by means of the micrometer screw, M, until the sensitive relay breaks contact. A pilot light (not shown) across the power relay is used to facilitate the setting.

When the relay breaks contact it signifies that the light from S is no longer focused through aperture O to the phototube compartment but, instead, is diffused by the empty glass tube. This change in light intensity is sufficient to operate the phototube, which is sensitive to a rise or fall of about 0.33 mm. of the oil column when at the level of the aperture. The sensitive relay operates a power relay (Figure 3) which intermittently interposes and cuts out a resistance, R, in series with the still-pot heater, H, as the oil level in the control arm of the manostat falls or rises indicating an increase or decrease in throughput. When using a 50-watt Chromalox bayonet-type heater immersed in an oil-filled center heater (a device to prevent bumping and promote boiling, *3*) in a 500-cc. round-bottomed insulated flask, an intermittent resistance of 75 ohms was found adequate to compensate for line voltage variations. A heater of larger wattage would call for a proportionately lower intermittent resistance.

At an extremely low throughput (30 cc. per hour) the percentage of fluctuation is naturally greatest (±14.8 per cent), as shown in Table I. Without automatic control, however, it had been found virtually impossible to maintain such low

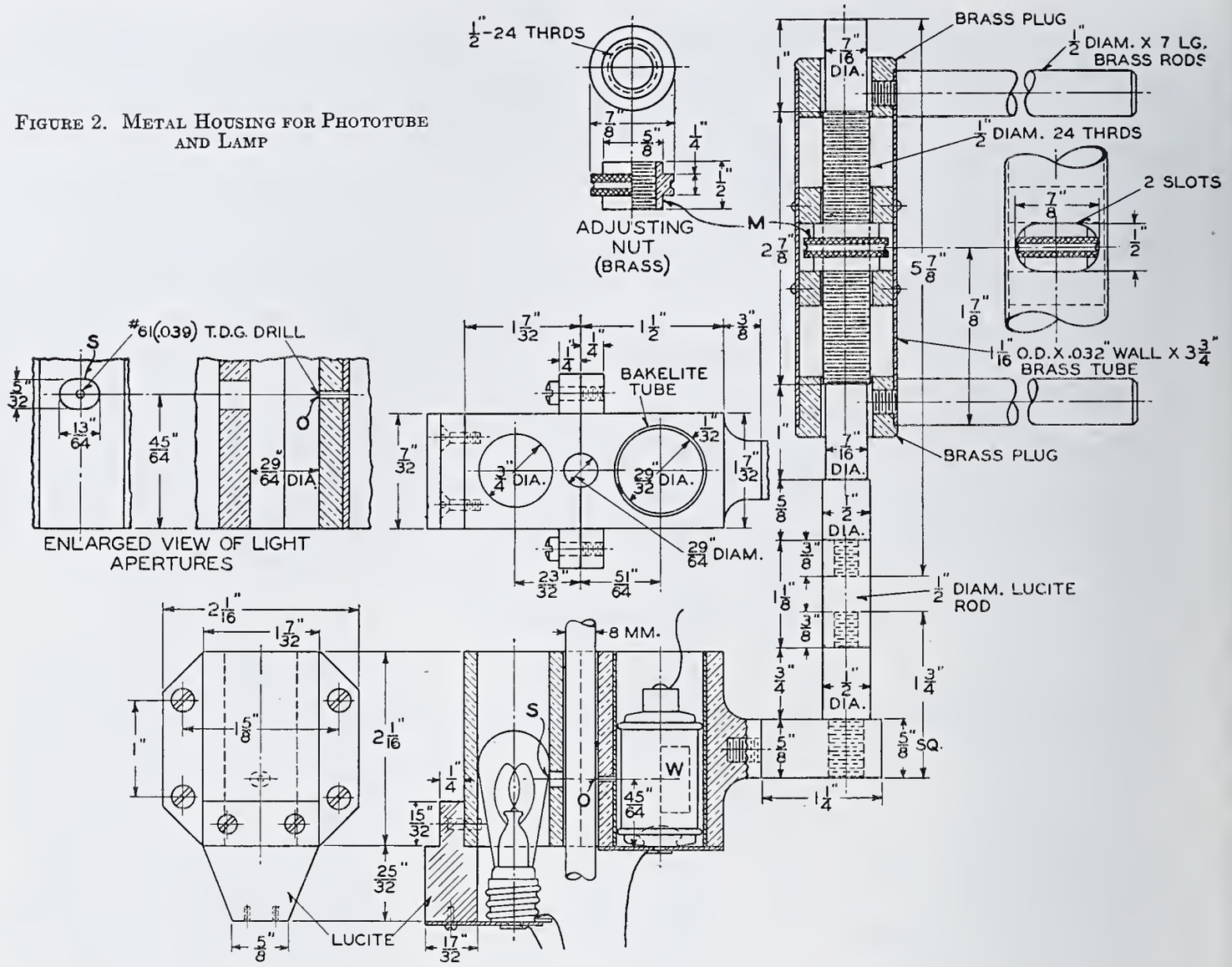

Figure 2. Metal Housing for Phototube and Lamp

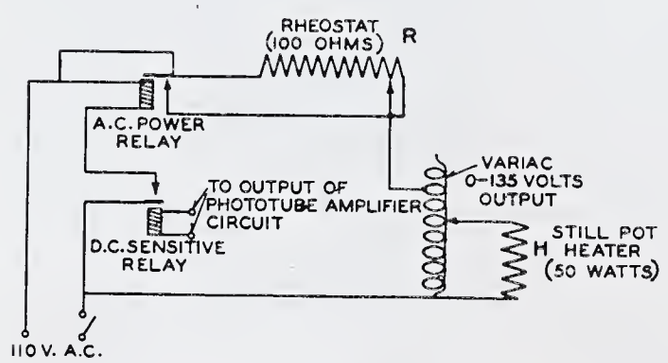
RHEOSTAT (100 OHMS) R
A.C. POWER RELAY
TO OUTPUT OF PHOTOTUBE AMPLIFIER CIRCUIT
D.C. SENSITIVE RELAY
VARIAC 0-135 VOLTS OUTPUT
STILL POT H HEATER (50 WATTS)
110 V. A.C.

Figure 3. Hookup

throughput with any degree of constancy. The fluctuation in Δp for a given throughput amounts to about 3 mm. of oil. This is due to the time lag between heat input change and throughput change.

Parts Used in Assembly

Photoelectric Mechanism. One R. C. A. 922 phototube with socket and cathode clip, one G. E. 6-watt, 115-volt Mazda lamp (candelabra screw base), and one Dialco pilot light mounting for lamp.

Phototube Amplifying Circuit. One G. E. 6 J 7 radio tube with socket, one G. E. 25 A 6 radio tube with socket, one Sangamo A1310 condenser (100 micro micro farads), one Mallory TP438 condenser (0.1 microfarad), one Mallory FPS120 condenser (20 microfarads), one I. R. C. BT-1 resistor (1 megohm), one Ohmite "Brown Devil" resistor (30 ohms 10-watt), one Ohmite "Brown Devil" resistor (50 ohms 10-watt), and one Ohmite 0568 variable resistor (250 ohms 50-watt) with 3 connecting clips.

Relays. One sensitive relay (12 milliamperes direct current), Type PC5 (Allied Control Co., New York, N. Y.), and one alternating current mercury plunger relay Type MP1-M (H. B. Electric Co., Philadelphia, Penna.).

The total cost of these parts was less than $17.

Acknowledgment

The writers wish to acknowledge the valuable assistance rendered by R. U. Bonnar of the Food and Drug Administration in the design of the phototube mechanism; also the helpful suggestions of J. J. Hopfield, physicist of the Naval Stores Research Division.

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Simplified Calibration of Dropping Mercury Electrodes

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THE diffusion current observed in polarographic measurements depends, among other factors, on the rate of flow of mercury from the dropping electrode, and hence this quantity (usually designated by m and expressed in the units mg. per second) is an important characteristic of a dropping electrode. In theoretical studies a knowledge of m is essential for interpreting diffusion current data by means of the Ilkovič equation (*1*, *2*). In practical analytical work frequent periodic determinations of m (and the drop time) under constant conditions serve as a check on the constancy of behavior of a given dropping electrode. Therefore it is highly desirable to have a method for determining m that is quicker and easier of execution than the rather laborious methods heretofore used, which involve weighing mercury collected from the dropping electrode over a measured interval of time (*2*). It was found that the determination of m can be expedited greatly by measuring the volume of mercury, rather than its weight, by means of the simple apparatus shown in Figure 1.

The instrument consists essentially of a calibrated glass capillary tube into which mercury, collected from the dropping electrode during a measured time interval, is drawn for measurement against a millimeter scale.

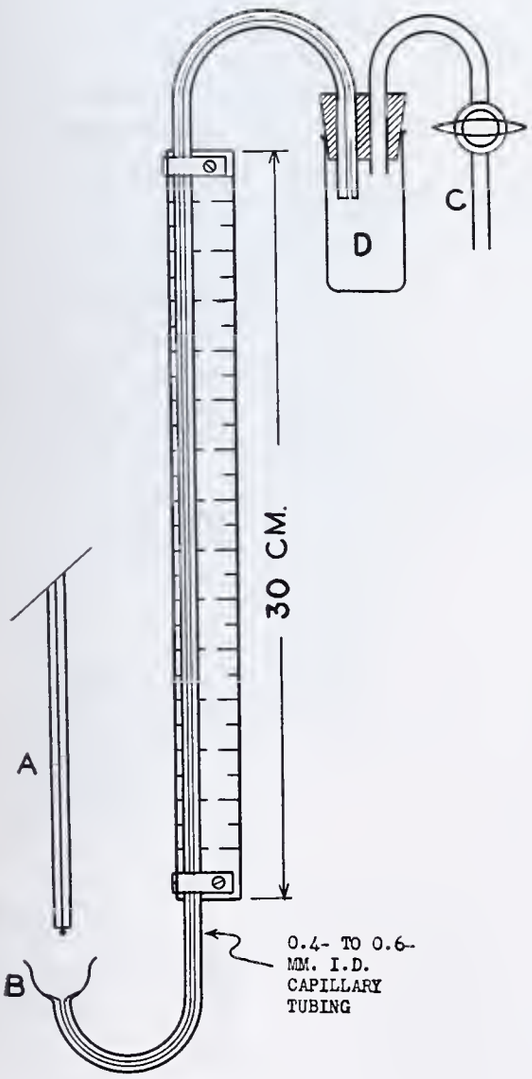

FIGURE 1

The mercury is collected from the dropping electrode, A, in the cup, B, which is so arranged that it can be rotated easily into or out of the path of the falling drops. The time of collection is noted with a stop watch, beginning and ending with the instant a drop falls. The globule of mercury is drawn up into the calibrated measuring tube by means of gentle oral suction at a rubber tube attached to tube C until the mercury thread is completely on the scale, and the stopcock is then closed. The length of the mercury thread is measured to ± 0.2 mm. against the scale (30-cm. section from a meter stick) and the corresponding weight of mercury is computed from previous calibration data. The mercury is then removed from the measuring tube by sucking it over into the weighing bottle, D, and the apparatus is ready immediately for another determination.

Pyrex capillary tubing of 0.4- to 0.6-mm. internal diameter and 6- to 8-mm. outside diameter is most suitable for the measuring tube. The tube is calibrated by measuring a thread of mercury in it, and then drawing the mercury over into the weighing bottle and weighing it. The capillary used by the author had an internal diameter of 0.470 mm. and 1 cm. on the scale corresponded to 23.45 mg. of mercury. Before fabrication the capillary should be tested for uniformity of bore by noting the length of about a 10-cm. mercury thread at various positions along the tube; the portion selected should be uniform within about ± 0.5 per cent over a 20-cm. length. To ensure an accuracy of ± 0.5 per cent in the value of m the mercury should be collected for a sufficient time to produce a thread at least 10 cm. long in the measuring tube. With the particular dropping electrode ($m = 2.63$ mg. per second) and measuring tube used by the author, only 90 seconds were required to produce a 10-cm. thread. A complete measurement can be made easily in 2 or 3 minutes, which is only a fraction of the time required to determine m by the gravimetric method.

Since m is independent of the medium in which the drops form (*2*, *3*), they can be collected either in water or in air. In a typical experiment the value of m with collection under water was 2.61 mg. per second, and 2.63 mg. per second when collected in air. Collection in air in a dry measuring tube is preferable, because when the measuring tube is wet water tends to be trapped in tiny droplets between the mercury thread and the glass, which makes accurate measurement difficult. The only disadvantage of collection in air is the lack of temperature control. The author found experimentally that the temperature coefficient of m is $+0.0031$ deg.$^{-1}$ between 0° and 25° C. Hence the error due to lack of temperature control will not exceed ± 1 per cent if the ambient temperature is within about $\pm 3°$ of that at which the electrode is to be used.

Incidentally, the temperature coefficient of m calculated by Kolthoff and Lingane (*2*, p. 75) is incorrect because of an error in the value used for the temperature coefficient of the density of mercury. Using the equation given by Kolthoff and Lingane (*2*, Equation 28, p. 75) and the correct values -0.000181 deg.$^{-1}$ and -0.0038 deg.$^{-1}$ for the temperature coefficients of the density and viscosity of mercury, respectively, the theoretical value of the temperature coefficient of m is $+0.0036$ deg.$^{-1}$. This agrees well with the foregoing experimental value.

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Laboratory Pressure Flowmeter

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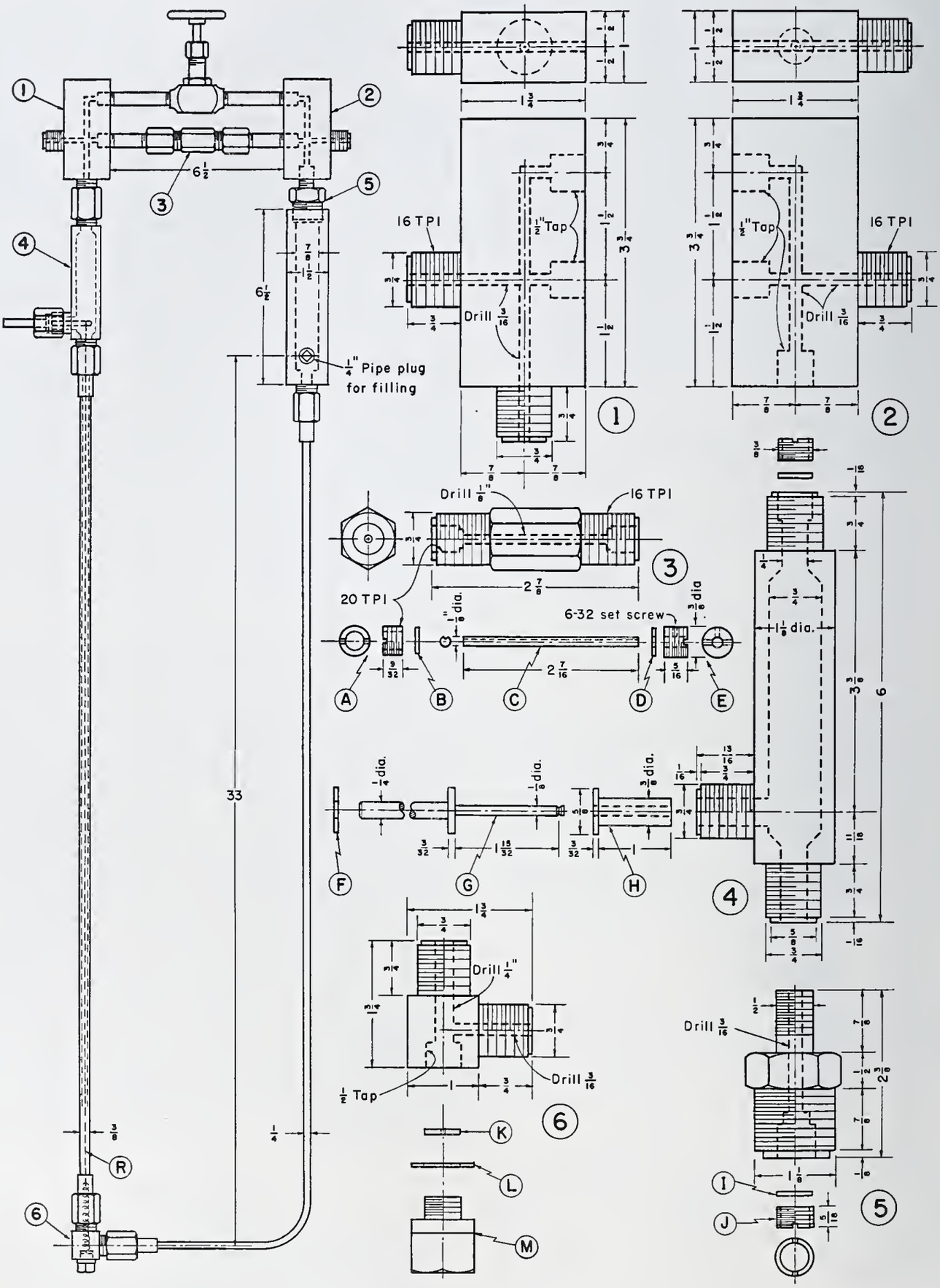

FIGURE 1 FLOWMETER

NO SATISFACTORY flowmeter, capable of measuring low gas rates under pressure, has been previously available, and, as a result, laboratory investigators who have studied gas and gas-liquid reactions under pressure have usually estimated the inlet gas rate from the products. This indirect procedure is unreliable because of the dissolved gas in the liquid products and because of side reactions which may take place. Heavy glass flowmeters have been used, but this practice is not to be recommended.

The authors have developed a metal flowmeter capable of measuring gas rates as low as 500 cc. per hour at normal temperature and pressure. The instrument described in this paper was designed for a maximum working pressure of 140 kg. per sq. cm., (2000 pounds per square inch) but this type of flowmeter can be designed for much higher pressures. In construction it is similar to the usual glass flowmeter, except that the orifice is a longitudinal scratch on the surface of a cylinder which fits snugly in a steel barrel, and the height of mercury is measured electrically.

Figures 1 and 2 show the construction of the flowmeter. All parts are of stainless steel unless otherwise stated.

FIGURE 2

A by-pass valve is connected to the distributing heads, 1 and 2. The orifice, 3, consists of a smooth cylinder, *C*, scratched longitudinally, which fits in the barrel (detail 3), a receptacle, *E*, which holds the cylinder in its place, and a hollow screw, *A*, for keeping a filled pulp-board filter, *B*, in position. The gasket, *D*, is copper.

In the top of the reservoir, 4, whose capacity exceeds by 30 per cent the volume of mercury used in the manometer, is placed a filter consisting of two single layers of filter paper sandwiched between three layers of 60-mesh nickel screen. An adapter, 5, is fitted with a similar filter, *I*, which is held in place by the hollow screw, *J*. These filters prevent mercury being blown out of the manometer. The left leg of the manometer (Figure 1) is glass-lined with 6-mm. Pyrex tubing approximately 0.8 of its length to insulate it.

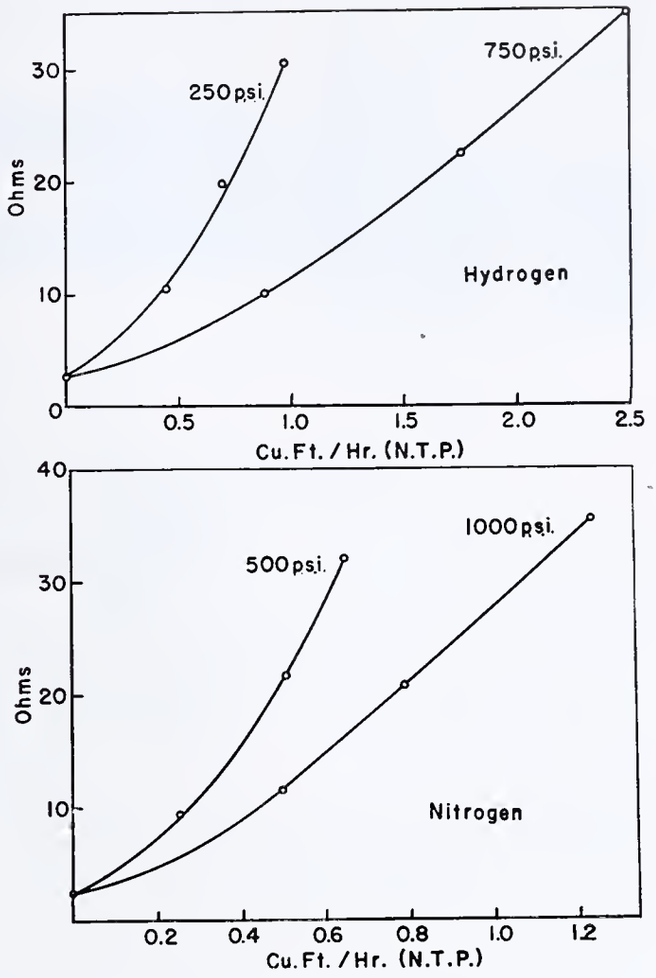

FIGURE 3

The reservoir, 4, is equipped with an insulated, electrical lead which consists of a shaft, *G*, fiber insulators, *F* and *H*, which also serve as gaskets, and a packing nut. The small end of the shaft is grooved and a small hole is drilled diametrically from the bottom of the groove through the shaft. This arrangement serves to anchor and guide a resistance wire, *R*, which extends from the shaft through the center of the glass-lined manometer leg and is fastened to a spring about 5 cm. (2 inches) long, the lower end of which is secured by the plate, *K*, in the tee, 6. The gasket, *L*, is copper. All slack is taken out of the wire by turning the shaft, *G*, sufficiently before the packing nut is tightened. *M* is a packing nut.

The resistance wire, *R*, forms one leg of a Wheatstone bridge, the resistance of which depends on the height of mercury in the manometer. An inexpensive resistance meter can be made from a 0–1 milliammeter, a 5000-ohm resistor, a 1000-ohm variable resistor, a switch, and a 6-volt battery. The resistors, switch, battery, and resistance wire are connected in series to form a closed circuit and the milliammeter is connected in parallel with the resistance wire. The current through the circuit is maintained at 1.0 milliampere by means of the variable resistance.

Figure 3 gives calibration curves for hydrogen and nitrogen, using an orifice cylinder with a 30-degree V-shaped groove, 0.075 mm. (0.003 inch) deep.

Acknowledgment

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Estimation of Magnesium with 8-Hydroxyquinoline

Microtitration and Photometric Procedures

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CERATE oxidimetry has been proved highly satisfactory for microtitration procedures in the determination of oxalate (*3*, *4*, *5*), has been employed in the estimation of glycerol (*7*), and has been applied by Nielsen (*6*) to the determination of magnesium in biological materials. The present status of the use of tetravalent cerium in volumetric analysis has been excellently summarized by Smith and Willard (*10*). In the application to magnesium the metal was precipitated as the 8-hydroxyquinolate and the organic portion oxidized with approximately 0.05 *N* ammonium hexanitrato cerate in 2 *M* perchloric acid.. Advantages claimed for the cerate oxidation are the elimination of the bromination of the hydroxyquinolate and the large cerate factor; one mole of magnesium required 59.7 equivalents of the oxidizing agent as compared with a factor of 8 in the bromination procedure.

A rapid sensitive method for the estimation of magnesium was needed in certain studies on distilled liquors. In view of the findings of Kirk and Tompkins (*4*), which favored the use of ceric sulfate over hexanitrato (perchlorato) cerate for the oxalate microtitration, a comparison of these two oxidizing reagents with respect to 8-hydroxyquinoline was carried out. Essentially, it was a balancing of the advantage of the high oxidation potential of the perchlorato cerate against the greater stability of the ceric sulfate, particularly in view of the dilute solutions employed. Since the procedure is admittedly empirical it was considered advisable to learn of the influence of such factors as time and temperature of heating and the concentration of cerate and perchloric acid on the extent and rate of oxidation.

The microtitration technique using either ceric reagent proved less convenient and less precise than the photometric procedure. Conditions for the development and characteristics of the color produced in an acetic acid solution of magnesium quinolinolate by the addition of a dilute (3 per cent) solution of ferric chloride were investigated by means of a spectrophotometer.

Reagents

HEXANITRATO CERATE, 0.01 *N*, IN 2 *M* PERCHLORIC ACID SOLUTION. Hexanitrato ammonium cerate (5.5 grams, commercial) was dissolved in 1 liter of 2 *M* perchloric acid (334 grams of 60 per cent perchloric acid per liter) and the mixture was allowed to stand on a steam bath for 24 hours. Dilution of the strong acid before addition of the salt is imperative. After cooling, the solution was clarified by centrifuging in Pyrex bottles and decanting the clear supernatant liquid, and standardized against sodium oxalate as described by Smith and Getz (*8*). Stronger solutions, prepared by using the appropriate amount of salt, were standardized against sodium oxalate solutions of corresponding strengths.

CERIC SULFATE SOLUTION, 0.01 *N*. Anhydrous ceric sulfate (3.5 grams) was warmed with a solution containing 28 ml. of concentrated sulfuric acid and 28 ml. of redistilled water. Complete solution was effected by the gradual addition of about 500 ml. of water with continued warming. The solution was placed on a steam bath overnight, diluted to 1 liter with redistilled water, and standardized against sodium oxalate.

SODIUM OXALATE SOLUTION, 0.01 *N*. Sodium oxalate obtained from the National Bureau of Standards (0.6700 gram) was dissolved in water to make 1 liter of solution.

INDICATOR SOLUTION. A 0.1 per cent aqueous solution of Setopaline C was found satisfactory. Frequently some of the indicator would come out of solution upon standing overnight. However, heating the mixture on the steam bath for an hour as recommended by Kirk and Tompkins (*4*) served to regenerate the indicator. The end point was very sharp with both oxidizing agents, although it was necessary to add the indicator towards the end of the titration when the perchlorato cerate was used. This device entails no real difficulty, inasmuch as the approach to the end point is readily observed, even in dilute solutions, as the color of the titrated solution fades to a very pale green. About 15 seconds were allowed at the end point to permit the reappearance of any pink color. The final color change was from pink to colorless in dilute solutions and to yellow with stronger cerate reagents. Ferroin is satisfactory as an indicator in titrating with the oxidizing agent; but in the back-titration of excess cerate with sodium oxalate solution this indicator is destroyed too rapidly to be of any service.

8-HYDROXYQUINOLINE SOLUTION. A 2 per cent solution in 95 per cent ethanol.

FERRIC CHLORIDE SOLUTION. A 3 per cent aqueous solution of sublimed ferric chloride.

Stability of Reagents

The relative stabilities of the perchlorato cerate and ceric sulfate solutions previously reported (*4*) were observed in the authors' series. No change was noted in the ceric sulfate solution held for 3 months in a clear glass bottle on the laboratory shelf. Hexanitrato cerate solutions in 2 *M* perchloric acid, stored in amber bottles in diffused light when not in use, changed as follows:

Table I. Oxidation of 8-Hydroxyquinoline by Ammonium Hexaperchlorato Cerate

Magnesium	8-Hydroxyquinoline				Cerate Factor (2 *M* $HClO_4$)[a]
Micrograms	*Mg.*				
Influence of Magnesium					
0	1				30.7
5	1				31.6
50	1				29.3
100	1				30.3
500	1				29.0
Effect of Time of Heating					
		Time at 95°–100° C. *Min.*			
	1	15			30.7
	1	30			32.1
	1	60			31.5
	1	90			31.3
	1	120			33.2
Influence of Perchloric Acid Concentrations. Heating 15 Minutes at 95° to 100° C.					
		Cerate Normality	Perchloric Acid Molarity	Blank *Ml. 0.05 N Cerate*	
...	1	0.063	2	0.55	30.7
...	1	0.080	4	1.38	28.5
...	1	0.081	6	2.56	26.5
Effect of Normality of Cerate on Factors for Magnesium and 8-Hydroxyquinoline					
					Range / Av.
100	...	0.4580	...	...	62.8–64.1 / 63.9
100	...	0.1003	...	...	61.6–62.2 / 61.9
100	...	0.0513	...	...	58.9–60.2 / 59.8
100	...	0.0109	...	...	58.7–60.0 / 59.5
...	1	0.4550	...	...	33.0–33.7 / 33.6
...	1	0.0994	...	...	30.2–30.9 / 30.6
...	1	0.0520	...	...	29.1–28.3 / 28.8
...	1	0.0097	...	...	27.0–27.9 / 27.2

[a] Equivalents of cerate per mole of 8-hydroxyquinoline

Time *Days*	Cerate Normality	Change %
0	0.4570	...
59	0.4510	1.31
0	0.1010	...
59	0.0996	1.40
0	0.0522	...
59	0.0512	1.92
0	0.00972	...
59	0.00962	1.05

These changes are similar to those reported in an extended study by Smith and Getz (*9*). Since standardization involved only a simple titration, this was carried out frequently in the present study.

Aqueous Setopaline C solutions were found to give satisfactory end points after standing under ordinary laboratory conditions for a month when heated on the steam bath just before using.

The alcohol solution of 8-hydroxyquinoline showed little discoloration and was serviceable at the end of 2 weeks if kept in the refrigerator when not in use.

Microtitration with Perchlorato Cerate

Magnesium of a purity estimated at 99.98 per cent, obtained through the courtesy of the Dow Chemical Company, was treated with sulfuric acid diluted with redistilled water in an amount just sufficient to give complete solution. The stock standard solution contained 1000 p. p. m. or 0.1 per cent of magnesium. Dilutions with redistilled water of this stock solution to 5 p. p. m. were made as required.

Titration of the perchloric acid solution of the magnesium quinolinolate obtained from the standard solutions (Table II) served to reveal the errors arising from all sources in the method and provided a direct means of ascertaining the cerate factor for magnesium. The purity of the sample of magnesium available was more definitely known than that of the 8-hydroxyquinoline; hence, the choice of the former for determining the cerate factors.

For the direct oxidation of the 8-hydroxyquinoline alone a definite volume of a carbon tetrachloride solution of known concentration of the compound was transferred to a 50-ml. Erlenmeyer flask. The solvent was removed by evaporation using a gentle air stream, and to the residue was added a known volume of the standard perchlorato cerate solution. The mouth of the flask was closed by means of an inverted beaker. The mixture was heated in an air oven at 95° to 100° C. for the periods described in Table I. After the solution had been cooled the excess cerate was titrated with standard oxalate solution; the indicator, Setopaline C, was added shortly before the end point was reached.

The magnesium quinolinolate was obtained by the following procedure:

A volume of the standard solution containing the required amount of magnesium was transferred to a 15-ml. conical centrifuge tube, a drop of phenol red was added, and the solution was adjusted to the first appearance of a yellow color using dilute hydrochloric acid and ammonium hydroxide. After the addition of 0.1 ml. of hydroxyquinoline reagent for each 10 micrograms of magnesium present, dilute ammonium hydroxide was added to a definite red color and the solution placed on a steam bath for 15 to 30 minutes. The mixture was cooled, 1 ml. of 95 per cent ethyl alcohol was carefully introduced to form a layer, and the precipitate was collected by centrifuging at about 3000 r. p. m. for 15 to 20 minutes. The sediment was washed three times with 3-ml. portions of *N* ammonium hydroxide, each time placing a layer of 95 per cent ethyl alcohol on the wash fluid and centrifuging as described. After complete removal of residual alcoholic vapors by means of a slow current of air was assured, the washed precipitate was dissolved by warming with 5 ml. of 2 *M* perchloric acid. An excess of cerate reagent was introduced, the oxidation allowed to proceed at 95° to 100° C., and the excess cerate then titrated with standard sodium oxalate solution after cooling of the oxidation mixture.

Factors Affecting Oxidation of 8-Hydroxyquinoline

The fact that cerate oxidation rests on an empirical rather than a stoichiometric basis does not militate against its application. The advantages of the cerate reagents encourage the study of the conditions under which, for a given substance, results of satisfactory accuracy and precision can be obtained.

Quantitative expressions of certain factors that might affect the oxidation of 8-hydroxyquinoline are given in Table I. Magnesium apparently exerts no catalytic effect on the reaction with hexaperchlorato cerate. When the reaction takes place at 95° to 100° C. no significant increase in oxidation is attained by prolonging the heating period from 15 to 90 minutes, though a slightly higher factor is obtained by continuing the oxidation for 120 minutes. In accord with the increase in oxidation potential with increase in molarity of perchloric acid as reported by Smith and Willard (*10*) a lower value for the cerate factor was found when the hexaperchlorato solution was made 4 and 6 molar with respect to acid concentration. There is no advantage in employing the higher acid concentrations in the cerate reagent. Indeed, the value for the blank became very large and the results extremely inconsistent at a perchloric acid concentration of 8 molar. Small but definite increases in the cerate factor resulted when the normality of the cerate solution was increased.

Table II. Titration of Magnesium Hydroxyquinolate with 0.01 N Ammonium Hexaperchlorato Cerate

(Heating period 15 minutes)

Magnesium	8-Hydroxyquinoline Equivalent of Mg	0.0109 N Cerate	Cerate Factor: Magnesium	Cerate Factor: 8-Hydroxyquinoline
Micrograms	*Micrograms*	*Ml.*	*Equivalents per mole*	
50	596	11.18	59.3	29.6
50	596	11.37	60.2	30.2
50	596	11.04	58.5	29.3
10	119	2.24	59.5	29.8
10	119	2.18	57.9	29.0
10	119	2.32	61.6	30.8
Blank	...	0.22	...	...
Av.	...	...	59.5	29.8

The difference in factors between the 0.01 N and 0.05 N reagents is not significant, while the factor for 0.1 N solution is very little higher.

In the authors' work the weaker solutions adequately cover the range of concentrations of magnesium encountered. The approximately 0.5 N solution represents a saturated solution of ammonium hexanitrato cerate in 2 M perchloric acid at room tempratures. Occasionally an exceptionally high titration value was obtained. If these aberrant values are to be ascribed to unclean glassware, scrupulous care must be given to the removal of all oxidizable contamination of vessels.

The average values for the perchlorato cerate factors—namely, 59.5 equivalents per mole of magnesium and 29.8 equivalents per mole of 8-hydroxyquinoline, as shown in Table II—are in good agreement with those reported by Nielsen (*6*). These values were reproducible (±3 per cent) over a range of 5 to 50 micrograms using 0.01 N and 0.05 N cerate. When the operations were carried out entirely in centrifuge tubes and the washing of the precipitate was accompanied by thorough sedimentation in the centrifuge, satisfactory results could be obtained with as little as 5 micrograms of magnesium. The upper limit of the method may be extended to cover a considerable range, as the washed precipitate containing the magnesium may be dissolved and diluted to any desired volume, and an aliquot taken for final oxidation and titration. The above technique also serves to hold the blank to a very small amount.

Microtitration with Ceric Sulfate

For the oxidation with ceric sulfate the precipitate of magnesium hydroxyquinolate was dissolved in sufficient 4 N sulfuric acid to give a solution about 1 N in sulfuric acid for the final titration. Similarly, the acid concentration for the titration of the 8-hydroxyquinoline alone was held at approximately 1 N in sulfuric acid. Ceric sulfate in excess was added to the acid solutions, the reaction allowed to proceed at 95° to 100° C. for the times specified in Table III, and the back-titration carried out with standard ferrous ammonium sulfate adding the indicator, Setopaline C, at the beginning of the titration.

The data presented in Table III show clearly that ceric sulfate was much less effective than perchlorato cerate as an oxidizing agent for 8-hydroxyquinoline. The increased rate and more extensive oxidation of the perchlorato reagent outweigh any advantage derived from a greater stability of the ceric sulfate.

Photometric Procedure

The requirement of standard solutions and the necessity for frequent restandardization of these solutions led to a search for a suitable photometric procedure. Trial of the dilute solution of ferric chloride as set forth by Wolff (*11*) proved the technique well suited to the determination.

The factors involved were studied, employing a Coleman Universal spectrophotometer, single monochromator type, with band width of 35 μ. The precipitate of magnesium quinolinolate was obtained as related above. Following the final washing the vapors were completely removed and the sediment was dried by means of a gentle current of air. Solution was effected by the addition of 10 ml. of 1 per cent acetic acid to the centrifuge tube, which was then placed in hot water for about 5 minutes. The solution was cooled to room temperature and 0.2 ml. of a 3 per cent solution of ferric chloride added. Upon mixing, a green color was produced; development of full color was complete in 5 minutes and was found stable for 24 hours. To ensure the removal of adventitious particles, the tubes in which the color was developed were centrifuged for a few minutes.

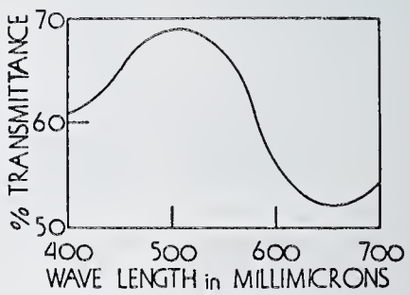

Figure 1. Spectral Transmittance Curves for Iron Hydroxyquinolate in Acetic Acid

The spectral transmittance curve for the colored solution is given in Figure 1. Absorption maxima occur at 650 mμ and, to a lesser degree, at 425 mμ. A transmittance-concentration curve, Figure 2, for wave length 650 mμ, was constructed from the data for 5, 10, 25, 50, 75, and 100 micrograms of magnesium. The relationship between optical density, D, and transmittance, T, has been expressed and defined by Ashley (*1*) as follows:

$$D_\lambda = \log \frac{I_{0\lambda}}{I_\lambda} = K_\lambda cl$$

$$\text{Since } \log \frac{I_{0\lambda}}{I_\lambda} = \log \frac{1}{T}$$

$$\text{then } D_\lambda = \log \frac{1}{T} = K_\lambda cl \qquad (1)$$

Density, D_λ, is then equal to the logarithm of the reciprocal of the transmittance and directly proportional to the concentration of a solution. When c is expressed in moles per liter, the value K_λ becomes the molecular extinction coefficient and may be calculated from the expression

$$\frac{D_\lambda}{cl} = K_\lambda \qquad (2)$$

Table III. Titration of Magnesium Hydroxyquinolate with Ceric Sulfate

(Heating periods, 1 hour and 4 hours)

Heating Period	Magnesium	8-Hydroxyquinoline Equivalent of Mg	0.00996 N Ceric Sulfate	Cerate Factor: Magnesium	Cerate Factor: 8-Hydroxyquinoline
Hours	*Micrograms*	*Micrograms*	*Ml.*	*Equivalents per mole*	
1	50	596	5.66	27.4	13.7
	50	596	5.60	27.1	13.6
	50	596	5.77	27.9	14.0
	10	119	1.28	31.2	15.5
	10	119	1.31	31.7	15.9
	10	119	1.27	30.8	15.4
				Av. 29.4	14.7
4	50	596	7.10	34.3	17.1
	50	596	7.12	34.4	17.2
	50	596	7.12	34.4	17.2
	10	119	1.38	33.5	16.7
	10	119	1.45	35.2	17.6
	10	119	1.42	34.4	17.2
	Blank	...	0.10	...	...
	Av.	...	...	34.4	17.2

Preference is given to this form of the Bouguer-Beer equation as the value for D may be read directly from the spectrophotometer used. In Figure 2 the optical density, D, is plotted against the concentration of magnesium. The points obtained fall on or very close to a straight line. Thus, within the precision of the instrument and in the range of 5 to 100 micrograms the solution obeys Beer's law. This concordance is further supported by the extinction coefficients calculated to a concentration of 1 microgram of magnesium per ml. (1 p. p. m.) with cuvette depth of 1 cm. at wave length 650 mμ as given in Table IV. The molecular extinction coefficient corresponding to the average value reported in Table IV is 1560.

As in the titration procedures, the upper limit of the photometric method may be extended by taking an aliquot of the acetic acid solution, adjusting the volume to 10 ml., and adding the ferric chloride solution. Concentration of an unknown solution may be taken from the curves relating transmittance and concentration, or density and concentration, or calculated from Equation 2. It is recognized that spectrophotometers are not available to many analysts; however, by choice of suitable concentrations of standard solutions and the appropriate filters the above procedure can be readily adapted to visual colorimeters of the Duboscq type and photoelectric comparators. To this end the combination, Wratten filter No. 29F plus Corning glass filter No. 430, 0.95 mm. in thickness, with a maximum transmission at wave length 650 mμ as established by Drabkin (*2*) should prove serviceable. Standard solutions are eliminated once the reference curve has been established for a given set of conditions.

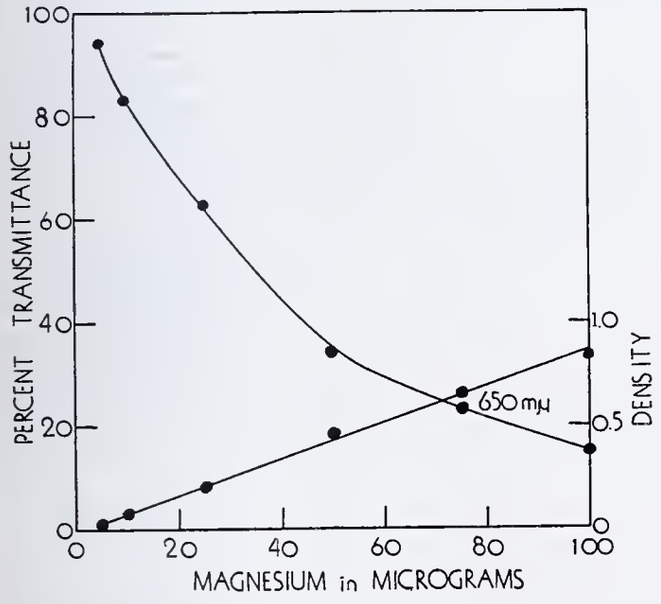

Figure 2. Transmittance Curves for Acetic Acid Solution of Magnesium Hydroxyquinolate and Ferric Chloride

Interfering Substances

The data presented in this paper, obtained on pure solutions, were the basis for subsequent application to the quantitative analysis of the ash of whisky for calcium, magnesium, iron, copper, lead, and tin. The scheme of separation and determination of these elements will be published later. Silica and members of the silver, copper, tin, iron, and aluminum groups must be removed; phosphate ion in large amounts results in loss of magnesium, and even in moderate amounts necessitates the use of excessive concentrations of ammonium chloride to keep the magnesium in solution during the preliminary separations. Since the authors' investigations required a determination of calcium, this element was removed before estimation of the magnesium. Wolff (*11*) states that magnesium may be separated from calcium by precipitation as the hydroxyquinolate at pH 8.2 to 8.5 and solution of the washed magnesium complex in 0.5 per cent acetic acid in which calcium quinolinolate is slightly soluble. Organic matter present in the original material is removed by wet- or dry-ashing.

Table IV. Extinction Coefficients

(Calculated for wave length 650 mμ from density of solutions of varying magnesium concentration)

Magnesium *Micrograms*	Density, 650 mμ	K (Extinction Coefficient)[a]
5	0.026	0.041
10	0.080	0.061
25	0.20	0.061
50	0.46	0.071
75	0.64	0.067
100	0.82	0.063
		Av. 0.064

[a] c = 1γ/ml.
l = 1 cm.

Summary

The determination of magnesium by precipitation with 8-hydroxyquinoline and oxidation of the organic part of the precipitate with ammonium hexaperchlorato cerate was found applicable to amounts of magnesium as small as 5 micrograms.

The factors 59.5 for the equivalents of cerate per mole of magnesium and 29.8 for the equivalents of cerate per mole of 8-hydroxyquinoline were reproducible when oxidation was carried out at 95° to 100° C. for not less than 15 minutes and with a cerate reagent 0.05 N or less at a perchloric acid concentration of 2 molar.

Ceric sulfate proved much inferior to the perchlorato cerate in rate and extent of oxidation of 8-hydroxyquinoline.

The conditions for development and characteristics of the color produced by ferric chloride when added to a solution of the magnesium hydroxyquinolate in 1 per cent acetic acid are described. In the range 5 to 100 micrograms the solution obeys Beer's law.

Advantages of the photometric procedure are the elimination of standard solutions that require relatively frequent restandardization, fewer operations once the washed precipitate has been prepared, and greater precision than the microtitration technique.

By means of the centrifuge all steps from the first precipitation up to the final reading in the photometric instrument are carried out in the same tube, thereby avoiding transfer losses and minimizing the correction for the blank.

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Evaporation of Standard Solution from the Tips of Microburets

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WHEN working with microburets having graduations on a tube of 2-mm. capillary bore or less (*3*, *4*), it is customary to immerse the tip in the solution to be titrated immediately after filling the buret and reading the position of the meniscus. With the buret of Schwarz (*6*) and with microburets using remote control (*1*, *2*, *5*, *7*) evaporation of standard solution may be prevented by keeping the opening of the tip inside the titration vessel and a little above the meniscus of the solution to be titrated. If, however, the tip is left in the open for any length of time, evaporation of standard solution becomes noticeable. The purpose of this investigation was to determine the consequences of such evaporation from the tip of microburets; it was proved that no error results with standard solutions containing a nonvolatile active agent, if some simple rules are followed.

The experiments were carried out with U-shaped burets with remote control (*5*), which may be described as measuring pipets having mechanical regulation of the air pressure in the space above the standard solution. Evaporation from the meniscus in the graduated capillary is highly improbable with these burets, for the air space above the meniscus is very small, of a shape to prevent convection currents, and in addition, is connected to a reservoir in which the air is saturated with water vapor.

From the outset it appeared obvious that no serious difficulties should be met with standard solutions containing a nonvolatile active agent, and most of the commonly used standard solutions belong in this category. When evaporation takes place from the tip of the buret, only solvent is lost. The corresponding active agent collects in the solution near the opening of the tip and is expelled with the next drop of standard solution. The decrease of volume is prematurely indicated in the graduated capillary, but no error will result, if the phenomenon is taken properly into consideration.

No error will result from evaporation between the time the zero reading is taken and the time at which the last portion of standard solution is added to the titrated solution. A buret reading, however, must not be taken following a period of significant evaporation. If an appreciable amount of time has lapsed after filling the buret, the solution in the tip must be rejected before the zero reading is taken. At the close of a titration the buret must be read a short time after addition of the last portion of standard solution from that buret. The appropriateness of these measures has been proved by a series of experiments in which evaporation was intentionally permitted to take place to an extraordinary extent.

Approximately 0.5 molar solutions of sodium hydroxide and hydrochloric acid were first titrated against one another in such a manner that no significant amount of evaporation could occur, and thus the correct ratio of centimeters of sodium hydroxide to centimeters of hydrochloric acid was obtained as the mean of three determinations equal to 1.046. In the following experiments one buret was used in a way to preclude a significant amount of evaporation. The other buret was read immediately after filling, and then was allowed to stand for 20 to 60 minutes, whereupon a second zero reading was taken just before starting the titration, which was conducted with proper precautions. The differences between the volumes corresponding to the first and second zero readings give the volumes of solvent which evaporated from the tip of the burets. In reading the burets, millimeter scales were placed behind the measuring capillaries which had a bore of approximately 0.8 mm. Thus, 1 cm. on the scales corresponded to approximately 5 cu. mm. of volume.

Table I indicates that even with 0.5 molar hydrochloric acid no significant amount of active agent is lost by evaporation from the tip of the buret. The ratios calculated with the use of the first zero readings are satisfactory, whereas those based on the second zero readings are grossly erroneous.

TABLE I. EVAPORATION FROM MICROBURETS

	Amount of Standard Solution NaOH *Cm.*	Amount of Standard Solution HCl *Cm.*	Ratio NaOH/HCl
0.5 *N* Acid and Base			
Evaporation prevented (mean of 1.044, 1.045, and 1.049)			1.046
Evaporation permitted from tip of NaOH buret			
Zero reading after filling	15.82	15.18	1.042
2nd zero reading after standing 60 minutes	14.82	15.18	0.976
Evaporation permitted from tip of HCl buret			
Zero reading after filling	14.90	14.30	1.042
2nd zero reading after standing 60 minutes	14.90	13.54	1.100
	I_2	$Na_2S_2O_3$	Ratio I_2/Na_2S_2O
0.1 *N* Iodine and Thiosulfate			
Evaporation prevented (mean of 1.131, 1.129, and 1.125)			1.128
Evaporation permitted from tip of iodine buret			
Zero reading after filling	15.70	13.50	1.163
2nd zero reading after standing 60 minutes	14.05	13.50	1.041

As is to be expected, standard solutions containing a volatile active agent are troublesome. If active agent and solvent would volatilize at rates that caused no change in the composition of the remaining solution, it would at least be possible to disregard evaporation occurring before the zero reading is taken. A fortunate coincidence of this kind will rarely happen, and in practice it must be expected that, in addition to a change of volume, some change of the composition of the standard solution will occur in the tip of the buret. This change of composition cannot be computed in a simple manner, and it becomes necessary to prevent loss of a significant amount of active agent during the whole time lapsing between last adjustment of meniscus previous to taking the zero reading and final establishment of the end point of the titration.

This statement is borne out by the experiment with 0.1 *N* iodine solution, the result of which is summarized in Table I. The ratio of centimeters of iodine to centimeters of sodium thiosulfate was determined. While care was taken to minimize evaporation from the tip of the thiosulfate buret, the tip of the iodine buret was left exposed to the atmosphere for 60 minutes after reading the zero position of the meniscus. A second zero reading was then taken immediately before starting the titration, and the table indicates that neither zero reading permits calculation of a satisfactory ratio.

Iodine solution and other standard solutions containing a volatile agent require filling the buret immediately before use or rejecting the contents of the tip immediately before taking the zero reading. The titrations must be started at once, and care must be taken to conduct the titration in a manner which assures that all active agents leaving the tip of the buret in either the dissolved or gaseous state will react with the titrated solution. The final reading must be made without much delay. If the titration is interrupted, a reading must be

taken before the buret is removed from the titrated solution, and when the titration is resumed the buret must be read again after rejection of the solution contained in the tip.

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Nicotinic Acid Content of Cereals and Cereal Products

Microbiological Method of Assay

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THE dietary position of cereals and cereal products in relation to their pellegra-preventive attributes has recently been given considerable prominence by the inclusion of nicotinic acid or niacin in the list of required components of enriched flour (*3*). (The word "niacin" has recently been accepted by the Federal Security Agency as a nontechnical synonym for the term "nicotinic acid".) This action has focused attention not only on the natural nicotinic acid content of flours designed for enrichment but also on the relative position occupied by the whole wheat from which these flours are derived, together with the other products obtained from milling.

Table I. Nicotinic Acid Assays of Extracts of Whole Wheat Flour

Type of Extraction	Nicotinic Acid
	μg./g.
Aqueous	37
1.5% sodium hydroxide	75
0.1 *N* sulfuric acid	37
3 *N* hydrochloric acid	72
Alkaline hydrolysis of aqueous extract	70

Search for information on this subject has been handicapped by the uncertainty of the suitability of the analytical methods used for estimating nicotinic acid, particularly chemical procedures. Kodicek (*4*) has emphasized the discrepancies observed in the analysis of cereals and pointed out the adverse role played by "chromogenic" substances of unknown composition. Similar observations have also been reported by Waisman and Elvehjem (*10*). Melnick, Oser, and Siegel (*6*) have applied an adaptation of the Melnick-Field chemical method (*5*) to the determination of flour and bread and reported fairly good agreement between the values thus obtained and those resulting from the application of the microbiological method of Snell and Wright (*9*). This observation lends support to the validity of the assay data and suggests a satisfactory specificity for the two types of procedures when the specified precautions are followed in the chemical method. This compatibility leaves the analyst with some latitude in the choice of methodology, with reasonable assurance that the selection will not introduce serious errors into the final assay results.

In the authors' laboratory the chemical and microbiological methods have been investigated for the purpose of comparing their applicability to cereal analyses, for both controlling the manufacture of enriched flours and evaluating other grain products. While in general fairly good agreement has been observed between the two types of procedures, values obtained chemically tend to be high. This is due to the difficulty of ensuring the complete removal of or compensation for "chromogenic" substances which are naturally present or are formed during extraction processes. For this reason microbiological assays have appeared to be more acceptable in instances where large discrepancies are observed.

Recent studies of the microbiological procedure have given rise to speculation about the accuracy of the nicotinic acid values thereby obtained. Depending on the procedure employed for extracting the cereal sample, assays varying more than twofold in magnitude can be produced.

Snell and Wright (*9*) in their original description of the microbiological method reported that alkaline extraction of cereals, in contrast to animal tissues, gives somewhat higher values than those obtained when water is employed for the extracting medium. They apparently attributed this to differences in extraction efficiency, since they found that finely ground samples gave comparable values by both extraction procedures. Oser, Melnick, and Siegel (*7*) also reported that alkaline extraction gives higher values but attributed this to hydrolysis rather than extraction since treatment of aqueous extracts with alkali raises the apparent nicotinic acid content to that found by direct extraction with sodium hydroxide. This explanation assumes that hydrolysis imparts greater availability to the test organism of some water-soluble derivative or precursor of nicotinic acid. The nature of this substance is unknown, since nicotinamide, cozymase, and nicotinuric acid all possess equivalent nicotinic acid activity to *Lactobacillus arabinosus 17–5* (*9*). The remaining derivative, trigonelline, is inactive and is not readily converted to an active form.

The authors' observations have shown even wider differences than those previously reported. These results have necessitated a careful inquiry into the relative behavior of various cereal products and a more extended evaluation of extraction procedures. The data presented in Table I demonstrate the influence of aqueous, acid, and alkaline solvents on the microbiological assay of a typical whole wheat flour.

Extraction Procedures

The aqueous extracts were prepared by autoclaving 1-gram samples with 90 ml. of water for 15 minutes at 6.8-kg. (15-pound) pressure. After cooling to about 50° C., 1 ml. of 6 per cent taka-diastase solution was added, and the mixture was allowed to cool at room temperature (30 to 40 minutes), made up to 100 ml., and filtered.

Alkaline extraction employed 50 ml. of 1.5 per cent sodium hydroxide. After autoclaving, the suspensions were neutralized to pH 6.5 with hydrochloric acid, made up to 100 ml., and filtered. Digestion with takadiastase after neutralization facilitates subsequent filtration and yields the same results. Where alkaline hydrolysis was carried out, 50 ml. of the filtered aqueous extracts were treated with 2.5 ml. of 30 per cent sodium hydroxide and autoclaved for 15 minutes at 6.8-kg. (15-pound) pressure. After neutralizing they were made up to 100 ml.

Acid extracts were prepared by autoclaving 1-gram samples with 50 ml. of 0.1 *N* sulfuric acid or 25 ml. of 3 *N* hydrochloric acid. The resulting suspensions were neutralized to pH 6.5 with sodium hydroxide, made up to 100 ml., and filtered. No digestion with takadiastase is required to facilitate filtration or improve extraction.

The assay values given in Table I show that water and dilute acid are comparable extraction media. Alkali and stronger acids are also comparable but yield twofold higher results. In confirmation of the observation reported by Oser, Melnick, and Siegel (*7*), alkaline hydrolysis of the clear aqueous extracts raises the nicotinic acid assays to essentially those obtained by direct extraction with alkali. Thus the factor responsible for the increase in apparent nicotinic acid is water-soluble but requires the action of alkali to exert its growth-promoting effect on the test organism.

The fact that 3 *N* acid also yields high values demonstrates that the unknown factor is also susceptible to acid hydrolysis. This observation is of interest since it suggests that trigonelline is not the substance responsible for the increased values. In order to confirm this suggestion trigonelline was subjected to the aqueous, acid, and alkaline extractions described above. In no instance was any nicotinic acid activity observed.

Since these studies indicated that fairly strong acid was required to produce the "hydrolytic effect", the action of alkali was examined. Autoclaved aqueous suspensions of a whole wheat flour were treated for various periods of time with different concentrations of sodium hydroxide at room temperature and on the steam bath. The results are shown in Table II.

Table II. Effect of Time, Temperature, and Concentration of Alkali on the Nicotinic Acid Assay

Concentration of NaOH	Nicotinic Acid: 5 minutes at room temperature	Nicotinic Acid: 30 minutes on steam bath
%	*Micrograms per gram*	
None	28	..
0.25	67	66
0.50	66	67
1.00	66	72
1.50	70	70

It is apparent that the effect of alkali is very pronounced, since the lowest concentration exerts in 5 minutes the same effect as six times this concentration for much longer periods at higher temperatures. Thus the factor produced by hydrolysis is formed easily and completely by alkali in contrast to the slower production by acid.

In order to gain some insight into the probable nature of this factor, recovery experiments were carried out using each of the several extraction procedures. While such experiments are of limited value for critically evaluating methodology, it was expected that variable recoveries would result if the factor was a growth-promoter unrelated to nicotinic acid. Similar studies demonstrated the presence of nonflavin growth factors in the microbiological assay of riboflavin in cereals (*2*).

Regardless of the type of extraction used, recoveries of added nicotinic acid were quantitative within the limits of experimental error. This finding was observed on a variety of cereal products, including whole wheat, patent and low-grade flours, wheat germ, starch, and gluten. For this reason it is doubtful if the effect of alkali noted above can be attributed to nonnicotinic acid growth-stimulating substances.

In view of the possibility that stimulators might exert a variable influence on the test organism over a prolonged growth period, a comparison of the assay values of aqueous and alkaline extracts of whole wheat flour was made at intervals during 78 hours' incubation at 37° C. Titration of acidity was carried out after 23, 30, 47, 54, 71, and 78 hours. The average for the aqueous extract was 31.5 micrograms per gram, and for the alkaline extract, 72 micrograms per gram. Since individual values did not show variations greater than ±5 per cent it is obvious that growth rates in the extracts are very similar to those in the nicotinic acid standards. If any stimulator is present in the extracts it is not revealed by this type of test.

Table III. Dialysis of Aqueous Extracts of Whole Wheat Flour

Time of Dialysis	Nicotinic Acid in Dialyzate: A[a]	B[a]	Ratio B/A	Nicotinic Acid in Undiffused Extract: A	B	Ratio B/A	Total: A	B
Hours	*μg./g.*	*μg./g.*		*μg./g.*	*μg./g.*		*μg./g.*	*μg./g.*
0	..	..	..	26.7	61.7	2.31	26.7	61.7
1	6.1	9.4	1.54	20.0	47.3	2.36	26.1	56.7
2	4.7	6.1	1.30	16.5	41.5	2.52	27.3	57.0
3	3.4	4.6	1.35	12.6	36.7	2.92	26.8	56.8
4	2.8	3.8	1.36	10.4	30.5	2.93	27.4	54.4
24	..	..	..	5.0	17.2	3.45	..	..

[a] A, before hydrolysis; B, after hydrolysis with alkali.

Several attempts have been made to separate the naturally active nicotinic acid compounds from the factor which is activated by hydrolysis. Extraction of whole wheat flour with alcohol has been tried by a variety of methods: Soxhlet extraction, direct heating with solvent, and addition of large volumes of alcohol to the sample after gelatinization in water and digestion with takadiastase. In all instances a separation into alcohol-soluble and alcohol-insoluble fractions was obtained but both exhibited nicotinic acid activity which was markedly enhanced by treatment with alkali.

In another experiment acidified aqueous and alkaline extracts were shaken with Lloyd's reagent. The adsorbates were washed thoroughly and assayed directly by suspending in the culture medium. That obtained from the aqueous extract gave an assay value of 38 micrograms per gram (based on the whole wheat flour) while that from the alkaline extract gave a value of 73 micrograms per gram. The solutions remaining after adsorption showed no nicotinic acid activity. Even after alkaline hydrolysis no growth was observed with the aqueous extract which had been treated with the Lloyd's reagent. This demonstrates that adsorption of the naturally active nicotinic acid compounds and the unknown factor, both before and after hydrolysis, occurs simultaneously and completely under the experimental conditions employed. For this reason separation of these constituents cannot be accomplished by such treatment.

Dialysis experiments have given the most promising results. Aqueous extracts of whole wheat flour were dialyzed against water by means of cellophane membranes. The data obtained from a typical experiment are shown in Table III.

It is evident that both nicotinic acid factors diffuse through the cellophane (No. 450), but at different rates. The naturally active components dialyze more rapidly, resulting in an appreciable increase in the ratio of the hydrolyzable factor to the "free" nicotinic acid compounds in the undiffused fraction.

The four dialyzates represent the amounts diffused during the first, second, third, and fourth hours, respectively. The slower diffusion rate of the hydrolyzable factor is reflected in

the lower ratio between this and the naturally active portion. Dialysis in these preliminary tests has thus reduced the concentration of hydrolyzable factor in the dialyzate to 40 per cent of the free nicotinic acid, and has concentrated it in the original extract to nearly 200 per cent. Studies are now under way to improve this separation further, so that each factor can individually be studied physiologically.

While most of the authors' studies have been confined to whole wheat flour, a few assays have been carried out on other cereal products, including a complete set of the fractions separated in the commercial milling of wheat (Table IV).

The values found by the two extraction procedures are of interest since they indicate the ratio of natural nicotinic acid activity to the total activity after hydrolysis. While the limit of experimental error urges caution in interpreting a significant differentiation between the three flours and the three feeds, the position of germ is definitely outstanding. The response shown by treatment with alkali is much below that observed for the other wheat fractions.

Since the germ sample contained appreciable quantities of other portions of wheat, which should contribute to the effect produced by alkaline extraction, it seemed probable that a more highly purified preparation would give the same assay value regardless of the extraction procedure. Such actually proved to be the case. A sample of germ of 85 to 90 per cent purity gave by aqueous extraction a value of 42.4 micrograms per gram; by alkaline extraction, 43.8 micrograms per gram. It seems rather significant that one part of the wheat berry should differ so markedly from all the rest, but no explanation can be offered in the light of our present knowledge.

This behavior of wheat germ is analogous to that exhibited by animal tissues, since Snell and Wright (*9*) have shown that alkali does not increase the assay values of a variety of animal organs. Nor is yeast influenced by the extraction procedure since practically identical values of 215 and 204 micrograms per gram were obtained by aqueous and alkaline extraction, respectively.

Table IV. Distribution of Nicotinic Acid in the Products of Wheat Milling

(As determined by microbiological assay of aqueous and alkaline extracts)

Mill Fraction	Nicotinic Acid		Ratio of Alkaline to Aqueous	% of Total N. A. in Mill Fraction
	Aqueous	Alkaline		
	μg./g.	μg./g.		
Patent flour	6.6	12	1.83	10.2
First clear flour	13.4	26	1.94	3.2
Second clear flour	46	83	1.80	4.7
Red dog	74	120	1.62	7.6
Shorts	96	159	1.66	17.8
Bran	197	330	1.68	56.3
Germ	56	68	1.21	0.2
Wheat	38	70	1.82	100.0

While in such materials as yeast, germ, and animal organs both vegetable and animal sources are represented and accordingly cannot be differentiated on such a basis, it is rather striking that in all cases we are dealing with "living" or potentially living tissues. On the other hand, such products as bran and flour entirely lack this attribute and are present in wheat primarily to protect the germ and supply it with food during the early stages of germination. This suggests a possible differentiation of two forms of nicotinic acid activity based on the cellular aggregates with which they are associated, biologically active tissues possessing a more readily available form than "storage" tissues where hydrolysis is required to liberate complete activity.

For the purpose of estimating the importance of cereals as dietary sources of nicotinic acid, it is of vital importance to decide the nutritional roles played by each of these types of activity. Are both fully utilized by animal organisms, or is only the naturally active form available? This question must be answered before present analytical methods can be applied to the evaluation of cereals and cereal products. The resistance of the hydrolyzable factor to dilute acids suggests that gastric digestion would fail to liberate activity while the sensitivity to alkali would operate favorably in subsequent digestive processes. This assumes, of course, that the unknown factor exerts activity comparable to that displayed toward the *Lactobacillus arabinosus*.

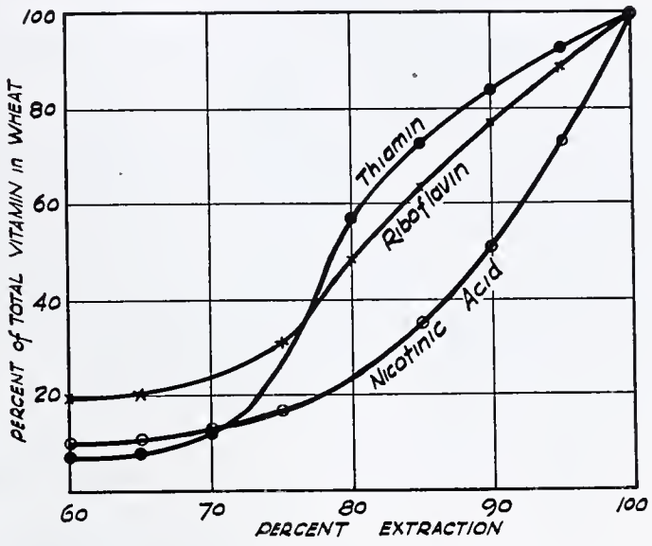

Figure 1. Distribution of Vitamins in Products of Wheat Milling

In the event that only the naturally active nicotinic acid and nicotinic acid derivatives are of nutritional value, careful consideration must be given to the condition employed for extraction. While the authors' data are not yet complete, it is apparent that the less active form is hydrolyzable even by water. In one experiment 15 minutes' autoclaving gave a value of 33 micrograms per gram; extending the autoclaving time to one hour increased the value to 45 micrograms per gram. For this reason more drastic extraction conditions may be expected to yield high results, intermediate between those representing natural activity and total activity after complete hydrolysis. If, on the other hand, the total activity is nutritionally available, extraction should be performed either with alkali or fairly strong acid in order to ensure complete utilization by the test organism.

Returning to the assays of the mill fractions, the values expressing per cent of total nicotinic acid are averages of those calculated from the assays of each type of extract. This seemed entirely justified, since the two sets were very similar. The greatest variation was observed in the case of patent flour, where the aqueous assay values showed it to contain 9.8 per cent of the total nicotinic acid; alkaline assay values were 10.6 per cent. This agreement between the two extraction procedures is further indication that a homogeneous relation exists between the two forms of nicotinic acid activity, since it is difficult to conceive of a condition where non-nicotinic acid factors would so closely parallel the nonuniform distribution of nicotinic acid activity in the various fractions. Once again, however, the evidence requires more direct substantiation.

The distribution of nicotinic acid (Figure 1) in the products of milling differs from that of either thiamin or riboflavin (*1*, *8*). The germ is relatively low in nicotinic acid content, since thiamin and riboflavin are present in six- to eight-fold concentrations of those existing in whole wheat. This is compensated for by the relatively higher concentration in the bran, which of all the commercial mill fractions is the richest

source of nicotinic acid activity. In patent flour the relation of thiamin and nicotinic acid is similar, since both contain approximately 10 per cent of the whole wheat total. However, because thiamin is more concentrated in the tissues represented by the second clear flour, red dog, and shorts, longer extraction flours will not show the large increases in nicotinic acid activity that they show in thiamin.

Summary

The nicotinic acid assay of cereals and cereal products by the microbiological method is influenced by the type of extraction employed. Water and dilute acids yield lower values than stronger acids and alkali. This discrepancy can be attributed either to the formation of growth-stimulating substances by the latter solvents or to the liberation of active nicotinic acid compounds by hydrolysis of a less active or inactive precursor. The evidence presented, while not conclusive, suggests that the second explanation is more likely to be correct. If this is true, the choice of extraction procedure required for evaluating cereals will depend on the nutritional availability of the activity liberated by hydrolysis.

Wheat germ, unlike bran and endosperm, does not exhibit enhanced activity on alkaline hydrolysis. In this respect it resembles yeast and animal tissues.

The distribution of nicotinic activity in the various fractions obtained by commercial milling differs from that of thiamin and riboflavin. Concentration is greatest in the bran.

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Growth Stimulants

In the Microbiological Assay for Riboflavin and Pantothenic Acid

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IT HAS been the observation of workers in this laboratory that a discrepancy in results exists when aqueous extracts of certain foodstuffs are assayed microbiologically for their riboflavin and pantothenic acid content with and without the inclusion of the extracted residues.

In the original microbiological riboflavin assay method, Snell and Strong (*6*) extracted the riboflavin by autoclaving the natural foodstuff in a large volume of water, or by boiling with dilute acid. The extracted residues were usually removed by centrifugation, but in some cases suspensions were assayed directly. Pennington, Snell, and Williams (*5*) in their microbiological pantothenic acid assay method make no definite suggestions for the removal of extracted residues from the autoclaved aqueous suspensions, but state that if necessary, fuller's earth or kieselguhr may be used to clarify the extract. On the other hand, Strong, Feeney, and Earle (*7*) state that autoclaved aqueous suspensions of finely ground samples may be used without the removal of the extracted residues, in their recent microbiological assay for the determination of pantothenic acid.

It is known that the extraction procedure varies in different laboratories employing these microbiological techniques, and while the present authors believe that little or no differences result from these varied extraction procedures in the case of many foodstuffs, they are convinced differences do occur in certain ones which are reported in this investigation.

Procedure

In the microbiological riboflavin assays reported herein the procedure of Snell and Strong (*6*) was carried out, while the pantothenic acid assays were conducted according to the microbiological procedure described by Pennington, Snell, and Williams (*5*), with modifications which included the shaking of all assay tubes after 16 to 20 hours of incubation and the use of a stab culture medium which more closely resembles the assay media:

Stab Culture Medium

Glucose, grams	2
Peptone solution, ml.	20
Cystine solution, ml.	20
Salt solution A, ml.	1
Salt solution B, ml.	1
Yeast extract (Difco), gram	1
Agar, grams	3.5
Adjust to pH 6.7, dilute to 200 ml., add agar, melt, and sterilize in tubes	

The preparation of the above ingredients has been described by Snell and Strong (*6*). The test organism used in these assays was *Lactobacillus casei*. The growth response was determined by the titer of the lactic acid produced after a 72-hour incubation period, except where an occasional turbidimetric evaluation was made, using the Coleman Universal spectrophotometer at a wave length of 650 mμ. Merck's synthetic riboflavin and synthetic dextrorotatory calcium pantothenate served as the assay standards.

Riboflavin Assay of Extracts with and without Residues. Samples of dried skim milk, distillers' dried solubles, meat scraps, fish meal, alfalfa leaf meal, and commercial liver meal in amounts of 0.5 to 1.0 gram were autoclaved with 25 to 35 ml. of distilled water for 15 minutes at 15 pounds' pressure in conical 50-ml. centrifuge tubes. After cooling, the tubes were centrifuged and the supernatant liquid was decanted. Another 25- to 35-ml. portion of water was added to the residue and the entire procedure was repeated. A third volume of water was then added to the residue and the tubes were recentrifuged. The clear centrifuged wash water was combined with the supernatant liquid from the two extractions and made to volume. Sometimes a filtration was necessary. The final extracted residues were resuspended in distilled water and made to volume. Equal parts of the clarified extract and the suspended residues were combined and designated as the water extract with residual solids suspended.

The results of the riboflavin assay of the clarified water extract, and the water extract with solids suspended, are presented in Table I.

Table I. Riboflavin Values for Foodstuffs

Foodstuff	Microbiological Assay: Clarified water extract	Water extract with solids suspended	Clarified water extract plus 40 to 60 micrograms of methyl palmitate[a] per tube	Water extract of ether-extracted foodstuff with solids suspended	Fluorometric Determination (3)
	Micrograms of riboflavin per gram				
Dried skim milk A	19.7	19.8	28.9	....	22.6
Dried skim milk B	16.1	16.9	..	16.4	17.2
Commercial liver meal A	57.4	56.8	55.3	34.5, 33.8[b]	31.6
Commercial liver meal B	55.7	83.5	84.0	42.2	33.8
Alfalfa leaf meal	14.1 14.7	14.4 16.0	19.2 18.7	13.6	15.4
Meat scraps	6.2	8.7	10.6	5.4	6.0
Fish meal	4.6	7.0	9.7	5.2	5.5
Distillers' dried solubles	15.9	22.0	23.8	16.9	15.6

[a] 0.95% ethyl alcohol solution.
[b] Carbon tetrachloride extracted.

Good agreement was obtained between the riboflavin values for dried skim milk, alfalfa leaf meal, and one sample of liver meal by the two methods of extract preparation. Higher riboflavin values were obtained on meat scraps, fish meal, distillers' dried solubles, and a second sample of liver meal by assay of the extracts with solids suspended as compared to the values on the clarified extracts.

Stimulatory Activity of Extracted Residues. In order to ascertain whether or not all the riboflavin was extracted from the last-mentioned foodstuffs, the water-extracted and washed residues were assayed for their riboflavin content in the presence and absence of added synthetic riboflavin. The results of this experiment are shown in Table II. The addition of the extracted residues to the basal medium produced no significant increase in the growth of *L. casei* over that of the basal medium, as shown by titration values, except in the case of meat scraps where apparently all of the riboflavin was not removed. In the presence of 0.10 microgram of riboflavin per tube the extracted residues of the liver meal and meat scraps produced an appreciable growth-promoting effect. It is therefore observed that these extracted residues, which appeared to contain no riboflavin, exerted a growth-stimulating action on the microorganism when incorporated in the medium with a suboptimum amount of synthetic riboflavin. The extracted residue of dried skim milk was microbiologically inert, while the residue of alfalfa leaf meal exerted a little stimulatory action.

The extracted residue of distillers' dried solubles will also produce the stimulatory phenomenon shown above.

Two samples of 4 grams each were extracted with a total of 800 ml. of water and a total of 660 ml. of 1.0 *N* hydrochloric acid, respectively, in four separate autoclaving extraction periods of 10 minutes each. After each autoclaving period, the suspensions were centrifuged, the supernatant liquid was decanted, and the residues were re-extracted with another portion of solvent. After the final extraction, the residues were dried and tested for their riboflavin content and stimulatory action. Portions of the aqueous extracted residues were extracted with acetone and methanol, respectively, for 12 hours and portions of the acid residues were extracted with acetone and ethyl ether, respectively, for 16 hours, the resulting extracts and residues of which, likewise, were assayed for their growth-stimulating effect.

The data are presented in Table III. It will be observed that the aqueous and acid-extracted residues exerted no growth-stimulating effect in the absence of riboflavin in the assay medium, but were found to have a considerable stimulatory action, being greater for the acid-extracted residues, in the presence of 0.10 microgram of riboflavin per tube. This stimulatory action was not due to the mere presence of suspended solids, for the stimulatory agent could be extracted from the aqueous and acid residues by organic solvents.

Additional experimentation on the saponification of an ether extract of distillers' dried solubles has revealed the stimulatory activity to be present in both the unsaponifiable and saponifiable fractions, the latter fraction, however, having the major part of the activity.

Stimulatory Activity of Photolyzed Extracts. The stimulatory phenomenon was also studied in another fashion. It has been known for some time that riboflavin in alkaline solution is destroyed by irradiation.

Aqueous suspensions of four foodstuffs mentioned above were autoclaved for 15 minutes, cooled, and adjusted to pH 8.0 to 9.0. The suspensions were then introduced into 1-liter Erlenmeyer flasks, plugged with cotton, and autoclaved for 5 more minutes. After cooling, these flasks were suspended over 100 to 200-watt electric light bulbs and irradiated through the bottom of the flasks for 24 to 30 hours. Following the sterile irradiation period, the contents of the flasks were adjusted to pH 6.6 to 6.8, and made to volume, and aliquots were assayed microbiologically.

The results are shown in Table II. Little or no growth response was obtained by incorporating the photolyzed

Table II. Stimulatory Action of Aqueous Extracted Residues and Photolyzed Aqueous Suspensions of Foodstuffs

(In the presence of suboptimum amounts of riboflavin)

Material	Mg. per Tube of Original Foodstuff	Titration Values as Ml. of 0.1 *N* Alkali: Basal medium	Basal medium plus preparation	Micrograms of Riboflavin: Added per tube	Recovered per tube	Per Cent Stimulation
	Aqueous Extracted Residues					
Dried skim milk	4.0	0.75	0.85	0.10	0.102	2
	6.0	0.75	0.90	0.10	0.103	3
Commercial liver meal B	1.0	0.80	0.80	0.10	0.159	59
	2.0	0.80	0.80	0.10	0.172	72
Alfalfa leaf meal	6.0	0.75	0.70	0.10	0.111	11
	9.0	0.75	0.70	0.10	0.114	14
Meat scraps	10.0	0.90	1.40	0.10	0.158	58
	15.0	0.90	1.40	0.10	0.158	58
	Photolyzed Aqueous Suspensions					
Dried skim milk	4.0	0.90	0.90	0.10	0.101	1
	6.0	0.90	0.90	0.10	0.104	4
Commercial liver meal B	1.0	0.90	1.15	0.10	0.146	46
	2.0	0.90	1.35	0.10	0.176	76
Alfalfa leaf meal	6.0	0.90	1.00	0.10	0.109	9
	9.0	0.90	0.90	0.10	0.122	22
Meat scraps	10.0	0.90	1.00	0.10	0.142	42
	15.0	0.90	1.00	0.10	0.156	56

Table III. Stimulatory Action of Extracted Residues of Distillers' Dried Solubles

(In the presence of suboptimum amounts of riboflavin)

No.	Extracted Residue	Mg. per Tube of Original Foodstuff Extracted	Titration Values as Ml. of 0.1N Alkali: Basal medium	Basal medium plus extraction residue	Micrograms of Riboflavin: Added per tube	Recovered per tube	Per Cent Stimulation
1	Distillers' dried solubles (extracted 4 times with water)	4.0	1.0	0.80	0.10	0.128	28
		6.0	1.0	0.80	0.10	0.134	34
2	Acetone extract of 1	4.0	1.0	0.80	0.10	0.148	48
		6.0	1.0	0.90	0.10	0.162	62
3	Acetone residue from 2	4.0	1.0	1.00	0.10	0.108	8
		6.0	1.0	..	..	...	...
4	Methanol extract of 1	4.0	1.0	0.70	0.10	0.134	34
		6.0	1.0	0.60	0.10	0.134	34
5	Methanol residue from 4	4.0	1.0	0.80	0.10	0.108	8
		6.0	1.0	0.90	0.10	0.106	6
6	Distillers' dried solubles (extracted 4 times with 1.0 N HCl)	4.0	0.95	0.80	0.10	0.137	37
		6.0	0.95	0.80	0.10	0.163	63
7	Acetone extract of 6	4.0	0.95	0.80	0.10	0.166	66
		6.0	0.95	0.60	0.10	0.180	80
8	Acetone residue from 7	4.0	0.95	1.00	0.10	0.112	12
		6.0	0.95	1.00	0.10	0.109	9
9	Ethyl ether extract of 6	4.0	0.95	0.90	0.10	0.177	77
		6.0	0.95	1.00	0.10	0.202	102
10	Ethyl ether residue from 9	4.0	0.95	0.70	0.10	0.106	6
		6.0	0.95	0.70	0.10	0.101	1

aqueous suspensions of the foodstuffs in the medium, thus indicating the absence of riboflavin. However, in the presence of the suboptimum amount of 0.10 microgram of riboflavin per tube, photolyzed aqueous suspensions of liver meal, and meat scraps, produced considerable stimulatory action, dried skim milk no stimulation, and alfalfa leaf meal a small amount. These results are in general agreement with those obtained on the aqueous extracted residues also reported in Table II.

Stimulatory Activity of Known Compounds. Based on the knowledge obtained in previous work a number of known compounds were tested for their stimulatory power. Water-insoluble salts, glass, etc., were finely ground before they were added to the medium. The water-soluble compounds were dissolved and made to volume. Water-insoluble, alcohol-soluble organics were first dissolved in ethyl alcohol and water was added until the solution was 0.95 per cent ethyl alcohol. Usually a colloidal solution resulted. The amount of alcohol introduced in this manner before sterilization of the assay tubes was tried repeatedly without the observation of significant stimulation or inhibition. The compounds were added in the absence of riboflavin and in the presence of 0.10 microgram of riboflavin per tube. Sufficient data are presented in Table IV on a few of the compounds to show the results of their addition under the conditions of the experiment.

The addition of 20 micrograms or more per 10 ml. of medium of calcium stearate, palmitic acid, linoleic acid, or diglycol laurate in the presence of suboptimum amounts of riboflavin produced marked stimulation, while in the absence of riboflavin no growth response occurred over that obtained on the basal medium alone. Calcium oxide, *n*-caproic acid, or ethyl laurate did not produce significant growth-stimulatory response either in the absence or presence of added synthetic riboflavin. The highest level of linoleic acid markedly inhibited growth. This marked inhibition has been observed in only one other instance when 160 micrograms per 10 ml. of medium of duodecyl alcohol were added in the presence of 0.10 microgram of riboflavin per tube.

Up to this point the effect of a stimulatory compound has been shown only in the absence of riboflavin and the presence of 0.10 microgram of riboflavin per 10 ml. of medium. Figure 1 demonstrates the typical reaction on the addition of graded amounts of a stimulatory compound (in this case Merck's 90 per cent lecithin was used) to the medium with increasing levels of riboflavin until the optimum riboflavin requirement for the growth of the organism was reached.

The figure shows that lecithin is not a vitamin or growth factor for *Lactobacillus casei*, for its addition to the medium in the absence of riboflavin or in the optimum amount of riboflavin resulted in no additional growth response. It does, however, appear to assist the microorganism to make more efficient use of suboptimum amounts of riboflavin and therefore may be said to have riboflavin-sparing action.

The space required for the manifestations of the effect of all compounds tried is not warranted here. However, all compounds used (Table V) have been divided into two groups: (1) those displaying appreciable stimulation when added to the medium in the presence of 0.10 microgram of

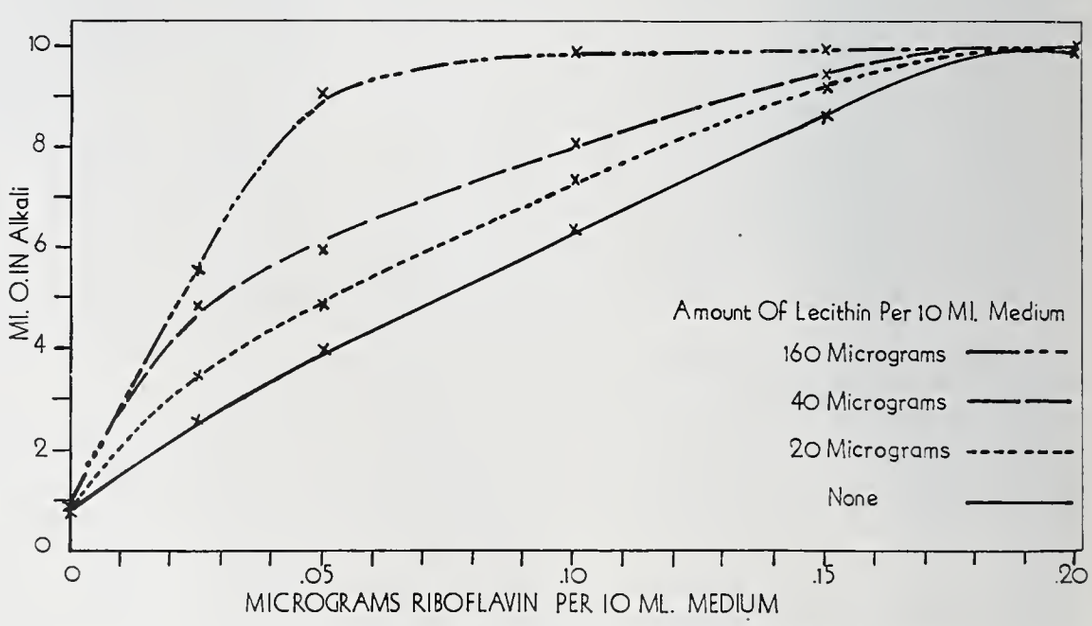

Figure 1. Response of *L. casei* to Lecithin in the Presence of Riboflavin

Table IV. Stimulatory Action of Known Compounds

(In the presence of suboptimum amounts of riboflavin)

Compound	Micrograms per Tube	Titration Values as Ml. of 0.1 *N* Alkali: Basal medium	Titration Values as Ml. of 0.1 *N* Alkali: Basal medium plus compound	Per Cent Stimulation in Presence of of 0.10 Microgram of Riboflavin per Tube
Calcium stearate	20	1.0	0.90	22
	160	1.0	0.70	49
Calcium oxide	20	1.0	1.00	2
	160	1.0	0.95	−8
Palmitic acid[a]	20	0.8	0.75	37
	160	0.8	0.70	80
n-Caproic acid[a]	20	0.9	0.95	0
	160	0.9	0.85	1
Linoleic acid[a]	5	0.8	0.70	8
	10	0.8	0.65	13
	20	0.8	0.60	28
	160	0.8	0.50	−33
Ethyl laurate[a]	20	0.8	0.75	2
	160	0.8	0.75	4
Diglycol laurate[a]	20	0.8	0.65	16
	160	0.8	0.60	108
Ethyl alcohol[b] (0.95%)	...	0.7	0.70	5
	...	0.7	0.70	3

[a] 0.95% ethyl alcohol solution.
[b] Ethyl alcohol added in same fashion as in [a].

Table V. Compounds Exhibiting Appreciable Stimulation on the Growth of *L. casei* in the Presence of Suboptimum Amounts of Riboflavin

(Assayed in amount of 20 to 160 micrograms per 10 ml. of medium)

Palmitic acid	Duodecyl alcohol[a]
Stearic acid	*N*-octadecyl alcohol
Undecylenic acid	Diglycol laurate
Oleic acid	Methyl palmitate
Eleostearic acid	Methyl stearate
Linoleic acid	Calcium stearate
Ricinoleic acid	Ethyl oleate
Fatty acid mixture[b]	Methyl oleate
Duodecyl aldehyde[a]	Lecithin (90%)
	γ-Undecalactone[c]

Nonstandard chemicals.

[a] Florasynth Laboratories, New York, N. Y.
[b] Neo fat No. 23, Armour & Co., Chicago, Ill.
[c] Felton Chemical Co., Inc., New York, N. Y.

riboflavin per tube and (2) those showing little or no stimulation.

The following materials exhibited little or no stimulation; ground glass, glass beads, asbestos, sodium chloride, calcium chloride, calcium oxide, calcium carbonate, calcium lactate, calcium phytate, calcium gluconate, butyric acid, valeric acid, *n*-caproic acid, malic acid, citric acid, pimelic acid, sebacic acid, salicyclic acid, cinnamic acid, furoic acid, furylacrylic acid, glutamic acid hydrochloride, glycerol, cinnamic alcohol, furfuryl alcohol, 2-alpha-furfuryl ethanol, tetrahydrofurfuryl alcohol, geraniol, inositol, cholesterol, acrolein, furfural, ethyl-*n*-caprylate, ethyl-*n*-caproate, isoamyl-*n*-caproate, ethyl laurate, ethyl sebacate, geranyl valerate, methyl cinnamate, enanthyl cinnamate, butyrolactone, alpha-angelica lactone, choline chloride, biotin concentrate, *p*-aminobenzoic acid, thiamin hydrochloride, calcium pantothenate, nicotinic acid, pyridoxine, maleic anhydride, tetrahydrofurfurylbenzyl ether, and asparagine monohydrate.

The stimulatory effects of known compounds have all been shown in the presence of synthetic riboflavin. In Table I the data demonstrate that the assay value of a clarified aqueous extract of a foodstuff can be enhanced by the addition of methyl palmitate to the assay tube. The results obtained thus simulate the effect of the water-extracted solids of those foodstuffs containing stimulants. One exception to this statement is the case of commercial liver meal A, where neither the extracted liver meal solids nor methyl palmitate had any stimulatory action on the assay results of the clarified aqueous liver meal extract. Failures of stimulation of the clarified extracts of this product occurred in five other trials using other known stimulants. It is suggested that already the value obtained on the clarified extract is a stimulated value (not a true riboflavin value) and that the addition of more stimulating agent is noneffective. It has been demonstrated many times that the per cent stimulation per unit of compound decreases sharply to the point where additional units are without effect.

The suggestion mentioned above also involves the water-solubility of these stimulating agents. Approximately 1 per cent of lecithin was mixed with samples of dried skim milk, meat scraps, and fish meal. These samples and controls without lecithin were given the usual autoclaving aqueous extraction, and after cooling, the solids were removed by gravity filtration through filter paper. The filtrates of all samples were assayed for riboflavin microbiologically. The samples containing lecithin assayed 13 to 52 per cent higher in apparent riboflavin content than the non-lecithin-containing samples, thus demonstrating the passage of the stimulant through the filter as a true or a finely colloidal solution. Another experiment with methyl palmitate showed no appreciable stimulation in any of the filtrates.

Since it has been demonstrated that the stimulatory agent of the acid residue of distillers' dried solubles could be extracted by ethyl ether, the six original dried foodstuffs were ether-extracted for 6 to 8 hours and dried, and the autoclaved (15 minutes at 15 pounds' pressure) aqueous extracts with residual solids suspended were microbiologically assayed for riboflavin content. The data of this study are presented in Table I as well as riboflavin determinations on the original foodstuffs by the fluorometric method of Hodson and Norris (*3*), using the Coleman Universal spectrophotometer with the mercury vapor lamp attachment.

The results show that the stimulatory agents are apparently extracted from the original dried foodstuffs with ethyl ether and that autoclaved aqueous suspension of ether-extracted material may be used in the bacterial assay procedure.

The microbiological assay results of ether-extracted liver meal and the fluorometric results on the original liver meal support the contention that stimulatory compounds may occur not only in the extracted residues of foodstuffs but in the extracts.

Several of the compounds which were shown to have stimulatory action in the microbiological riboflavin assay were also tested in the microbiological pantothenic acid assay, in the absence of pantothenic acid, and in the presence of the suboptimum amount of 0.08 microgram of pantothenic acid per 10 ml. of medium. Table VI contains the results of this experiment.

The titration values obtained when fusel oil residue, methyl palmitate, oleic acid, and lecithin were introduced in the basal medium are more irregular than were obtained in the microbiological riboflavin assays heretofore presented. When these compounds were assayed with the suboptimum amount of pantothenic acid, their stimulatory action was significant, thus indicating that the same type of stimulatory compounds which interfere in the microbiological riboflavin assay also interfere in the microbiological pantothenic acid assay. The

Table VI. Stimulatory Action of Certain Compounds

(In the presence of suboptimum amounts of pantothenic acid)

Compound	Micrograms per Tube	Titration Values as Ml. of 0.1 N Alkali: Basal medium	Basal medium plus compound	Per Cent Stimulation in Presence of 0.08 Microgram of Pantothenic Acid per Tube
Fusel oil residue[a]	20	1.1	2.4	29
	120	1.1	2.4	131
Methyl palmitate[a]	40	2.0	2.1	15
	120	2.0	3.2	25
Oleic acid[a]	20	1.1	1.7	12
	120	1.1	1.6	66
Lecithin[a]	40	2.0	1.6	34
	120	2.0	1.4	76

[a] 0.95% ethyl alcohol solution.

latter part of this statement is also supported by other assays not reported here, in which the aqueous extracted residues of liver meal, fish meal, distillers' dried solubles, and meat scraps have been demonstrated to possess stimulatory activity in suboptimum amounts of pantothenic acid.

To demonstrate the effect of adding graded amounts of a stimulatory compound (lecithin) to the assay medium in the presence of graded levels of pantothenic acid, Figure 2 is given. The greatest effect of the stimulant occurs in the presence of suboptimum amounts of the vitamin, as was also shown in Figure 1.

Discussion

Certain foodstuffs contain compounds which exert a stimulating effect on the growth of *L. casei* in suboptimum concentrations of riboflavin and pantothenic acid. Usually these compounds are not extracted in the aqueous autoclaving extraction procedure and therefore can exert their maximum effects only when aqueous extracts with residual solids suspended are assayed.

This stimulatory action on the growth of the microorganism is evidenced by either criteria of growth response, lactic acid titration, or turbidity determination (cell production).

Snell and Strong (*6*) state that when recoveries of pure riboflavin were attempted in the presence of the residue from a hydrochloric acid extraction of liver, the results were high and variable, although the residue showed a riboflavin content of zero. In view of the authors' results, it is believed that their observation can be explained as the action of these growth stimulants. The authors' research confirms the observations of Snell and Strong (*6*) that the incorporation of inert solid material or milk solids in the assay medium did not affect the recovery of added riboflavin.

Pennington, Snell, and Williams (*5*) reported that urine, oysters, and mushrooms offered difficulty in that the pantothenic acid assay figures did not agree satisfactorily when calculated from different levels of assay, but that this difficulty could be overcome by the incorporation of an acid-treated supplement of the material to be assayed in the basal medium. Research has been reported by Isbell, Wooley, and Fraser (*4*) demonstrating the inhibiting effect of urea on the growth of *L. casei* in the presence of riboflavin.

It has been the authors' experience that agreement of results at different levels of assay is not always a good index of the presence of stimulatory action. Apparently, the amount of stimulatory agent present in the foodstuff is a deciding factor. If similar stimulation occurs at different levels of assay along the linear portion of the curve, agreement of results between levels of assay may occur. If these conditions are not met disagreement is evident.

Asparagine produced no stimulation even when included at a concentration of 5 mg. of the monohydrate per 10 ml. of medium and therefore the stimulation herein described differs from that reported by Feeney and Strong (*2*).

The presence of a factor in blood which enhances bacterial growth activity of riboflavin has been reported by Eckhardt, György, and Johnson (*1*). These workers have irradiated diluted alkaline blood to destroy its riboflavin and then attempted to recover added synthetic flavin by the Snell and Strong microbiological assay method. They found that the growth of the microorganism was stimulated in the presence of suboptimum amounts of riboflavin. The authors are able to offer confirmatory evidence of this stimulatory phenomenon. At the time of this investigation they had in the laboratory a sample of dried blood meal which was extracted with water, and the extracted residue incorporated into the medium equivalent to 5 to 20 mg. of the original meal along with suboptimum amounts of riboflavin. Stimulation was observed to the extent of 34 to 46 per cent.

Eckhardt, György, and Johnson (*1*) suggested that substances to be assayed for riboflavin should be tested for this stimulatory action through irradiation or fuller's earth treatment of extracts. The authors wish to repeat their advice for the microbiological procedures and state that after a material is known to contain growth stimulants, it may be avoided through one or more of the following treatments, depending on the nature of the foodstuff:

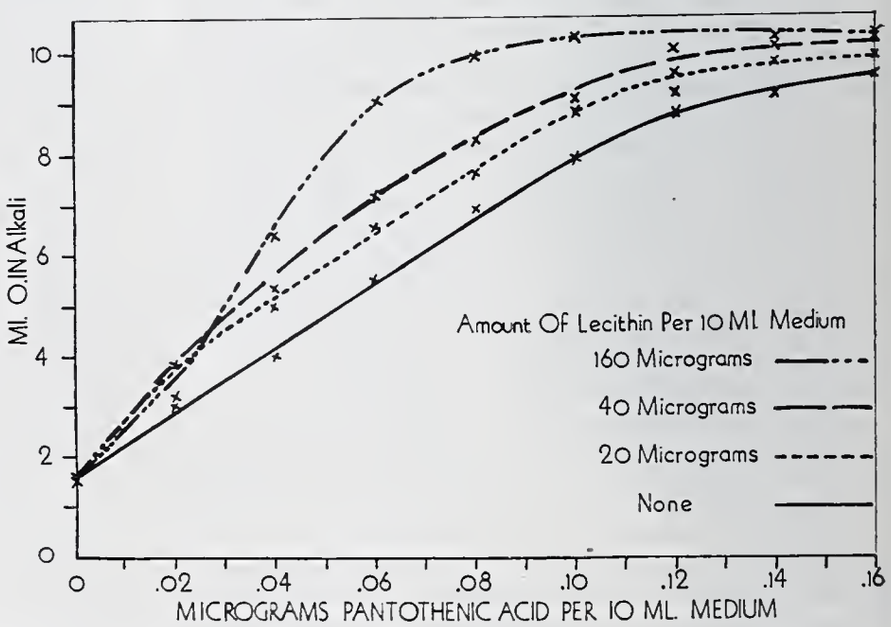

Figure 2. Response of *L. casei* to Lecithin in the Presence of Pantothenic Acid

The use of clarified aqueous extracts.

The preliminary extraction of the dried sample with lipoid solvent, after which an autoclaved aqueous suspension of the product may be used for assay.

The inclusion of photolyzed extracts of the product to be assayed in the riboflavin assay medium, or the inclusion of an alkaline or acid-treated extract of the product to be assayed in the pantothenic acid assay medium, as has been previously suggested by other workers.

Summary

Growth stimulants have been shown to exist in certain foodstuffs for the growth of *L. casei* in the microbiological assays for riboflavin and pantothenic acid when suboptimum amounts of the vitamin are present in the assay medium. Attention is directed to the presence of the stimulants and treatments are discussed to avoid them.

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Extraction and Assay of Nicotinic Acid from Animal and Plant Tissues

Comparison of Methods

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SINCE the discovery of the vitamin properties of nicotinic acid in 1937 (*6*) considerable interest has been focused upon the estimation of this substance. Its widespread importance to clinicians and students of nutrition has made obvious the desirability of having standardized procedures for its quantitative determination wherever it may occur. The practical use of such methods for the control and the manufacture of enriched bread and enriched flour is especially timely at present.

At the present time some 25 methods or modifications of methods for the determination of nicotinic acid have appeared in the literature, and several points of controversy exist as to what experimental steps are to be preferred.

The present paper is a report of progress with various aspects of this problem as applied to a number of natural substances.

Enzyme Digestion

For the liberation of nicotinic acid from possible complexes, autolysis or digestion with added enzymes seemed an attractive procedure. Accordingly, a number of materials were treated with enzymes and the extracts were assayed according to the Snell and Wright microbiological method (*19*). Seven enzymes were employed: takadiastase, malt diastase, pancreatic amylase, papain, pepsin, trypsin, and pancreatin.

Procedure

Samples of fresh tissue were ground several times in a meat chopper, mixed thoroughly, and weighed into sterile tubes or flasks. Each portion was then suspended in ten times its weight of 0.1 per cent buffer solution, the pH of which was chosen to coincide with the supposed "optimal pH" for activity of the enzyme. A weighed amount of enzyme equal to 2 per cent of the weight of the tissue sample was added to each flask, a few drops of benzene were added, and the samples were allowed to digest under specified conditions (see Table I).

After digestion the samples were steamed 30 minutes to inactivate the enzymes and remove the benzene. Each sample was filtered through a very thin layer of kieselguhr on a Hirsch filter and the residue was washed with a volume of water equal to about twice that of the filtrate. The combined washings and filtrates were diluted, usually to a concentration of 1 ml. per 25 mg. of fresh tissue. The extracts were placed in tubes or flasks stoppered with cotton plugs, steamed 5 to 10 minutes, and stored in the refrigerator until used.

Materials which were received in a homogeneous state, such as milk powder, flour, and other cereals, were not ground or mixed thoroughly prior to the preparation of extracts.

The samples of whole wheat flour and bread, white flour, enriched white bread, and dry yeast were obtained as "collaborative samples" from the Research Corporation Committee on Assay Methods. The other materials were obtained in nearby stores and markets.

Results of Enzyme Digestion

Table I shows that in general maximum amounts of nicotinic acid are extracted from tissues by digesting with takadiastase and papain, either separately or in combination. (Since this combination has been found effective in releasing other vitamins from tissues, *2*, the authors have employed it routinely in the preparation of extracts for nicotinic acid assays.) Other means, such as autolysis or brief hot water extraction, are somewhat less effective.

Extraction with hot water has been deemed insufficient for chemical analysis, because of incomplete hydrolysis of nicotinamide or nicotinuric acid (*7*, *12*, *14*). This objection does not hold for the microbiological assays by the Snell and Wright method, since both of the above compounds as well as cozymase are as effective as nicotinic acid in promoting bacterial growth (*19*), but it would seem, then, that nicotinic acid may exist in non-growth-promoting combinations from which it cannot be freed completely by hot water.

The utility of nicotinuric acid as a pellagra preventive is at present uncertain, owing to the contradictory findings of Woolley and co-workers (*22*) and of Dann and Handler (*5*) regarding the antiblacktongue potency of this compound. Since its presence in measurable quantities seems to be con-

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fined to urine, this point is of small importance to assays of plant and animal tissues.

Other Methods of Preparation of Extracts

In order to convert the nicotinic acid present in natural materials into a form available for assay, two other methods have been used generally—namely, hot extraction with sulfuric acid or sodium hydroxide.

Although animal tissues and milk powder show essentially the same apparent nicotinic acid content when determined microbiologically after treatment by the three methods discussed (Table II), acid or alkaline extraction of cereals or cereal-containing diets has in the authors' hands given generally higher results than those obtained by treatment with enzymes. The observations with regard to alkali treatment are in agreement with data obtained by other workers (*8*, *16*, *19*).

The increases due to treatment with alkali, although in some cases small, are often appreciable. A possible source of these increases seemed to be trigonelline. Melnick, Robinson, and Field (*14*) noted that alkaline hydrolysis of trigonelline in the presence of urine produced a substance in yields varying up to 30 per cent which gave the color reactions for nicotinic acid in their test. Sarett, Perlzweig, and Levy (*18*) found further that these yields could be increased to 70 per cent in the presence of 6 *N* alkali and a high concentration of ammonium salts or urea. That the conversions are genuine is indicated by similar results with the microbiological technique. The conversion of samples of trigonelline to nicotinic acid (or its equivalent) was studied under varying conditions by microbiological estimations. The results are listed in Table III.

It is apparent from Table III that, although trigonelline alone is unaffected by autoclaving 30 minutes with 2 *N* sodium hydroxide, it may under sufficiently drastic treatment with alkali be converted to nicotinic acid in rather high percentage yields. However, under the conditions of hydrolysis ordinarily employed only a slight error is to be expected. This may sometimes assume greater significance in the presence of high concentrations of substances which promote the reaction, such as asparagine, which are probably present in the framework of plant proteins in fairly large amounts. The authors are of the opinion that the higher conversions obtained in the presence of asparagine or beta-alanine may be due to slow production of ammonia (or $-NH_2$ groups) in the reaction mixture.

The increases due to acid hydrolysis parallel in most cases the increases with alkali hydrolysis within the limits of experimental error. However, they cannot be explained by conversion of trigonelline, as may be seen from the experiments with whole wheat bread listed in Table IV. Large amounts of trigonelline failed to affect the apparent nicotinic acid content when heated with hydrochloric or sulfuric acid. The addition of reducing agents, cystine and hydrogen sulfide, also failed to produce significant increases.

If the assay values obtained after acid extraction can be assumed to represent no converted trigonelline, the method of Sarett, Perlzweig, and Levy (*18*) can then be used to indicate the amount of trigonelline present. This has been done for whole wheat bread.

Table I. Effect of Enzyme Treatment on Yields of Nicotinic Acid from Various Tissues

(Micrograms per gram of fresh tissue)

Material	Treatment	pH	Nicotinic Acid Content	Relative Yield, Maximum 100%
Hog heart	Autolyzed 24 hours at 37°, natural pH	...	85	98
	Digested 24 hours at 37° with malt diastase	4.5	77	89
	With pancreatic amylase	7.0	77	89
	With papain	5.0	82	94
	With pepsin	2.1	77	89
	With trypsin	8.4	87	100
	With pancreatin	8.4	74	85
	Digested with takadiastase 3 hours, then with papain 5 hours at 45–47°	4.7	81	93
	Steamed 30 minutes with 0.1 *N* HOAc	...	62	71
	Autoclaved 30 minutes with steam at 15-pound pressure	...	79	91
Beef leg muscle	Autolyzed 24 hours at 37°, natural pH	...	41	89
	Digested 24 hours at 37° with takadiastase	3.0	44	96
	With malt diastase	4.5	46	100
	With pancreatic amylase	7.0	46	100
	With pepsin	1.8	46	100
	With trypsin	8.4	46	100
	With pancreatin	8.4	44	96
	Digested with takadiastase 3 hours, then with papain 5 hours at 45–47°	4.7	42	91
	Autoclaved 30 minutes with steam at 15-pound pressure	...	40	87
Beef brain	Autolyzed 24 hours at 37°, natural pH	...	30	86
	Digested 24 hours at 37° with takadiastase	4.5	30	86
	With malt diastase	4.5	29	83
	With pancreatic amylase	7.0	35	100
	With papain	5.0	35	100
	With pepsin	2.1	34	97
	With trypsin	8.4	30	86
	With pancreatin	8.4	35	100
	Digested with takadiastase 3 hours, then with papain 5 hours at 45–47°	4.7	35	100
	Autoclaved 30 minutes with steam at 15-pound pressure	...	29	83
Green peas	Autolyzed 24 hours at 37°, natural pH	...	20	91
	Digested 24 hours at 37° with takadiastase	4.5	20	91
	With malt diastase	4.5	20	91
	With pancreatic amylase	7.0	22	100
	With papain	5.0	20	91
	With pepsin	2.1	22	100
	With trypsin	8.4	16	73
	With pancreatin	8.4	21	96
	Digested with takadiastase 3 hours, then with papain 5 hours at 45–47°	4.7	35	91
	Autoclaved 30 minutes with steam at 15-pound pressure	...	20	91
Milk powder	Autolyzed 24 hours at 37°, natural pH	...	7.6	100
	Digested 24 hours at 37° with takadiastase	4.5	7.5	99

(*Table I continued top of next page*)

Table II. Effect of Extraction with Sulfuric Acid or Sodium Hydroxide on Microbiological Assays for Nicotinic Acid in Various Substances

Material	Nicotinic Acid Content: Digested with takadiastase 3 hours, then with papain 5 hours at 45–47°	Autoclaved 30 minutes with 1 *N* H_2SO_4	Autoclaved 30 minutes with 2 *N* NaOH	Increase Due to Treatment with: H_2SO_4	NaOH
	Micrograms per gram of fresh tissue			%	%
Beef muscle	36	35	..	−3	..
Beef brains	37	38	38	3	3
Milk powder	7.5	7.8	8.2	4	9
Green peas	22	22	22	0	0
"Average American diet"					
Batch 31	13.8[a]	16.0[a]	16.4[a]	16[a]	19[a]
35	15.0[b] (10.9)[c]	21 (12.5)[c]	16.8 (11.8)[c]	40 (15)[c]	12 (8)[c]
36	13.8 (11.2)[c]	18.2 (11.2)[c]	15.8 (14.8)[c]	32 (0)[c]	15 (32)[c]
Corn meal	6.4	9.4	8.4	47	31
Whole wheat bread	42	62	60	47	43
Whole wheat flour	44	60	65	36	48
Takadiastase	21[b]	..	25	..	19
Papain	19[b]	..	20	..	5
Blackeyed peas	13.2	18.4	..	40	..
Germinated lima beans	8.5	11.5	..	35	..

[a] Samples assayed after 2 months' storage at 0–5°.
[b] Autoclaved 30 minutes with steam at 15-lb. pressure.
[c] Values in parentheses represent assays on same samples after 8 months' storage at 0–5°.

TABLE I. (*Continued*)

(Micrograms per gram of fresh tissue)

Material	Treatment	pH	Nicotinic Acid Content	Relative Yield, Maximum 100%
Milk powder (*Contd.*)	Digested 24 hours at 37° with papain	5.0	7.1	93
	Autoclaved 30 minutes with steam at 15 to 20 pound pressure	6.2	6.2	82
	Autoclaved, then digested with takadiastase 24 hours at 37°	4.5	7.2	95
	Autoclaved, then digested with papain 24 hours at 37°	5.0	7.2	95
Corn meal	Autolyzed 24 hours at 37°, natural pH	...	5.6	85
	Digested with takadiastase 3 hours, then with papain 3 hours at 37°	4.7	4.5	68
	Digested with takadiastase and papain together, 24 hours at 37°	4.7	5.7	86
	Autoclaved 30 minutes with steam at 15-pound pressure	...	5.9	89
	Autoclaved, then digested with takadiastase 12 hours, then with papain 12 hours at 37°	4.7	6.1	92
	Autoclaved, then digested with takadiastase 3 hours, then with papain 5 hours at 45°	4.7	6.6	100
Whole wheat bread	Autoclaved with steam 30 minutes at 15-pound pressure	Natural	24	57
	Autoclaved, then digested with takadiastase 3 hours, then with papain 5 hours at 45–47°	4.7	42	100
	Autoclaved, then digested with takadiastase 12 hours, then with papain 12 hours at 37°	4.7	42	100
	Digested 24 hours + takadiastase at 37°	4.5	37	88
	Digested 24 hours + clarase at 37°	4.5	39	93
	Digested 24 hours + papain at 37°	5.0	37	88
	Digested 24 hours + pepsin at 37°[a]	1.5	35	83
	Digested 24 hours + trypsin at 37°[a]	8.5	37	88
	Digested 3 hours + takadiastase, then papain 5 hours at 45–47°	4.7	42	100
	Autolyzed 24 hours at 37°	Natural	23	55
White flour	Autolyzed 24 hours at 37°	Natural	4.3	51
	Autoclaved, then digested with takadiastase 3 hours, then with papain 5 hours at 45–47°	4.7	8.5	100
Brewer's yeast, dry	Autolyzed 24 hours at 37°	Natural	490	100
	Autoclaved 30 minutes with steam at 15-pound pressure	...	490	100
	Autoclaved, then digested with takadiastase 3 hours, then with papain 5 hours at 45–47°	4.7	490	100

[a] pH adjusted to approximately 5.5 before steaming.

TABLE III. CONVERSION OF TRIGONELLINE TO NICOTINIC ACID UNDER VARYING CONDITIONS WITH SODIUM HYDROXIDE

Concentration of NaOH	Treatment	Other Reagent	Total Volume	Weight of Trigonelline	Theoretical Conversion
N		*Mg.*	*Ml.*	*Micrograms*	*Per Cent*
2	Autoclaved 30 minutes at 15-pound pressure	None	40	4000	0.11
		$(NH_4)_2SO_4$, 100	25	1000	2.4
			22.5	500	3.4
			21	200	4.0
	Autoclaved 30 minutes in stoppered flask	$(NH_4)_2SO_4$, 50	12.5	500	2.8
		Urea, 50	12.5	500	2.5
		Urea, 100	12.5	500	3.1
		Asparagine, 50	12.5	500	6.4
		Glutamine, 50	12.5	500	1.2
		β-Alanine, 50	12.5	500	18
	Heated 2 hours at 75°	Urea, 50	12.5	500	0.6
5	Heated 2 hours at 75°	$(NH_4)_2SO_4$, 50	12.5	500	8.3
		Urea, 50	12.5	500	5.3
		Asparagine, 50	12.5	500	34
		α-Alanine, 50	12.5	500	4.4
		β-Alanine, 50	12.5	500	33
6	Heated 45 minutes at 75° in stoppered flask	$(NH_4)_2SO_4$, 300	2.5	200	42
		Urea, 300	2.5	200	52
		Asparagine, 300	2.5	200	52
		β-Alanine, 300	2.5	200	69
		Acetamide, 300	2.5	200	51
		Glutamine, 300	2.5	200	10
		Urotropine, 300	2.5	200	2
					γ/g. equivalent of nicotinic acid
2	Autoclaved 30 minutes at 15-pound pressure	Asparagine, 100	10	0	<0.1
		β-Alanine, 50	10	0	<0.2
	None	Asparagine	..	0	6
		β-Alanine	..	0	2.5

The apparent trigonelline content was thus found to be 15 micrograms per gram, which is rather low compared to the amount of nicotinic acid present. It is evident then that the increase due to alkaline treatment over the enzyme-treated extract must arise chiefly from some source other than trigonelline.

Additional studies of the effect of extraction and hydrolysis upon the apparent contents of nicotinic acid in whole wheat bread and flour are recorded in Tables V and VI.

Both the whole wheat flour and bread yield approximately 45 micrograms of nicotinic acid per gram when assayed either in the form of an autoclaved suspension or as extracts prepared by digestion with takadiastase and papain. Autolysis, like a single autoclaving, apparently is somewhat less effective in releasing all of the vitamin present. Hydrolysis with acid or alkali, on the other hand, gives values considerably higher, as indicated previously. Intermediate values may be obtained by autoclaving with water under varying conditions, with the highest of these values resulting from the most drastic treatment. The questions arise whether enzymatic liberation of the vitamin is complete and whether the effect of acid or alkaline hydrolysis is exerted on the tissue or on dissolved substances. These are answered by the data of Table VI, wherein it may be seen that clear extracts obtained by enzymatic digestion of bread or flour show increased nicotinic acid contents after hydrolysis with alkali or acid, and to a smaller degree after prolonged hydrolysis with water. The increases are as large, within experimental error, as are those obtained by hydrolyzing the tissue. Enzymatic liberation of the hydrolyzable substance appears to be complete.

The facts could be accounted for by assuming the presence in wheat of a substance which can be converted to nicotinic acid or its equivalent by hydrolysis with acid or alkali, and only slowly but with measurable velocity by hot water. Whether this substance is of antipellagric value per se can be determined only by animal assays.

Hydrolysis of the precursor substance appears to proceed most rapidly in the presence of alkali. Thus in Table I the value of 37 micrograms of nicotinic acid per gram of whole wheat bread, obtained after trypsin digestion, may be increased to 62 micrograms per gram by merely steaming the mixture after digestion at the pH of 8.0 to 8.5 employed for digestion. On the other hand, heating whole wheat bread briefly with 0.25 *N* hydrochloric acid (Table IV) does not seem to effect hydrolysis.

Comparison of Assay Procedures

A large number of comparative assays of similar materials are available in the literature (*1, 4, 8, 9, 10, 12, 15, 19, 20, 23*).

Table IV. Apparent Nicotinic Acid Content of Whole Wheat Bread

(After treatment with HCl, reducing agents, trigonelline, and H_2SO_4)[a]

	Before H_2SO_4 Autoclaving	After H_2SO_4 Autoclaving	Increase Due to H_2SO_4	Increase Due to H_2SO_4
	Micrograms per gram			%
Heated at 75° 45 minutes with 0.25 *N* HCl	34	53	19	56
Heated with HCl + H_2S + cystine	37	55	18	49
+ 200γ of trigonelline per gram, heated + H_2S + cystine	40	54	14	35
+ 1000γ of trigonelline per gram, heated + H_2S + cystine	41	58	17	41
+ 200γ of trigonelline per gram, heated with HCl but no reducing agents	36	51	15	42
Autoclaved 30 minutes with 1 *N* H_2SO_4	..	62	..	..
Autoclaved 30 minutes with 1 *N* H_2SO_4 + 200γ of trigonelline per gram	..	60	..	..

[a] All assays were made on centrifuged extracts.

In general, results of different workers are in relatively close agreement for animal tissues, but the agreement is not so good for cereals and milk. Such information is of great interest but does not furnish crucial tests of the credibility of analytical values obtained by a variety of methods.

Comparisons of identical materials using dog assays as one criterion have been reported (*19, 21*). Doubt has been expressed as to the suitability of the basal diet used in these experiments with dogs, so that assays performed by this method are of questionable value (*17*).

Waisman and Elvehjem (*21*) have assayed several samples of meat by the Snell and Wright method and by a modification of the Melnick and Field chemical method (*12*). The agreement between the two methods is generally good except in the case of liver.

Melnick, Oser, and Siegel (*13*) have recently shown their modification of the method of Melnick and Field to be in reasonably good agreement with the Snell and Wright method when applied to the assay of flour. On the other hand, these workers reported orally (*16*) the results of several comparative assays of "average American diets" by the above methods wherein they obtained consistently higher values by the chemical method, usually about double those obtained microbiologically. Checks of some of their microbiological values have shown good agreement with the authors' own.

In order to check the chemical assay values obtained by these workers, the authors have employed the method of Dann and Handler (*4*), which in the hands of its authors gave values in close agreement with the microbiological method for rat tissues (*23*) and corn meal. It seemed to be specific for nicotinic acid, since it possessed the advantage of yielding colorless extracts for subsequent color development. The results of chemical and microbiological assays of a number of extracts of various materials are listed in Table VII. The extracts of average American diets are especially suitable for comparisons of this sort because of the diverse character of their constituent foods.

The "average American diets" were prepared by Lane, Johnson, and Williams (*11*). Each batch contained the 14 most staple foods together with a selection of 15 other minor food articles, all in the proportions in which they are consumed by the American population as a whole. Cereals, dairy products, various meats, vegetables, and fruits were included in suitable proportions. The mixtures were desiccated to 10 to 22 per cent moisture. On a dry basis the preferred nicotinic acid values range from 13 to 17 mg. per kg. The nicotinic acid values of these mixtures, from the standpoint of national nutrition, need interpretation in the light of losses incurred during the storage of the samples (Table II), losses of nicotinic acid in the cooking of meats (*3*), etc. This will be attempted in a later paper.

The results of Table VII are reassuring as to the agreement between the present chemical method and the microbiological method of Snell and Wright for assay of a wide variety of materials. The close similarity of results in a number of cases by the two methods after enzymatic digestion suggests that combined forms of the vitamin may be converted to the free acid by this procedure. This is borne out by the results of enzymatic digestion of nicotinamide. On the other hand, the failure of this enzyme mixture to liberate all of the vitamin from yeast as free nicotinic acid indicates that enzyme digestion may not be a practicable extraction procedure for all foodstuffs when a chemical method is to be employed.

The results also serve to emphasize the fact, mentioned previously (*4*), that when a chemical method is employed it is essential that all extracts be rendered colorless prior to the addition of the color-forming aromatic amine. The presence of colored substances in liver may have been responsible for the higher results obtained by Waisman and Elvehjem by their chemical method (*20*). The extracts of average American diets which were used were very highly pigmented, owing presumably to the presence of fried foods, gravies, etc., which were extensively browned in cooking. These

Table V. Effect of Extraction and Hydrolysis upon Apparent Nicotinic Acid Contents of Bread and Flour

Method of Extraction	Whole Wheat Flour	Whole Wheat Bread	White Flour	Enriched White Bread[b]
	Micrograms per gram			
Autolyzed	22	23	..	..
Autoclaved once, 30 minutes, 10 cc. of H_2O per gram	26	29	..	..
2 times, as above, centrifuged; solids resuspended after first autoclaving	33	36	..	..
3 times, as above, centrifuged; solids resuspended after first and second autoclaving	43	44	..	..
3 times, as above, but 2 hours each	57	53	..	..
Once, not filtered	45	44	..	..
Once, not filtered, 50 cc. of H_2O per gram	..	49	..	..
3 times, 50 cc. of H_2O per gram	..	49	..	..
Enzyme digested[a], 10 cc. of H_2O per gram	..	42	8.5	43
Enzyme digested, 50 cc. of H_2O per gram	..	44	..	..
Autoclaved, then enzyme digested, 10 cc. per gram	45	44	8.3	..
Autoclaved with 1 *N* H_2SO_4 30 minutes, 10 cc. per gram	60	62	10.6	46
Autoclaved with 2 *N* NaOH 30 minutes, 10 cc. per gram	65	60	9.9	41

[a] Enzyme digests were prepared by incubating samples 3 hours with 2% (by weight) of takadiastase, then 5 hours with 2% of papain, at 46–48° under C_6H_6. Samples were then steamed 30 minutes, filtered, diluted to desired volume.

[b] White bread was enriched with 33γ of nicotinic acid per gram.

Table VI. Apparent Nicotinic Acid Content of Clear Enzyme Extracts of Whole Wheat Bread and Flour after Treatment with Other Agents

(Microbiological assays)

	Whole Wheat Bread		Whole Wheat Flour	
	Content	Increase due to hydrolysis	Content	Increase due to hydrolysis
	Micrograms per gram			
Enzyme extract[a]	39	..	45	..
Enzyme extract, autoclaved 30 minutes with 2 *N* NaOH, 10 cc. per gram	57	18	68	23
Enzyme extract, autoclaved 30 minutes with 1 *N* H_2SO_4, 10 cc. per gram	56	17	67	22
Enzyme extract, autoclaved with H_2O 2 hours, 50 cc. per gram	44	5	52	7

[a] Enzyme extracts were prepared by successive digestion with takadiastase and papain at 46–48° (see Table V).

Table VII. Comparative Assays of Identical Extracts

Material	Treatment	Nicotinic Acid Content: Microbiological	Nicotinic Acid Content: Chemical	Deviation of Chemical from Microbiological
		Micrograms per gram		%
Beef muscle	Autolyzed	35	30	−14
	H_2SO_4 autoclaved	35	31	−11
Beef brain	Autoclaved	31	28	− 9.7
	H_2SO_4 autoclaved	38	32	−16
	NaOH autoclaved	38	32	−16
Whole wheat bread	Enzyme digested[a]	38	41	+ 7.9
	H_2SO_4 autoclaved	62	64	+ 3.2
	NaOH autoclaved	60	60	0
Whole wheat flour	Autoclaved, then enzymes	44	42	− 4.5
	H_2SO_4 autoclaved	60	64	+ 6.7
	NaOH autoclaved	65	56	−14
Average American diet				
Batch 35	Enzyme digested	15.0	15.6	+ 4.0
	H_2SO_4 autoclaved	21.0	22.2	+ 5.7
	NaOH autoclaved	15.4	16.8	+ 9.1
Batch 36	Enzyme digested	13.8	15.6	+13
	H_2SO_4 autoclaved	18.2	18.0	− 1.1
	NaOH autoclaved	15.6	14.7	− 5.8
Batch 30	Enzyme digested	10.4	11.1	+ 6.7
Batch 31	Enzyme digested	14.0	15.0	+ 7.1
	H_2SO_4 autoclaved	16.0	16.8	+ 5.0
	NaOH autoclaved	16.4	15.1	− 7.9
Dry yeast	Autoclaved	490	250	−48
	Enzyme digested	490	250	−48
	H_2SO_4 autoclaved	..	510	...
			γ/γ	
Nicotinamide	Enzyme digested 12 hours at 50[a]	..	0.99	...

[a] Enzyme digests prepared as in Table V.

pigments were not completely removed in all instances even by precipitation with lead hydroxide, thus necessitating the use of "blanks" which contained no cyanogen bromide or metol. In one or two extreme cases it was necessary to repeat the adsorption procedure in order to remove essentially all of the colored material from the extract.

Summary

Digestion of foodstuffs with takadiastase and papain appears to liberate nicotinic acid completely from a variety of materials.

Enzymatic digestion or acid or alkaline extraction gives the same values for nicotinic acid content of meats and milk.

Extraction of cereals with acid or alkali, or similar treatment of clear cereal extracts, gives higher nicotinic acid values than enzymatic liberation alone.

The increases caused by acid treatment of cereals or cereal extracts are apparently due to the presence in cereals of some substances other than trigonelline which is convertible to nicotinic acid.

The increases due to alkaline treatment of cereals or cereal extracts are apparently largely due to the conversion of the substance mentioned in (*4*) but perhaps partly to conversion of trigonelline into nicotinic acid.

That the biologically active substance formed by hydrolysis of the hypothetical precursor is actually nicotinic acid is indicated by consonant determinations of it by both microbiological and chemical methods, provided in the latter case preformed color is removed prior to coupling with metol.

Whether the hypothetical precursor substance has antipellagric value is unknown.

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Technique and Applications of Industrial Microradiography

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Because of widespread interest in the possibilities of the technique of microradiography as an industrial testing and research method, this paper presents details of the best equipment, procedures, and applications developed at present. Since it is impossible to magnify x-ray images in the same sense that optical images are magnified by suitable lenses, it is necessary to register the x-ray image on a photographic plate and then enlarge the photographic image. By use of the Lipmann emulsion with extremely fine grain size for the silver halide, a magnification up to 300 may be made without loss of detail from graininess. Successful radiography also depends upon the choice of x-ray wave lengths which will give a suitable differentiation between constituents in a specimen, such as aluminum and copper in an alloy used in the construction of aircraft.

A new technique employing two or more characteristic radiations to bring out details of polycomponent metal systems is presented and illustrated with a variety of alloys such as copper-beryllium, aluminum-magnesium, aluminum-copper, bronze, leaded iron, rolled beryllium, silicon steel, graphitic steel, season-cracked brass, and lead-antimony type metal. Instead of employing very soft x-rays generated with special equipment at a few thousand volts and applicable to a very limited number of materials, and without vacuum cameras, very successful results were obtained with characteristic Kα-rays of molybdenum, copper, iron, cobalt, chromium, etc., as produced in ordinary diffraction apparatus.

It is demonstrated that microradiography supplements the microscope in metallurgical and other industrial applications, with the advantages that it gives a three-dimensional view of the specimen; does not depend upon differential action of etchants but only on the variations in absorbing power of constituents, including internal voids and cracks; requires no special polishing of the specimen; and is directly and incontrovertibly interpreted. A few striking biological applications, especially in wood technology, are illustrated.

THE most familiar branch of x-ray science is termed radiography. This depends essentially upon the differential absorbing power of different materials for x-rays as they penetrate through a given specimen to produce the resulting "shadowgraph" which delineates the gross structural details of any heterogeneous material. Thus medical diagnostic radiographs show very clearly the shadows and gross structural details of bones or foreign bodies in contrast with soft tissues. In industrial applications cracks, blow holes, and other defects may be shown in metal castings and welds, foreign bodies in packages of breakfast food or candy, slate in coal, and in general the internal structure of any material. Similarly in the field of art, old paintings may be tested for retouching, hidden pictures, and characteristic technique of masters.

A logical extension of the practical uses of this property of x-rays is to the examination under high magnification of the gross structure of various types of small specimens as revealed by means of x-rays, analogous to the familiar science of photomicrography, in which the microscopically enlarged image of a surface can be examined and photographed in visible light. However, one very serious obstacle presents itself—namely, the absence of any lenses for the enlargement of x-ray images corresponding to those which form the essential optical system of a microscope. Thus all radiographs are representations of the exact size of the object irradiated by the beam from an x-ray tube, since the object is in direct contact with the photographic film. The science of microradiography must, therefore, depend upon the exact registration on a photographic film and the subsequent microscopic enlargement of the image recorded in the developed silver emulsion of the film.

Because of the present extraordinary interest in the possibilities of the technique of microradiography in research and testing of a variety of important defense materials, this paper outlines details of the best procedures which have been developed, and illustrates applications to metallurgical and biological specimens.

Historical

Microradiography grew out of the desire to obtain and view radiographic images of heterogeneous objects of small size.

As early as 1913, Goby (*5*), using x-ray radiation of very long wave length and working in a vacuum, obtained radiographs of fossils and minerals. These radiographs were then enlarged to such an extent that they could be viewed with the unaided eye. In 1930, Dauvillier (*3*), using a technique similar to that employed by Goby, obtained radiographs of vegetable cells, such as those of elder pith. He used voltages as low as 8 kilovolts. The sample and photographic plate were both mounted in a vacuum connected directly with the x-ray tube in order to prevent absorption by the air of the very soft radiation used. Lamarque and associates (*7*) in 1936, using 5000 volts and currents as high as 100 milliamperes, studied plant and animal cells. The method was found very useful for studying the differences in densities of the various cell components. Stereoscopic microradiography was suggested by Goby (*6*) in 1925 and Yoshida and Tanaka (*9*) in 1934. The use of very soft x-rays on grenz-rays to differentiate inorganic and organic constituents in a sample has been rather common. For example, Schupp and Boller (*8*) in 1938 studied the sodium silicate bonds in corrugated fiberboard with low-voltage rays; but the maximum enlargement used for any radiograph was only 15 diameters, so that the technique could scarcely be classed as microradiography.

In 1938 the senior author had the privilege of witnessing attempts to adapt microradiography with x-rays of very long wave lengths to aircraft light alloys in the French Air Ministry in Paris. Very intense x-ray beams from special tubes were generated at 4000 volts and passed through very thin specimens in vacuum cameras. Some success was achieved, as reported by Fournier (*4*). However, it was evident from all previous work that, because of the special requirements of apparatus and technique and the limitation to so few types of specimens consequent upon the use of very soft radiation, there was little hope of practical use of the method in industry. Successful results with metal specimens were reported in 1939 by Clark (*1*) and Clark and Shafer (*2*), with a technique involving the use of ordinary x-ray diffrac-

tion tubes operating at 30,000 or 40,000 volts and the elimination of all vacuum cameras.

Experimental Technique

The principal features of the practical microradiographic technique which has been developed and tested are:

1. Preparation of the sample in the form of a small piece with a thickness of 0.1 mm. or less for metals or other dense materials, or considerably greater if necessary for biological specimens.
2. Registration of the radiograph by a very simple technique involving a special photographic emulsion.
3. Enlargement of the radiograph to a suitable size.

The two chief essentials are: (1) a photographic emulsion of sufficiently small grain size to stand enlargements of 100 to 300 diameters without loss of definition from graininess; and (2) the selection of x-radiation of suitable wave length and intensity.

Photographic Emulsions. Tests on microradiographs and electron microscope photographs at 60,000 diameters magnification have demonstrated that the Lipmann photographic emulsion alone is sufficiently fine-grained so that enlargements may be made of the radiograph up to 300 diameters without serious loss in definition. To physicists this transparent emulsion is generally familiar. It has been rather widely used in aerial photography when great enlargement is desired.

The first work on microradiography in this laboratory in 1938 was carried out with Belgian Gevaert film. When this supply was cut off by the war, Eastman Type 548-0 spectrographic plates with Lipmann emulsion became available and have proved entirely satisfactory. There is some advantage in the film, since it may be cut into small circles for each exposure. Multiple exposures, however, may be made on a plate by a simple translation after each exposure. The plate has the further advantage of no shrinkage or warping.

The Lipmann emulsion has a resolving power of 2 microns or 500 lines per millimeter. The limit may be approached for sufficiently thin sections. As expected, the Lipmann emulsion is very slow in comparison with the usual type of x-ray films. As a matter of fact, it may be developed in any light safe for ordinary chloride photographic papers.

The contrast of the developed films or plates, as measured by the slope of the density *vs.* log exposure curve, is used to determine the effect of various times of development in Eastman regular x-ray developer at 18°. The densities of the steps were determined with the aid of a Leeds & Northrup microphotometer and plotted against log E. The values of γ graphically determined were as follows:

Development Time *Min.*	γ
0.5	0.93
1.0	1.07
2.0	1.48
4.0	1.80
8.0	2.05

The thin emulsions may be considered fully developed in 1 minute, but it is actually advantageous to develop 5 minutes. Eastman developer D-8 as recommended by the company develops the Eastman 548-0 plates satisfactorily in 1.5 minutes. A number of fine-grained developers have been tried but no advantage was observed; in fact, they tended to increase fogging. Attempts have been made to increase the sensitivity of the Lipmann emulsion by prefogging and by treatment with mercury, ammonia, and ammoniacal silver tungstate. The last named does increase the sensitivity very markedly but the treatment is so difficult to control and the possibilities of introducing artifacts are so great that it is not recommended until further investigations are carried out.

Preparation of the Sample. The thickness of the sample is dependent upon the intensity of the available radiation and upon the linear absorption coefficient of the material being investigated. Using commercial diffraction x-ray tubes as radiation source, it has been found advisable for best results to take steel samples down to about 0.075 mm. (0.003 inch) in thickness; copper alloys are usually suitable up to 0.125 mm. (0.005 inch) in thickness, magnesium up to 0.25 mm. (0.010 inch) thick. When the sample is too thick, the microradiograph will show ordinarily only diffuse resolution of detail rather than well-defined structural effects. The metal sample may be cut to approximate size by any method, and the only finishing necessary is a final treatment with 2(0) emery paper, moistened with oil to remove pits and tool marks.

Radiographic Technique. Cameras may easily be made for holding the sample and film, or the assembly can be improvised by binding the sample against the fine-grained film or plate with a piece of black paper and simply exposing to the x-ray beam.

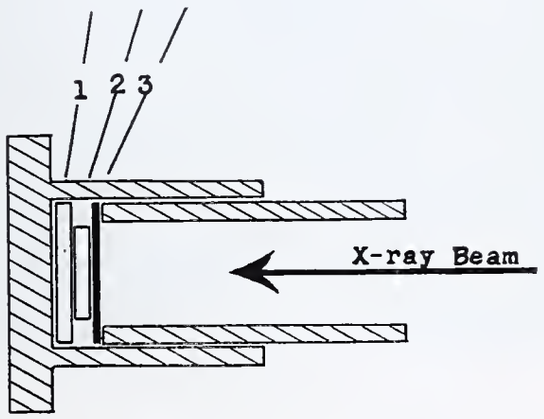


Figure 1. Microradiographic Camera Designed to Fit as Inset in Collimating Systems of Commercial X-Ray Equipment

1. Fine-grain film. 2. Sample. 3. Black paper.

In this laboratory, it has been convenient to make small cylindrical cameras (Figure 1) which will fit in the collimating tube system of the regular x-ray diffraction equipments. This particular method employs a cup of the proper dimensions to fit the collimating system, which holds the film. A close-fitting cylinder, with the sample fastened on one end, is then slipped into place, bringing the sample in contact with the film emulsion. The x-radiation passes down the cylinder and impinges on the sample. It is essential that the sample be as close to the emulsion of the film as possible; otherwise there is an appreciable decrease in definition. The radiation employed does not have to be collimated with any especial care. If the sample and film are about 12.5 to 15 cm. (5 to 6 inches) from the target of the x-ray tube, no collimation is really required, although it is preferred to use a 0.15- to 0.31-cm. ($^1/_{16}$ to $^1/_8$ inch) opening near the window of the x-ray tube to define the beam. The close contact of sample and film makes this use of essentially noncollimated radiation possible and also permits comparatively rapid exposures.

Clark and Shafer (*2*) described a microradiographic camera adapted from a flat diffraction cassette holder in which a circular disk cut from film can be rotated for making multiple exposures. When Eastman plates are used, small pieces are cut and wrapped tightly with black paper or aluminum foil on which the specimen is placed, preferably inside in close contact with the emulsion. A camera is now being designed in which the specimen is directly against the plate emulsion without intervening paper or foil layers, and in which the plate may be moved laterally for multiple exposures.

The period of exposure required is dependent upon the nature of the sample, its thickness, and the intensity of the x-ray beam, and no generalizations can be made. This subject is discussed in a quantitative sense in the following section.

Characteristic Radiation *vs.* Soft General Radiation for Metal Microradiographic Examination; Microradiographs of Specific Elements in a Polycomponent Metal Section. In all earlier work on microradiography, as outlined above, very soft general x-radiation produced at voltages as low as 4000 volts was used simply because there

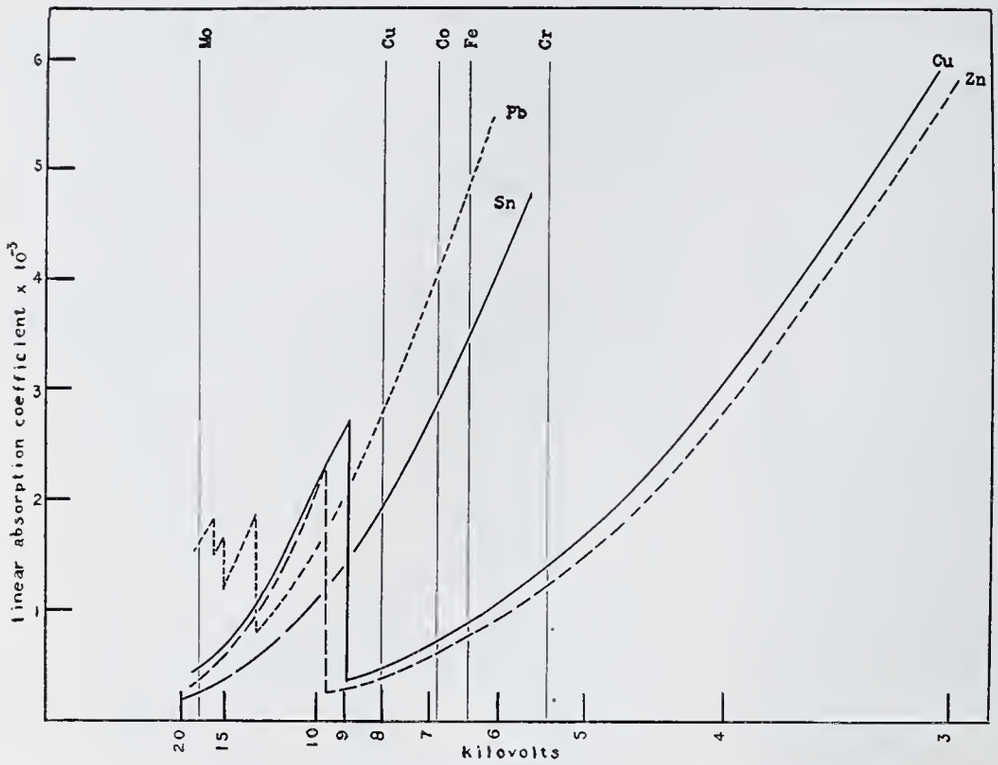


FIGURE 2. LINEAR ABSORPTION CURVES FOR METALLIC ELEMENTS IN THE ALLOY BRONZE
The vertical light lines represent the critical voltage above which the characteristic Kα-rays from the various x-ray tube targets are generated.

is the greatest differential in absorbing power of different constituents at these long wave lengths. However, the technique was a failure from the standpoint of practical use because of the extraordinary precautions required in making extremely thin specimens and in the necessity of working in vacuum to avoid scattering effects of air. Hence, there was no success whatever with metals and only a very limited number of biological specimens showed any promise. Where x-ray diffraction equipment is available, its use should not be ignored for microradiography, since the "characteristic" radiation obtained from such equipment is present in very considerable intensity, far greater than that usually obtained with low voltage and general radiation; furthermore, these characteristic radiations may be considered as essentially monochromatic, while radiation obtained at low voltages is always spread over a considerable band of wave lengths, even when filters are employed. The microradiography of such a system as magnesium-aluminum is ordinarily considered to require general radiation, but the print shown in Figure 5 for an alloy containing 10 per cent aluminum, 0.2 per cent manganese, with the balance magnesium, is from a microradiograph obtained in 15 minutes from a commercial Machlett cobalt-target diffraction tube operating at 15 milliamperes and 40,000 volts, with the $K\alpha_1$ characteristic ray of cobalt with a wave length of 1.785 Å. predominating. Copper or iron characteristic radiations are as suitable. Clark and Shafer (*2*) first demonstrated the successful use of the α-rays of molybdenum for a variety of alloys.

In Figure 2 are plotted the linear absorption coefficients for the elements usually found in brass and bronze. The light vertical lines correspond to various specific radiations obtained with commercial x-ray diffraction tubes. The heavy lines represent the linear absorption coefficients; therefore, the vertical distance between any two lines is related to the maximum intensity ratio obtainable on the microradiograph by the relation:

$$I_a/I_b = e^{-\left(\frac{\mu_a}{\rho_a}\rho_a - \frac{\mu_b}{\rho_b}\rho_b\right)x}$$

where x is the sample thickness in centimeters, μ_m/ρ_m is the mass absorption coefficient, and ρ_m is the density of the metal. The greater the vertical distance between any two curves on the graph, the greater the sensitivity of the microradiographic method for the detection of inhomogeneities, etc. Another factor to be considered is that the required exposure to the x-ray beam is fixed by the height of the curves above the base line. This height is the linear absorption coefficient μ (also listed in Table I) and is related to the proper exposure by the formula on the next page.

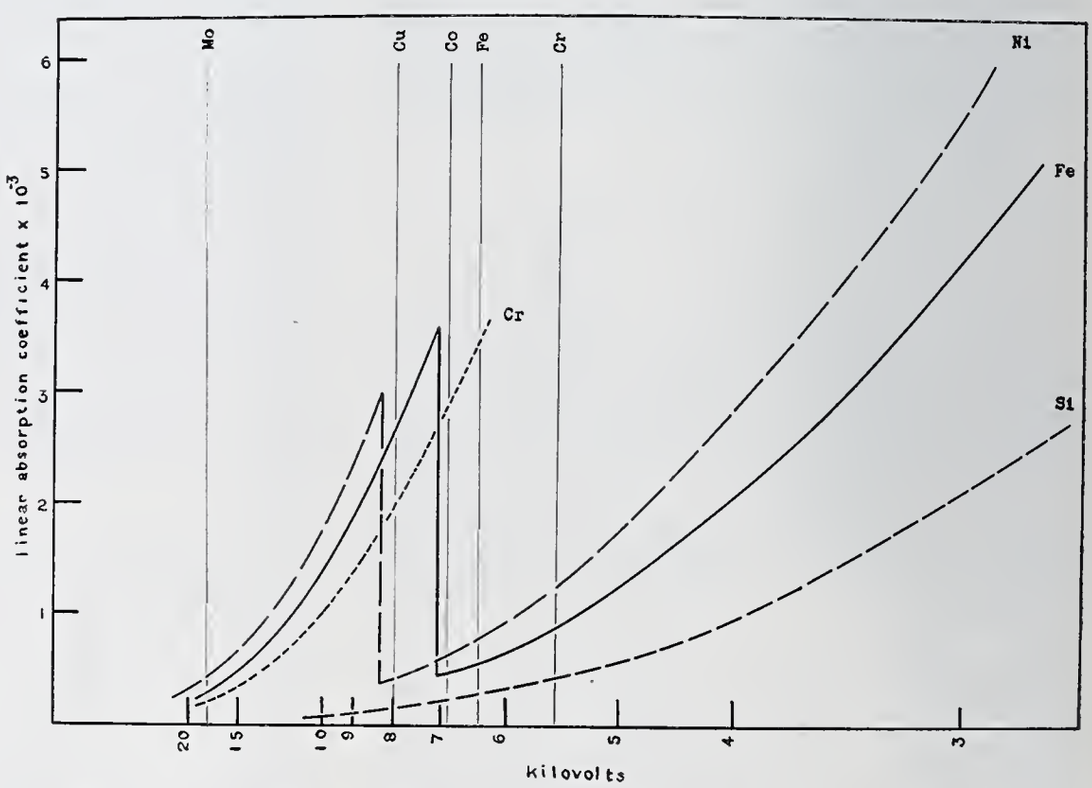


FIGURE 3. LINEAR ABSORPTION CURVES FOR METALLIC ELEMENTS IN NICKEL-CHROMIUM STEEL

A *B*

FIGURE 4. MICRORADIOGRAPHS SHOWN AS NEGATIVE PRINTS OF COPPER-BERYLLIUM ALLOY, MADE WITH CHARACTERISTIC COPPER RADIATION

Magnifications (*A*) ×80, (*B*) ×300 (enlargement of area indicated in *A*). Copper-rich phase light, beryllium dark

$$\text{Time of exposure} = Ke^{\mu x}$$

where x is the thickness of the sample in centimeters, μ is the linear absorption coefficient for the material predominant in the sample, e is the base of natural logarithms, and K is a constant for any given source of x-radiation. K varies inversely as the intensity of the x-ray beam.

FIGURE 5. MICRORADIOGRAPH (NEGATIVE PRINT) OF MAGNESIUM-ALUMINUM ALLOY MADE WITH CHARACTERISTIC COBALT RADIATION

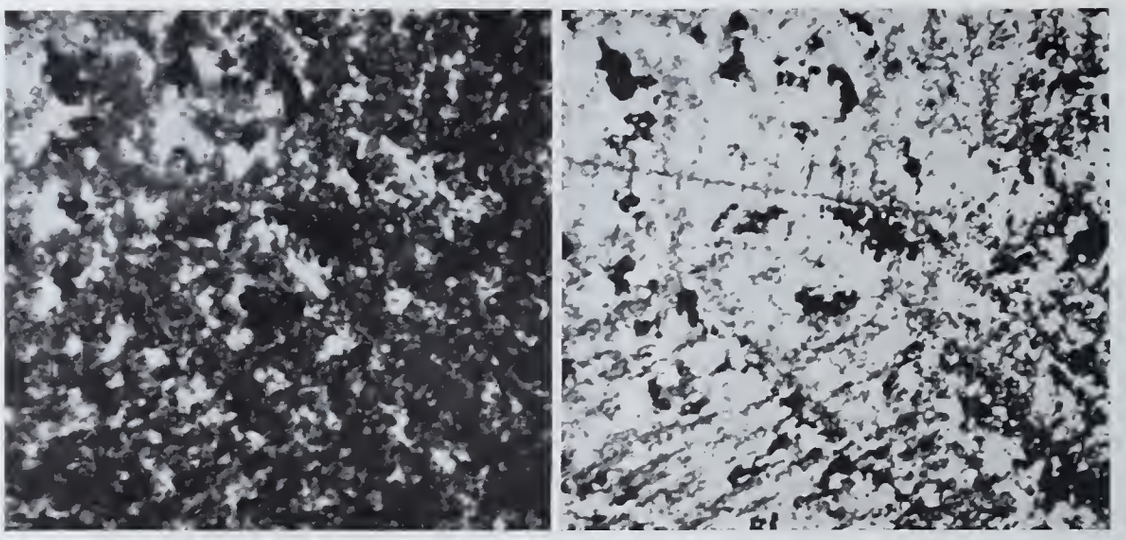
Sample thickness 0.010 inch, exposure time 15 minutes, magnification ×90. Copper light, magnesium dark.

For a partial illustration of the possible use of such a graph as that given above, we may consider a bronze with the composition about 80 per cent copper, 10 per cent lead, 10 per cent tin, and 0.75 per cent phosphorus. Examination of the graph shows that molybdenum radiation would tend to show the distribution of the lead in the sample; but that use of copper radiation would show not only the lead but also the tin distribution. Therefore, if two microradiographs were taken, such as those shown in Figure 6, using the same metal section, and successively with molybdenum and copper radiation, it is possible to learn considerably more from the sample than has previously been indicated.

The above method of using characteristic radiations for the purpose of identifying the material present may be employed generally for any favorable case. For example, a more complicated alloy might be considered—a steel containing iron, chromium, nickel, and silicon. Examination of the graph of linear absorption coefficients for these materials (Figure 3) shows that we could expect the radiographs listed below:

Exposure	Light Regions on Negative	Dark Regions
	Molybdenum characteristic radiation	
Short	Elements of high atomic number—e. g., Mo., etc.—or very dense regions	.....
	Cobalt characteristic radiation	
Short	Chromium, also the effects observed with molybdenum radiation	.....
	Copper characteristic radiation	
Long	As with molybdenum	Large amounts of chromium, nickel, silicon

It is apparent that the use of several characteristic radiations can be of considerable value in the interpretation of the enlargements obtained; this is rather generally true for practically any polycomponent system of metals.



A *B*

FIGURE 6. MICRORADIOGRAPHS (NEGATIVE PRINTS) OF ONE AREA OF BRONZE 0.0035 INCH THICK

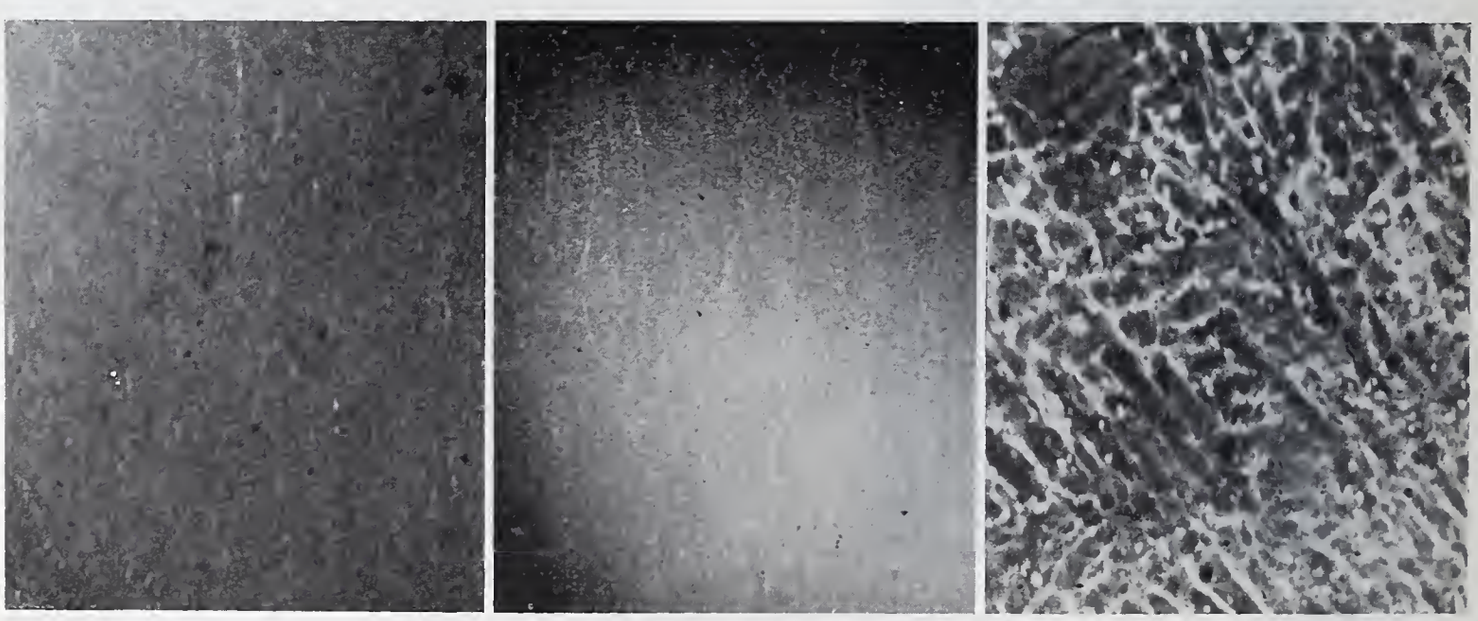
A. With characteristic molybdenum radiation. *B*. With characteristic copper radiation. Magnification ×90. Lead light in *A*. Lead and tin light in *B*

A table has been arranged of some of the common elements, listed in the order of their absorption for the various linear absorption values for given characteristic radiation values. The linear absorption value, estimated by taking the best values of mass absorption coefficients from the literature and multiplying them by reasonable density values for the elementary materials, is given along with the atomic symbol. Table I may, therefore, be used to ascertain whether these particular characteristic radiations are suitable for the examination of any particular binary system of metals. Thus, nickel and cobalt may be easily differentiated with characteristic copper radiation although a rather thin sample would probably be required; however, iron or molybdenum radiation would not be suitable. Beryllium and magnesium are well differentiated by iron radiation, and even better by the characteristic chromium radiation. Lead and iron could not be distinguished by copper radiation, but molybdenum radiation (Figure 7*A*) would furnish an exceedingly sensitive method of detecting lead in iron. Such examples could be multiplied at length on the simple principle that the sensitivity for differentiation is greater, the greater the difference in linear absorption.

Metallurgical Examples

Figure 4 illustrates the remarkable results obtained with a copper-beryllium alloy which now has such great industrial importance. The microradiograph at 80 diameters enlargement shows three distinct phases—essentially beryllium in black, copper in white, and an intermediate phase in gray showing a peculiar beaded structure of the matrix. The area enclosed with ink lines is shown in beautiful detail at 300 diameters in Figure 4*B*.

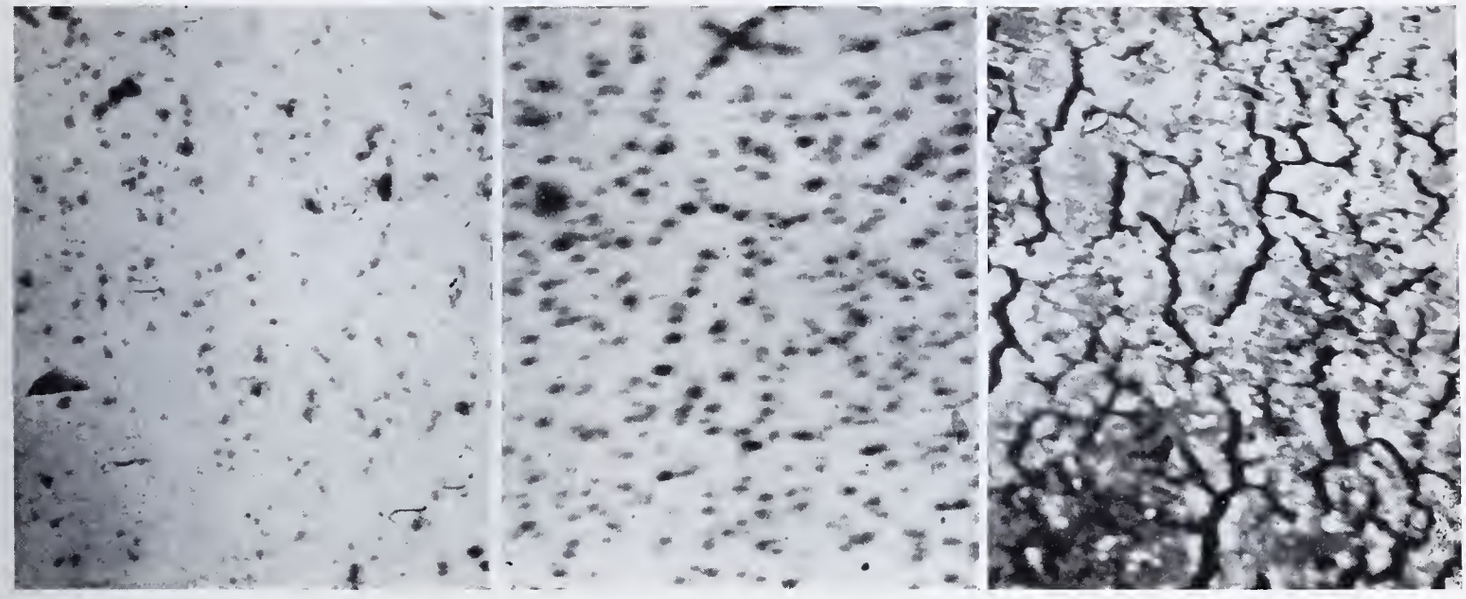
Figure 5 for a magnesium-aluminum alloy has been discussed above. The more highly absorbing aluminum phase is shown as needlelike crystals.



A *B* *C*

FIGURE 7. MICRORADIOGRAPHS (NEGATIVE PRINTS)

A. Leaded iron 0.003 inch thick, made with characteristic molybdenum radiation. Magnification ×90. Lead light, iron dark
B. Rolled beryllium foil containing microporous streaks. Made with characteristic chromium radiation. Magnification ×100
C. Aircraft aluminum-copper alloy, made with characteristic molybdenum radiation. Magnification ×100. Aluminum dark, copper light



A *B* *C*

FIGURE 8. MICRORADIOGRAPHS (NEGATIVE PRINTS)

A. Silicon steel with inclusions (dark), made with characteristic copper radiation. Magnification ×200
B. Graphitic steel showing graphite dark, made with characteristic molybdenum radiation. Magnification ×100
C. Season-cracked brass with cracks black and lead white, made with characteristic molybdenum radiation. Magnification ×100

TABLE I. RADIATION

Copper Radiation		Iron Radiation		Molybdenum Radiation	
Be	2.96	Be	5.64	Be	0.58
Mg	71.4	Mg	135	Mg	7.53
Al	132	Al	252	Al	14.1
Si	144	Si	266	Si	15.2
S	182	S	346	S	19.8
Ag	228	Fe	560	Ti	109
Zn	418	Zn	785	Mo	203
Ni	427	Ni	797	Cr	221
Cu	454	Cu	868	Sn	248
Ti	958	Ti	1777	Ag	289
Cr	1659	V	2612	Fe	303
Sn	1798	Sn	3422	Co	383
Mn	2124	Cr	2460	Zn	421
Pb	2609	Ag	4253	Ni	427
Fe	2578	Pb	4854	Cu	455
Co	3186	W	5790	Pb	1537
W	3397			W	2007

Figure 6 for a bronze has also been discussed in connection with the technique of two or more characteristic radiations in showing the marked difference in using molybdenum and copper Kα rays for diagnostic purposes. Reference to Figure 2 demonstrates that with the former radiation the tin should not appear (Figure 6*A*) but will be disclosed with copper Kα rays (Figure 6*B*). The two figures correspond exactly and any chosen area may be compared to show the effect of tin on the general structure. Actually in Figure 6*A*, some slight evidence of the tin areas appears in gray, from which we might conclude that there is some solid solution of the tin in lead or that it is in the form of an intermetallic compound.

Figure 7*A*, represents the difficult case of leaded iron (lead being introduced for improved machinability without otherwise affecting physical properties). The specimen is cold-rolled in fabrication, so that the lead appears as extended white streaks parallel with the rolling direction.

The microradiographic technique has been successfully used in perfecting the process of rolling beryllium into foil for use as windows in Machlett x-ray diffraction tubes. Because of the very great transparency of this element of atomic number 4 it presents perhaps the most difficult possible case. Furthermore, it is almost impossible to prepare satisfactory optical photomicrographs for this metal. The sample in question was found unsatisfactory in maintaining a high Coolidge vacuum. The microradiograph in Figure 7*B*, with characteristic chromium Kα radiation (2.285 Å.) shows clearly the streaks of inclusions responsible for the microporosity.

Figure 7*C*, is a typical microradiograph for aircraft age-hardening aluminum alloy. Copper, supposedly in solid solution, actually appears, as white streaks at grain boundaries, and in this case in lamellar layers. This alloy was unsatisfactory in performance.

Figure 8*A*, illustrates inclusions which were missed entirely on photomicrographs in very large grains of silicon steel for high-duty electrical use. This is an excellent example of the value of the three-dimensional information of the microradiograph in contrast with the two-dimensional view of a polished surface in the photomicrograph.

Figure 8*F*, illustrates the distribution of graphite (black)

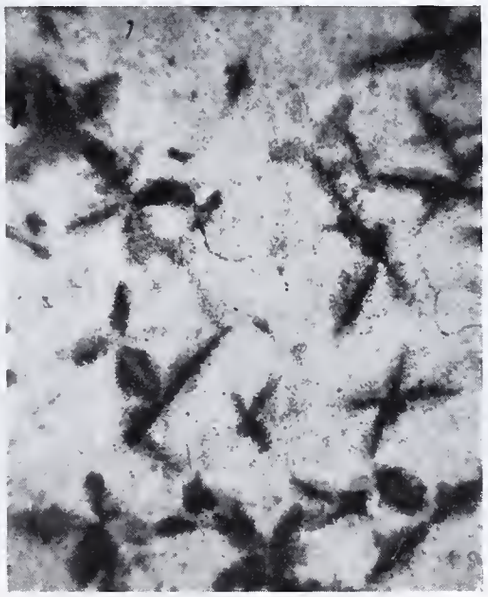
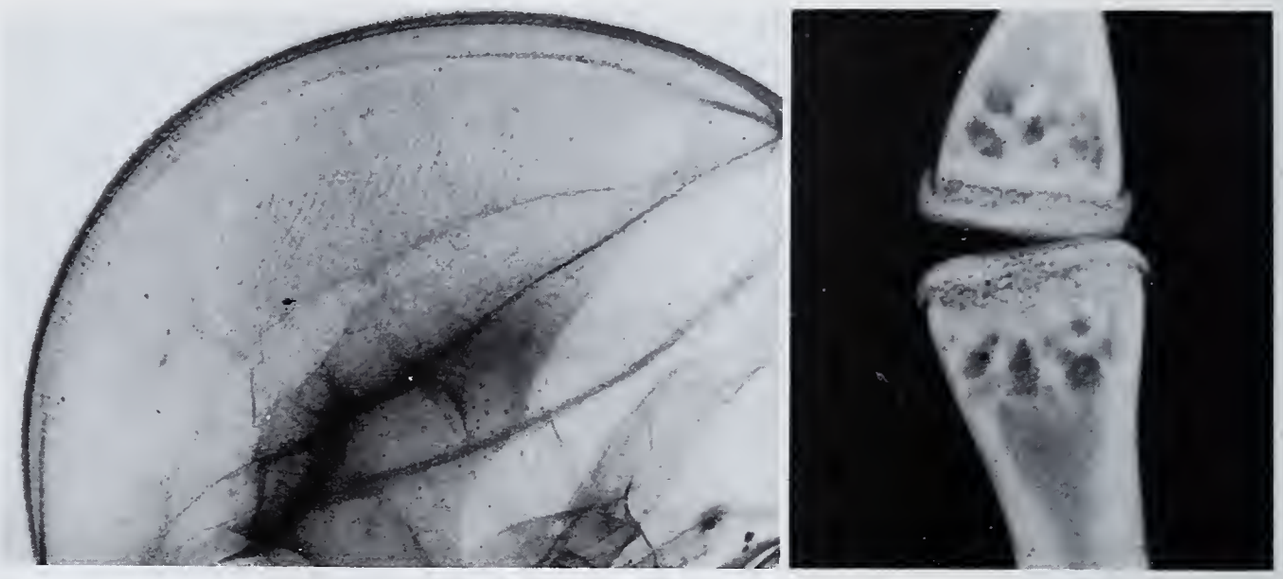


FIGURE 9. MICRORADIOGRAPH (NEGATIVE PRINT) OF TYPE METAL (LEAD-ANTIMONY CRYSTALS DARK), MADE WITH CHARACTERISTIC MOLYBDENUM RADIATION. MAGNIFICATION ×200



A B

FIGURE 10. MICRORADIOGRAPHS

A. Positive print of the eye of a fly made with characteristic copper radiation. Magnification ×150
B. Negative print of the smallest bone in the foot of a very small frog taken through the web, made with characteristic copper radiation. Magnification ×50

in some of the new graphitic steels now being widely used for machining operations.

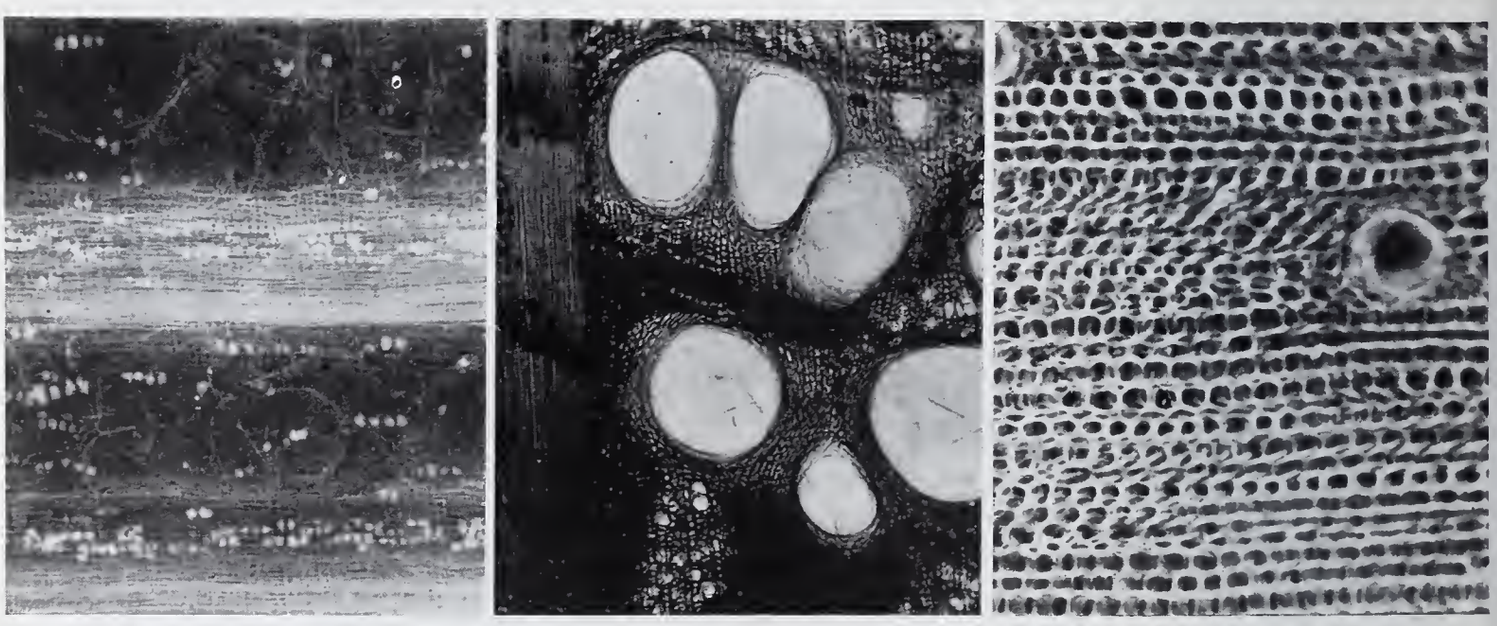
Figure 8*C*, is typical of season-cracked cartridge brass, a long recognized and dreaded failure. Numerous cases have been found similar to this of the incipient formation of cracks and other failures in metals as a result of fatigue or temperature conditions.

Figure 9 is a striking example of magnification at 200 diameters of intermetallic crystals in type metal (essentially lead-antimony) which serve to preserve the type against deformation of the soft lead of the matrix.

In similar fashion a wide variety of alloys, metals such as tungsten which are very difficult to use in photomicrography, ceramic materials, minerals, powder mixtures, fillers in rubber and plastics, bone structures especially in cases of lead poisoning, soil and clay sections, fillers in paper, foreign particles in insulators, and many other materials have been subjected successfully to this technique.

Biological Microradiography

An extended account of this application by Clark and Bicek will appear in an appropriate journal, but a few of the choicest photographs are illustrated here. For the most part a successful radiograph for very small biological specimens depends very largely on a difference in density of a given material such as cellulose, chitin, protein, etc., where oxygen is ordinarily the heaviest element present in the compound. In such cases there is no segregation of different chemical ele-



A B C

FIGURE 11. MICRORADIOGRAPHS OF SECTIONS OF WHITE OAK WOOD, MADE WITH CHARACTERISTIC COPPER RADIATION. MAGNIFICATIONS ×50

A. Negative print of radial section showing in white rows calcium oxalate crystals
B. Positive print of transverse section (note fibers in large channel openings)
C. Negative print of tangential section

ments or phases as in alloys, unless, for example, a tissue is injected with thorotrast or some other heavily absorbing substance. The photographs here were made with the characteristic Kα radiation of copper exactly as with metals. Very soft general radiation generated at low voltages is useful for this type of specimen depending upon very slight differences in thickness of the same material in adjacent areas. Figure 10*A*, shows in amazing detail the eye of an ordinary house fly; Figure 10*B*, is the microradiograph of the smallest bones in the foot of a very small frog, taken through the web of the foot; Figure 11 illustrates the striking structures of white oak wood taken through radial, transverse, and tangential sections. The three photographs are highly characteristic of each type of wood at different ages, conditions of growth, drying, impregnation, and other treatments to which wood may be subjected. It is evident that very accurate measurements can be made even of cell-wall thicknesses.

Acknowledgments

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Identification of Sugars

By Microscopic Appearance of Crystalline Osazones

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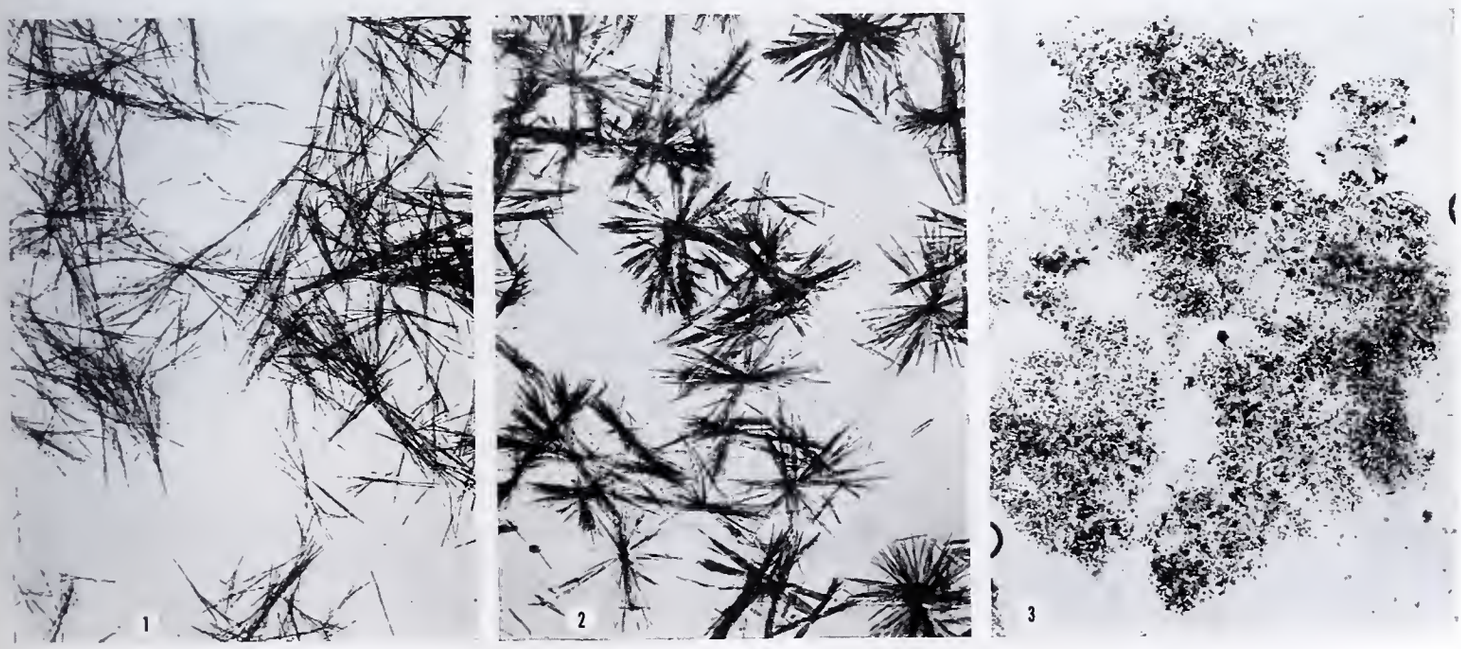
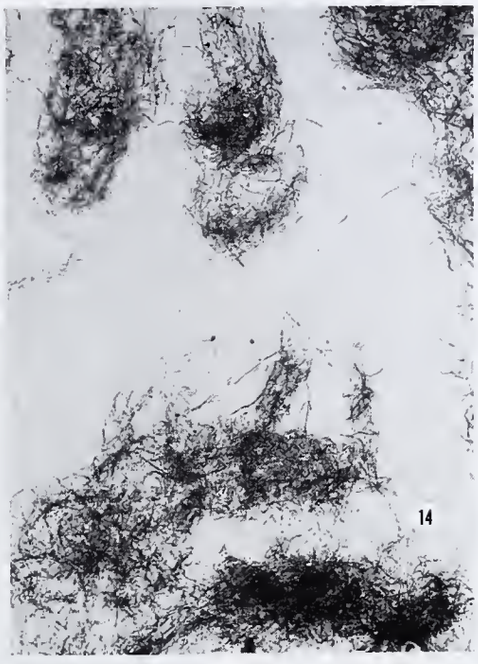
THE carbohydrates as a group form a number of chemical derivatives and give a variety of reactions greater perhaps than any other class of chemical compounds. However, because of the close similarity in properties of the various sugars, the identification of the different members is often difficult.

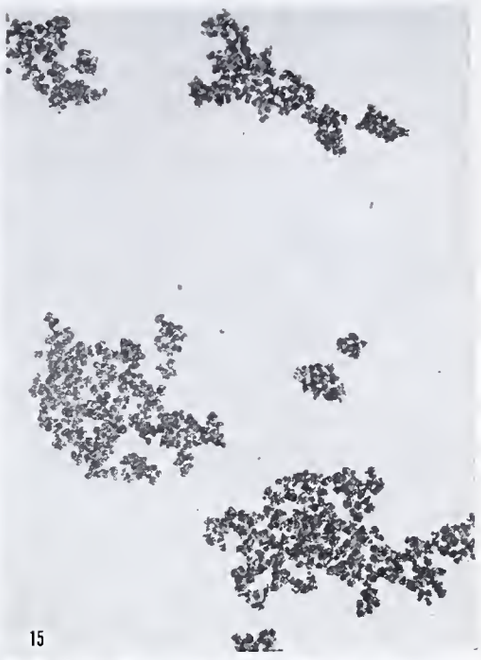
One of the many tools available to the chemist for the identification of pure sugars and their crystalline derivatives is the microscope. The identification of pure sugars and certain sugar mixtures has been accomplished by crystallizing the sugar from saturated aqueous solutions upon the addition of precipitating agents (*4*, *12*). The sugar is then identified by its characteristic crystal habit (*2*, *12*), and further confirmation is made by a study of the optical properties



1 Glucosazone 2 Galactosazone 3 Sorbosazone (amorphous)

4 Sorbosazone (crystalline)
5 Mannose phenylhydrazone
6 Arabinosazone
7 Xylosazone
8 Rhamnosazone
9 Fucosazone
10 Maltosazone
11 Lactosazone
12 Cellobiosazone





13 Melibiosazone
14 Gentibiosazone
15 Phenylhydrazine phenylhydrazone galacturonate
16 Phenylhydrazine phenylhydrazone glucuronate
17 Diphenylhydrazine fructose diphosphate phenylhydrazone
18 Diphenylhydrazine glucose monophosphate osazone
19 Mucic acid
20 Potassium acid saccharate

(*6*, *12*, *14*, *15*). However, this method is objectionable in that impure samples often fail to crystallize, and a study of optical properties requires the use of the petrographic microscope, which may not be readily available in many laboratories.

The phenylhydrazine reactions first introduced by Fischer (*3*) are still one of the most important tests for the identification of sugars. The osazones produced by various sugars are crystalline and give definite melting points by which the identity of the sugar may be established.

In dealing with unknown sugars, impurities may be present and if the available quantity is insufficient for further purification, advantage can be taken of the phenylhydrazine reaction. The osazones possess characteristic crystalline forms when observed under the microscope. Not more than 5 mg. of sugar is needed for this purpose.

In the identification of a sugar, the unknown osazone may be compared with one prepared simultaneously from known sugars or with photomicrographs of osazones of different sugars. By observing the crystal form of the osazone under the low power of the ordinary microscope, a particular sugar may be tentatively identified. Further confirmation is desirable from melting point determinations or from a study of the optical properties, if a petrographic microscope is available (*9*, *16*, *17*).

Although photomicrographs of a number of sugar osazones are shown by Morrow and Sandstrom (*10*), the present authors found it desirable to present a more extended list of phenylhydrazine derivatives of reducing sugars and of several important sugar derivatives. For this reason photomicrographs of phenylhydrazine derivatives of the commonly occurring hexoses, pentoses, methylpentoses, disaccharides, uronic acids, and hexosephosphates were prepared and are shown here (×45).

Procedure

To prepare the osazone 2 cc. of a neutral clear solution, containing 5 to 20 mg. of the sugar, are placed in a 15 × 150 mm. test tube with 0.3 gram of powdered sodium acetate and 0.2 gram of phenylhydrazine hydrochloride, and the tube is shaken until the contents are dissolved. The loosely stoppered test tube is then immersed in a boiling water bath for 30 minutes and after reacting is allowed to cool slowly at room temperature. Sudden cooling by immersion in cold water should be avoided, since this causes distortion of the crystals. The osazones of the monosaccharides usually crystallize while hot. If the amount of the sugar is too small, however, crystallization occurs only after cooling. Since the osazones of disaccharides are soluble while hot, they can be separated from those of the monosaccharides by taking advantage of this difference in solubility. The monosaccharide is first identified when the solution is still warm; the solution is then filtered, and slowly cooled to allow crystallization of the disaccharide osazone.

The osazone is identified by placing a drop of the solution with a pipet (a wide tip pipet should be used in order not to break up the crystals) on a slide and observing the shape of the crystals with a microscope at low power.

There are certain limitations to the identification of sugars by the procedure. The osazone reaction with phenylhydrazine is not always an absolute test of the identity of a sugar, as a number of sugars, having a common enolic form, yield the same osazone. Thus, the hexose sugars, *d*-glucose, *d*-mannose, and *d*-fructose, yield the same phenylosazone. Similarly, the same osazone is obtained from *d*-galactose, *d*-talose, and *d*-tagatose. Other examples of sugars giving the same osazone are *d*-allose, *d*-altrose, and *d*-pseudofructose; *d*-idose, *d*-gulose, and *d*-sorbose. The pentose sugars, *d*-arabinose and *d*-ribose, yield one osazone; *d*-xylose and *d*-lyxose give another. Furthermore, the corresponding groups of sugars belonging to the *l*-series, having the same configuration beyond the second carbon atom, also yield one and the same osazone, differing from the *d*-osazone only in the direction of optical rotation. Additional tests should therefore be made in order to identify a sugar belonging in a particular enolic series.

Glucose, fructose, and mannose, for example, may be distinguished from one another as follows:

Glucose on oxidation with nitric acid (*10*) and neutralization with potassium carbonate or hydroxide produces crystals of potassium acid saccharate (No. 20); mannose forms an insoluble phenylhydrazone when treated with phenylhydrazine hydrochloride and sodium acetate, as previously described, and allowed to remain in the cold (No. 5); fructose may be identified by the Seliwanoff reaction (*1*). Galactose can be distinguished from its other enolic forms by preparing the *l*-tolylhydrazone derivative (*5*). *d*-Gulose may be distinguished from *d*-sorbose and *d*-idose by the fact that, when oxidized with nitric acid, potassium acid saccharate is obtained. Sorbose, being a ketose sugar, gives the Seliwanoff reaction. In general, when a sugar is identified by the characteristic shape of the crystal of its osazone, the identification should be confirmed by some other test.

Since in the last decade the hexosephosphates gained much prominence in the intermediary metabolism of animals and plants, photomicrographs of the phenylhydrazine derivatives of fructose diphosphate (Harden Young ester, *7*), and the glucose-6-phosphate (Robison ester, *13*) are included. The fructose-6-monophosphate (Neuberg ester) gives the same osazone as the Robison ester. The latter is oxidizable with hypoiodite and thus can be distinguished from the former (*8*).

Remarks

Sorbose produced an amorphous osazone (No. 3). However, when this osazone was allowed to remain for 24 hours, the amorphous mass changed into crystalline needles (No. 4).

When fructose diphosphate was heated with phenylhydrazine hydrochloride and sodium acetate for 15 minutes, diphenylhydrazine fructose diphosphate phenylhydrazone (No. 17) was formed (*7*). Prolonged heating (one hour or more) caused hydrolysis of the phosphoric acid group on the first carbon atom, producing the same phenylhydrazine hexose monophosphate osazone as the Robison ester (No. 18).

Galacturonic acid, when heated with the same reagents for 30 minutes, produced a derivative (No. 15) which was identical with the phenylhydrazine phenylhydrazone of galacturonate described by Niemann, Schoeffel, and Link (*11*).

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INDUSTRIAL AND ENGINEERING CHEMISTRY

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Tabulated Diffraction Data for Cubic Isomorphs

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CHEMICAL analysis by the Debye-Scherrer-Hull method consists of matching the diffraction pattern of an unknown material with one or more standard powder patterns (*1–6, 11–13, 15, 16, 18–22*). In applying this empirical method to a large number of actual analyses, one encounters cases where the patterns sought are not found in a collection of 1–2000 catalogued patterns. In those instances the matching method can be augmented by systematically comparing the diffraction pattern of an unidentified phase with representative patterns of the various known crystal structures in an attempt to establish isomorphism between the unknown phase and one of the standard structures. This comparison method is readily applicable in the case of cubic substances, since their diffraction patterns are recognized by the absence of "splitting" of lines—i. e., in the isotropic system the interplanar distance d_{hkl} is independent of the sign and order of the indices h, k, and l. The procedure for comparing the diffraction patterns of isomorphous substances has been described previously (*8*); hence only the tabulated diffraction data for cubic isomorphs are presented here.

Figures 1 and 2 contain representative diffraction patterns of 33 cubic crystal structures designated as in the "Strukturbericht" (*7, 9, 10, 14*). Within each group—e. g., the A-structures—the patterns are arranged in increasing order of complexity. The averaged relative intensities, based predominantly on the Dow file of standard patterns, refer to MoK_α radiation; for other radiations well-known corrections for the polarization factor and absorption factor must be considered. The logarithmic scale and index scale of Figures 1 and 2 are useful in the rapid identification of cubic diffraction patterns.

Table I. X-Ray Diffraction Data

[Filtered MoK_α radiation was used to obtain the powder diffraction data. d = interplanar spacing. I is the intensity of a powder reflection (in arbitrary units) while I/I_1 is the relative intensity. The lattice constants for the two cubic phases, A and B, are 6.964 ± 0.005 Å. and 6.643 ± 0.009 Å., respectively.]

		hkl		I/I1	
d, Å.	I	Phase A	Phase B	Phase A	Phase B
4.02	10	111	...	0.50	..
3.83	6	...	111	..	0.30
3.48	20	200	...	1.00	..
3.315	20	...	200	..	1.00
2.460	17.5	220	...	0.88	..
2.350	17.5	...	220	..	0.88
2.100	6	311	...	0.30	..
2.010	8	222	311	0.40	..
1.920	4	...	222	..	0.20
1.740	2	400	...	0.10	..
1.660	2	...	400	..	0.10
1.599	1.5	331	...	0.08	..
1.559	6	420	...	0.30	..
1.487	6	...	420	..	0.30
1.421	4	422	...	0.20	..

Table II. X-Ray Diffraction Data

[Filtered MoK_α radiation was used to obtain the powder diffraction data. d = interplanar spacing. I is the intensity of a powder reflection (in arbitrary units), while I/I_1 is the relative intensity. The lattice constant of Ag(Br, I) is 5.916 ± 0.009 Å. and corresponds to the approximate composition 60 AgBr.40 AgI.]

		hkl		I/I1	
d, Å.	I	Ag(Br, I)	AgI	Ag(Br, I)	AgI
3.77	3	...	111	..	1.0
2.96	75	200	...	1.00	...
2.29	2	...	220	..	0.7
2.087	40	220	...	0.53	...
1.961	1	...	311	..	0.3
1.710	10	222	...	0.13	...
1.479	3	400	...	0.04	...
1.324	7	420	...	0.09	...
1.207	4	422	...	0.05	...

General Procedure for Identifying a Noncatalogued Pattern. (1) Plot the log d values and corresponding relative intensities of the unidentified pattern on a strip of translucent paper; (2) use the index scale of Figure 1 to check if the pattern is cubic; (3) find an isomorphic prototype from Figures 1 and 2, also check Table III; (4) compute the lattice constant of the unidentified cubic phase and check Table IV or "Tables of Cubic Crystal Structure" (*17*); (5) confirm the identification of the unknown phase by a qualitative spectroscopic analysis or by spot tests.

In case the substance to be identified is not listed in Table IV, one may still establish its type of structure and then synthesize the unknown phase on the basis of the elements present. The following example illustrates the procedure.

Columns 1 and 2 of Table I give the diffraction data of an unknown material. On plotting the log d values and checking the index scale of Figure 1, one recognizes that the material consists of two cubic phases, both of which are found to be isomorphous with sodium chloride (structure B 1). The respective lattice constants for phases A and B are 6.96 Å. and 6.64 Å., and according to Table IV the compounds BaTe (6.99 Å.) and SrTe (6.65 Å.) are indicated. However, a qualitative spectroscopic analysis shows potassium to be the only metallic element present. An examination of Table IV for potassium salts with the sodium chloride structure suggests a solid solution of potassium iodide for phase A and a solid solution of potassium bromide for phase B. The silver nitrate test with an aqueous solution of the unknown yields a typical silver halide precipitate, the diffraction pattern of which is identified as a solid solution of silver bromide and silver iodide (see Table II). Assuming approximate validity for Vegard's rule and using the crystal radii of the iodide ion (2.20 Å.) and of the bromide ion (1.96 Å.), one calculates the composition of the mixed silver halide circa 60 AgBr.40 AgI (spot tests on the original unknown for chloride and cyanide being negative). Having con-

Table III. Comparison of Structures

[The structures having comparable powder patterns are listed in rows. In the column below each structure are listed examples and criteria distinguishing it from the structure in the first column. Z_A is the atomic number of element A. I_{hkl} refers to the intensity of reflection (hkl). For some structures, such as B 1 two special cases are considered—namely, the CsH type for which the odd reflections (111, 311, 331, etc.) are strong and the RbBr type for which the odd reflections are extremely faint.]

Structures Having Comparable Powder Patterns

A 4	B 32 $Z_B >> Z_A$ NaTl, LiIn	B 3 $Z_A \approx Z_B$ InSb, CdTe	C 1 $Z_A \approx 2Z_B$ $SrCl_2$ $Pt(In, Ga)_2$	.....	.....	.....	...
A 1	B 1 $Z_A >> Z_B$ KH, RbH, CsH, TaC LiI	B 3 $Z_B >> Z_A$ BeSe, BeTe $I_{220} > I_{230}$	C 1 $Z_A >> 2Z_B$ UO_2, PbF_2 ThO_2, ZrO_2 BaF_2, CeO_2 Mg_2Pb	C 3 $Z_A >> Z_B$ Check (110)	J 1_1 $a_{J1_1} > a_{A1}$	J 2_1 $a_{J2_1} > a_{A1}$	...
A 2	B 2 $Z_A \approx Z_B$ CsI, CuZn	D 2_1 CaB_6	.....	.....	.....	.....	...
B 1	$Z_A >> Z_B$ CsH, RbH, KH See A 1	$Z_A \approx Z_B$ RbBr, KCl, NaF, SrSe, SnTe (111), (311) very weak	C 1 $I_{200} > I_{111}$	.....	.....	.....	...
B 3	$Z_B >> Z_A$ BeSe, BeTe, HgS See A 1	$Z_A \approx Z_B$ InSb, CdTe See A 4	B 32	C 1	C 3 $Z_A >> Z_B$ Check (110)	.....	...
B 32	$Z_B >> Z_A$ NaTl See A 4	$Z_A >> Z_B$	B 3 InSb, CdTe	C 1 CaF_2, $SrCl_2$	.....	.....	...
B 20	$Z_A >> Z_B$	$Z_A \approx Z_B$	F 1 Check I_{200}, I_{220}	GO_3 Check I_{200}			
B 2	$Z_A >> Z_B$ HgLi, PdBe, AuMg	$Z_A \approx Z_B$ CsI, CuZn See A 2; CaB_6 See D 2_1	E 2_1 Check I_{200}	.....	.....	.....	...
C 1	$Z_A >> 2Z_B$ UO_2, ThO_2, ZrO_2, PbF_2 BaF_2, Li_2Te Mg_2Pb See A 1	$Z_A \approx 2Z_B$ CaF_2, $SrCl_2$ See A 4	B 3	B 32	C 3 $Z_A >> Z_B$ Check (110)	J 1_1 $a_{J1_1} > a_{C1}$	...
C 15	$Z_A >> Z_B$ Au_2Na	$Z_B >> Z_A$ (Be_2Cu) See A 4	H 1_1	.....	.....	.....	...
C 2	$Z_A >> Z_B$ OsS_2 See A 1	F 1 Check (110)	K 6_1	G 2_1	H 4_{13} $a_{H4_{13}} > a_{C2}$	.....	...
C 3	$Z_A >> Z_B$ Ag_2O See A 1	$Z_B >> Z_A$ See A 2	B 3 (110) absent	C 1 (110) absent	J 1_1 $a_{J1_1} > a_{C3}$	.....	...
F 1	B 20 Check I_{200}	G 0_3	H 2_1 (111), (221), (311) absent	.....	.....	.....	...
D 5_3	D 1_1 Check (111), (210), (320)	Tetrahedrite (110), (310) very weak	.....	.....	.....	.....	...
D 6_1	C 15 Check I_{311}	D 1_1 Check (210), (211), (321)	H 1_1 Check I_{311}	.....	.....	.....	...
D 1_1	D 5_3 (111), (210), (320) absent	D 6_1 (210), (211), (321) absent	Tetrahedrite (111), (210) absent	.....	.....	.....	...
E 2_1	B 2	.....	.....	.....	.....	.....	...
G 0_3	B 20 Check I_{200}	F 1	.....	.....	.....	.....	...
G 2_1	C 2 Check I_{200}, I_{222}	K 6_1 Check I_{200}	.....	.....	.....	.....	...
H 1_1	C 15 Check I_{111}, I_{220}, I_{222}	.....	.....	.....	.....	.....	...
H 5_8	C 1 Check I_{111}, I_{222}	J 1_1 Check I_{111}, I_{200}	J 2_1 Check I_{111}, I_{200}	.....	.....	.....	...
H 4_{13}	C 2 Check I_{200}, I_{221}	G 2_1 Check I_{221}, I_{222}, I_{321} $a_{H4_{13}} > a_{G2_1}$	H 2_4 $a_{H4_{13}} > a_{H2_4}$	K 6_1 Check I_{200}, I_{221}	.....	.....	...
H 2_1	B 20 Check (111), (311)	F 1 Check (111), (311)	S 6_2 Check I_{210}, I_{320}	.....	.....	.....	...

TABLE III (*Continued*)

H 2_4	C 1 (210), (221) absent	H 4_{13} Check I_{321}	.....	.....	.....	.....	...
J 1_1	A 1 $a_{J1_1} > a_{A1}$	B 1 $a_{J1_1} > a_{B1}$	B 3 $a_{J1_1} > a_{B3}$	C 1 $a_{J1_1} > a_{C1}$	C 15 (200) absent $a_{J1_1} > a_{C15}$	C 3 Check (110) $a_{J1_1} > a_{C3}$	J 2_1
J 2_1	A 1 $a_{J2_1} > a_{A1}$	B 1 $a_{J2_1} > a_{B1}$	B 3 Check I_{200}, I_{222}, I_{400} $a_{J2_1} > a_{B3}$	C 1 Check I_{400} $a_{J2_1} > a_{C1}$	C 3 Check I_{222}, I_{400} Check (110) $a_{J2_1} > a_{C3}$	H 5_8 Check I_{200}	J 1_1
K 6_1	C 2	G 2_1 Check I_{200}, I_{222}	.....	.....	.....	.....	...
S 1_4	.....	.....	.....	.....	.....	.....	...
S 6_1	.....	.....	.....	.....	.....	.....	...
S 0_8	C 15 Check I_{400}, I_{422}, I_{440}	H 1_1 Check I_{111}, I_{400}, I_{440}	J 1_1 Check I_{200}, I_{220}, I_{400}	J 2_1 Check I_{200}, I_{220}, I_{400}	.....	.....	...
S 6_2	H 2_1 Check I_{210}, I_{320}	.....	.....	.....	.....	.....	...
Tetrahedrite		D 5_3	.....	.....	.....	.....	...

TABLE IV. CUBIC SUBSTANCES

[Table IV lists 705 cubic substances including some pseudocubic cases such as perovskite (E 1_1) and structures related to J 2_1. For each isomorphous group the members are arranged in the ascending order of their lattice constants. (To avoid repetition the various literature values have been averaged.) For specific references prior to 1938 see "Strukturbericht" Vols. I to V; for later references see *Chemical Abstracts*, **32** to **35** (1938–41).]

a, Å.	Substance
	A 4
3.56	C (diamond)
5.42	Si
5.62	Ge
6.46	α-Sn
	A 1
3.517	Ni
3.554	α-Co
3.60	Taenite (57.7% Fe, 40.8% Ni, 0.5% P)
3.608	Cu
3.63	γ-Fe (1370° K.)
3.797	Rh
3.831	Ir
3.880	Pd
3.88–4.04	Pd-H
3.912	Pt
4.041	Al
4.070	Au
4.077	Ag
4.30	Co-N
4.40–4.46	Ti-H
4.52	Ne (4° K.)
4.66	Zr-H
4.84	β-Tl
4.939	Pb
5.08	Th
5.14	α-Ce
5.296	β-La
5.43	A (4° K.)
5.56	Ca
5.59	Kr (20° K.)
5.70	Kr (92° K.)
6.05	Sr
6.20	X (88° K.)
	A 2
2.861	α-Fe
2.875	α-Cr
2.90	β-Fe (1070° K.)
2.93	δ-Fe (1700° K.)
3.03	V
3.03–3.41	V-C
3.140	Mo
3.157	W
3.295	Cb
3.30	Ta
3.32	β-Ti (1200° K.)
3.46	Li (~80° K.)
3.50	Li
3.61	β-Zr (1120° K.)
4.24	Na (~80° K.)
4.29	Na
5.02	Ba
5.20	K (120° K.)
5.33	K
5.62	Rb (~80° K.)
6.05	Cs (~80° K.)
	B 1
4.018	LiF
4.065	LiD
4.08	VO
4.09	LiH
4.12	(Li_2TiO_3)
4.12–4.20	(Li_2TiO_3-MgO)
4.13	VN
4.14	CrN
4.14	VC
4.142	($63Li_2Fe_2O_4.37Li_2TiO_3$)
4.173	NiO
4.207	MgO
4.282	MgO (1570° K.)
4.225	TiN
4.235	TiO
4.24	80 TiN - 20 TiC
4.27	CoO
4.28	V-N
4.283	FeO (160° K.)
4.290	FeO (299° K.)
4.30	VC (ε-phase)
4.315	TiC
4.40	CbC
4.41	CbN
4.426	MnO (117° K.)
4.436	MnO (299° K.)
4.44	ScN
4.446	TaC
4.458	HfC
4.615	NaF
4.62	ZrN
4.69	CdO
4.69	ZrC
4.80	CaO
4.82	(Na_2CeO_3)
4.84	(Na_2PrO_3)
4.88	NaH
4.92	AgF
5.006	CaNH
5.13	SrO
5.14	LiCl
5.14	NdN
5.19	MgS
5.192	MnS (130° K.)
5.210	MnS (299° K.)
5.33	KF
5.45	MgSe
5.45	MnSe
5.45	SrNH
5.49	LiBr
5.52	BaO
5.545	AgCl
5.55–5.76	AgCl-AgBr
5.627	NaCl
5.63	RbF
5.68	CaS
5.69	SnAs
5.70	KH
5.755	AgBr
5.76–5.92	AgBr-AgI
5.83	NdP
5.83	NaCN
5.84	BaNH
5.87	SrS
5.91	CaSe
5.94	PbS
5.95	NaBi
5.957	EuS
5.96	NdAs
6.00	PrAs
6.00	LiI
6.01	CsF
6.04	RbH
6.05	β-NaSH (>360° K.)
6.06	CeAs
6.13	LaAs
6.14	PbSe
6.23	SrSe
6.278	KCl
6.285	SnTe
6.31	NdSb
6.345	CaTe
6.35	PrSb
6.36	BaS
6.38	CsH
6.40	CeSb
6.44	PbTe
6.462	NaI
6.45	PrBi
6.48	LaSb
6.49	CeBi
6.53	KCN
6.53	NH_4Cl (> 457° K.)
6.56	RbCl
6.57	LaBi
6.58	KBr
6.59	BaSe
6.60	β-KSH (>440° K.)
6.65	SrTe
6.82	RbCN
6.86	RbBr
6.90	NH_4Br (>411° K.)
6.93	β-RbSH (470° K.)
6.99	BaTe
7.052	KI
7.10	β-CsCl (>730° K.)
7.24	NH_4I (>255° K.)
7.325	RbI
	H 0_5
6.96 ± 0.04	$AgClO_4$ (453 ± 20° K.)
7.16 ± 0.10	$NaClO_4$ (618 ± 35° K.)
7.49 ± 0.02	$KClO_4$ (598 ± 15° K.)
7.65 ± 0.05	$TlClO_4$ (553° K.)
7.65 ± 0.02	NH_4ClO_4 (528 ± 15° K.)
7.68 ± 0.03	$RbClO_4$ (583 ± 10° K.)
7.97 ± 0.01	$CsClO_4$ (513 ± 10° K.)
	B 3
4.255	CuF
4.36	CSi IV
4.855	BeS
5.10	BeSe
5.304	$(Cu, Fe, Mo, Sn)_4(S, As, Te)_{3-4}$, cousite
5.41	CuCl
5.425	β-ZnS
5.43	AlP
5.44	GaP
5.58	BeTe

(*Continued top of next page*)

TABLE IV (*Continued*)

a, Å.	Substance
	B 3 (*Contd.*)
5.60	MnS (red)
5.63	AlAs
5.635	GaAs
5.655	ZnSe
5.68	CuBr
5.82	β-CdS
5.84	HgS
5.86	InP
6.04	CdSe
6.04	InAs
6.05	CuI
6.07	HgSe
6.08	ZnTe
6.103	α-Cu_2HgI_4
6.12	AlSb
6.12	GaSb
6.13	SnSb
6.383	α-Ag_2HgI_4
6.40	HgTe
6.43	CdTe
6.45	InSb
6.48	AgI
	B 32
6.195	LiGa
6.209	LiZn
6.36	LiAl
6.687	LiCd
6.786	LiIn
7.297	NaIn
7.373	($CeMg_3$
7.373	($PrMg_3$
7.473	NaTl
	B 20
4.437	NiSi
4.438	FeSi
4.438	CoSi
4.548	MnSi
4.620	CrSi
	B 2
2.603	NiBe
2.606	CoBe
2.69	CuBe
2.813	PdBe
2.82	AlNi
2.945	CuZn
2.989	CuPd
3.146	AuZn
3.156	AgZn
3.168	AgLi
3.259	AuMg
3.275	AgMg
3.287	HgLi
3.325	AgCd
3.34	AuCd (670° K.)
3.424	LiTl
3.442	HgMg
3.628	MgTl
3.67	PrZn
3.70	CeZn
3.73	AlNd
3.74	α-RbCl (83° K.)
3.75	LaZn
3.82	TlCN
3.82	PrCd
3.84	TlSb
3.835	TlCl
3.847	CaTl
3.86	NH_4Cl (< 457° K.)
3.86	CeCd
3.88	MgPr
3.90	LaCd
3.97	TlBr
3.98	TlBi
4.024	SrTl
4.05	NH_4Br (< 411° K.)
4.112	CsCl
4.20	TlI
4.20	CsCl (< 720° K.)
4.25	CsCN
4.287	CsBr
4.29	CsSH
4.37	NH_4I (290° K.)
4.56	CsI
	D 2_1
4.07	YB_6
4.07	ErB_6
4.10	NdB_6
4.12	GdB_6
4.12	PrB_6
4.13	CeB_6
4.13	YbB_6
4.14	CaB_6
4.15	LaB_6
4.15	ThB_6
4.19	SrB_6
4.33	BaB_6
	C 1
4.33	Be_2C
4.619	Li_2O
5.06	($3ZrO_2.MgO$)
5.07	ZrO_2
5.08	($95ZrO_2.5CeO_2$)
5.13	($95HfO_2.5CeO_2$)
5.38	PrO_2
5.40	CeO_2
5.40	CdF_2
5.406	CuF_2
5.45	CaF_2
5.47	UO_2
5.526	($66CaF_2.33YF_3$)

a, Å.	Substance
	C 1 (*Contd.*)
5.53	($91CaF_2.9ThF_4$)
5.54	HgF_2
5.55	Na_2O
5.58	ThO_2
5.59	Cu_2S
5.704	Li_2S
5.749	Cu_2Se
5.782	SrF_2
5.796	EuF_2
5.838	($66SrF_2.33LaF_3$)
5.91	$PtAl_2$
5.91	$PtGa_2$
5.935	β-PbF_2 (520° K.)
5.99	Al_2Au
6.005	Li_2Se
6.06	$AuGa_2$
6.19	BaF_2
6.34	Mg_2Si
6.35	$PtIn_2$
6.368	RaF_2
6.379	Mg_2Ge
6.436	K_2O
6.50	Li_2Te
6.50	$AuIn_2$
6.526	Na_2S
6.763	Mg_2Sn
6.809	Na_2Se
6.81	Mg_2Pb
6.98	$SrCl_2$
7.314	Na_2Te
7.38	K_2S
7.65	RbS_2
7.676	K_2Se
8.152	K_2Te
	C 15
5.94	Be_2Cu
6.287	Be_2Ag
6.435	Be_2Ti
6.96	MgNiZn
7.03	Cu_2Mg
7.61	W_2Zr
7.79	Au_2Na
7.91	Au_2Pb
7.94	Au_2Bi
8.02	Al_2Ca
8.04	Al_2Ce
8.16	Al_2La
9.50	Bi_2K
	C 2
5.41	FeS_2
5.42	(Fe, Ni)S_2 (6.5% Ni)
5.57	RhS_2
5.57	RuS_2
5.57	Bravoite (53.8% NiS_2, 39.1% FeS_2, 7.1% CoS_2)
5.62	OsS_2
5.64	CoS_2
5.65	(Cu, Ni, Co, Fe)(S, Se)$_2$
5.68	PtP_2
5.74	NiS_2
5.85	$CoSe_2$
5.92	$RuSe_2$
5.93	$OsSe_2$
5.94	$PtAs_2$
5.97	$PdAs_2$
6.02	$NiSe_2$
6.096	MnS_2
6.36	$RuTe_2$
6.37	$OsTe_2$
6.43	$PtSb_2$
6.44	$PdSb_2$
6.64	$AuSb_2$
6.94	$MnTe_2$
	C 3
4.25	Cu_2O
4.73	Ag_2O
	F 1
5.55	CoAsS
5.68	NiAsS
5.90	NiSbS
	(Ni, Fe)AsS, plessite
	Ni(As, Sb)S, corynite
	Ni(Sb, Bi)S, kallilite
	(Co, Ni)SbS, willyamite
	D 5_3
8.13	Be_3N_2
9.37	(Mn, Fe)$_2O_3$
9.42	Mn_2O_3
9.74	Zn_3N_2
9.79	Sc_2O_3
9.94	Mg_3N_2
10.12	In_2O_3
10.15	Be_3P_2
10.37	Lu_2O_3
10.39	Yb_2O_3
10.52	Tm_2O_3
10.54	Er_2O_3
10.57	Tl_2O_3
10.58	Ho_2O_3
10.60	Y_2O_3
10.63	Dy_2O_3
10.70	Tb_2O_3
10.79	Gd_2O_3
10.79	Cd_3N_2
10.84	Eu_2O_3
10.85	Sm_2O_3
11.05	Nd_2O_3

a, Å.	Substance
	D 5_3 (*Contd.*)
11.40	α-Ca_3N_2
12.02	Mg_3P_2
12.33	Mg_3As_2
	D 6_1
11.05	As_4O_6
11.14	Sb_4O_6
	D 1_1
10.32	$ZrCl_4$
11.25	$TiBr_4$
(11.34)	(CBr_4) (> 320° K.)
(11.62)	(CI_4)
11.89	GeI_4
11.99	SiI_4
12.00	TiI_4
12.23	SnI_4
	E 2_1
3.67	$YAlO_3$
3.75	$CdTiO_3$
3.78	$LaAlO_3$
3.80	$CaTiO_3$
3.83	$NaWO_3$
3.85	(Na, Ce, Ca)(Ti, Cb)O_3, loparite
3.88	$NaTaO_3$
3.89	$LaGaO_3$
3.89	$NaCbO_3$
3.91	$SrTiO_3$
3.92	$CaSnO_3$
3.97	$BaTiO_3$
3.98	$KTaO_3$
3.99	$CaZrO_3$
4.00	$KMgF_3$
4.005	$KNiF_3$
4.01	$KCbO_3$
4.03	$SrSnO_3$
4.05	$KZnF_3$
4.07	$KCoF_3$
4.07	$SrHfO_3$
4.09	$SrZrO_3$
4.18	$BaZrO_3$
4.35	$BaPrO_3$
4.38	$BaCeO_3$
4.46	KIO_3
4.48	$BaThO_3$
4.5	NH_4IO_3
4.52	$RbIO_3$
4.66	$CsIO_3$
5.12	$MgZrO_3$
5.20	$CsCdCl_3$
5.33	$CsCdBr_3$
5.44	$CsHgCl_3$
5.77	$CsHgBr_3$
	G 0_3
6.57	$NaClO_3$
6.71	$NaBrO_3$
	G 2_1
7.60	$Ca(NO_3)_2$
7.81	$Sr(NO_3)_2$
7.84	$Pb(NO_3)_2$
8.11	$Ba(NO_3)_2$
	H 1_1
8.045	$NiAl_2O_4$
8.07	$CuAl_2O_4$
8.07	$CoCo_2O_4$
8.07	$MgAl_2O_4$
8.08	$CoAl_2O_4$
8.08	$ZnAl_2O_4$
8.10	$FeAl_2O_4$
8.11	(Ni, Co)(Co, Ni)$_2O_4$
8.11	(Zn, Co)Co_2O_4
8.11	$MgCo_2O_4$
8.27	$MnAl_2O_4$
8.27	(Mn, Co)(Co, Mn)$_2O_4$
8.28	$MgGa_2O_4$
8.30	$NiCr_2O_4$
8.30	$MgCr_2O_4$
8.31	$ZnCr_2O_4$
8.32	$CoCr_2O_4$
8.32	$ZnGa_2O_4$
8.35	$NiFe_2O_4$
8.35	$Cu_2Cr_2O_4$
8.35	$FeCr_2O_4$
8.36	$MgFe_2O_4$
8.38	$CoFe_2O_4$
8.38	$NiMn_2O_4$
8.40	$ZnFe_2O_4$
8.40	$FeFe_2O_4$
8.42	(Mn, Mg)Fe_2O_4
8.42	$TiCo_2O_4$
8.43	$MnCr_2O_4$
8.43	$TiMg_2O_4$
8.43	$TiZn_2O_4$
8.44	$CuFe_2O_4$
8.47	FeV_2O_4
8.49	$MnCr_2O_4$
8.50	$TiFe_2O_4$
8.54	$MnFe_2O_4$
8.58	$CdCr_2O_4$
8.58	$SnMg_2O_4$
8.61	$SnCo_2O_4$
8.63	$SnZn_2O_4$

TABLE IV (*Continued*)

a, Å.	Substance
	H 1_1 (*Contd.*)
8.67	$CdFe_2O_4$
8.67	$TiMn_2O_4$
8.81	$MgIn_2O_4$
9.26	Ag_2MoO_4
9.4	$CoCo_2S_4$
9.45	$(Co, Ni)_3S_4$
9.46	$CuCo_2S_4$
9.5	NiN_2S_4
9.92	$ZnCr_2S_4$
10.05	$MnCr_2S_4$
10.19	$CdCr_2S_4$
12.54	$K_2Zn(CN)_4$
12.76	$K_2Hg(CN)_4$
12.84	$K_2Cd(CN)_4$
	H 5_8
10.08	$2Na_2SO_4.NaCl.NaF$
	H 4_{13}
12.11	$KCr(SO_4)_2.12H_2O$
12.12	$KAl(SO_4)_2.12H_2O$
12.15	$NH_4Al(SO_4)_2.12H_2O$
12.15	$NH_4Fe(SO_4)_2.12H_2O$
12.20	$RbAl(SO_4)_2.12H_2O$
12.21	$TlAl(SO_4)_2.12H_2O$
12.31	$CsAl(SO_4)_2.12H_2O$
12.44	$NH_3.CH_3Al(SO_4)_2.12H_2O$ (β-alum)
	Langbeinite
9.93	$K_2Mg(SO_4)_3$
10.2	$K_2(Ca, Mg)(SO_3)_3$
	H 2_1
6.00	Ag_3PO_4
6.120	Ag_3AsO_4 (90° K)
6.130	Ag_3AsO_4 (380°K)
	H 2_4
5.37	Cu_2VS_4
	J 1_1
8.17	K_2SiF_6
8.35	$(NH_4)_2SiF_6$
8.38	$Rb_2CrF_6H_2O$
8.41	$Tl_2CrF_6H_2O$
8.42	$(NH_4)_2VF_6H_2O$
8.42	$Rb_2VF_6H_2O$
8.45	$Tl_2VF_6H_2O$
8.45	Rb_2SiF_6
8.58	Tl_2SiF_6
8.87	Cs_2SiF_6
8.99	Cs_2GeF_6
9.73	K_2PtCl_6
9.73	K_2OsCl_6
9.76	Tl_2PtCl_6
9.84	$(NH_4)_2PtCl_6$
9.86	K_2ReCl_6
9.88	Rb_2PtCl_6
9.92	Rb_2TiCl_6
9.94	$(NH_4)_2SeCl_6$
9.97	K_2SnCl_6
9.97	Tl_2SnCl_6
9.98	Rb_2SeCl_6
10.02	Rb_2PdBr_6
10.04	$(NH_4)_2SnCl_6$
10.08	$Ni(NH_3)_6Cl_2$
10.10	Rb_2SnCl_6
10.10	$Co(NH_3)_6Cl_2$
10.11	Tl_2TeCl_6
10.14	$(NH_4)_2PbCl_6$
10.14	K_2TeCl_6
10.15	$Fe(NH_3)_6Cl_2$
10.16	$Mg(NH_3)_6Cl_2$
10.17	Cs_2PtCl_6
10.18	Rb_2ZrCl_6
10.18	$(NH_4)_2TeCl_6$
10.20	$Mn(NH_3)_6Cl_2$
10.20	Rb_2PbCl_6
10.22	Cs_2TiCl_6
10.23	Rb_2TeCl_6
10.25	$Zn(NH_3)_6(ClO_4)_2$
10.26	Cs_2SeCl_6
10.30	K_2OsBr_6
10.35	Cs_2SnCl_6
10.36	K_2SeBr_6
10.36	K_2PtBr_6
10.39	$Co(NH_3)_6Br_2$
10.4	$Ni(NH_3)_6Br_2$
10.41	Cs_2ZrCl_6
10.42	Cs_2PbCl_6
10.45	Cs_2TeCl_6
10.45	$Co(NH_3)_6H_2OSO_4Br$
10.46	$(NH_4)_2SeBr_6$
10.46	$Zn(NH_3)_6Br_2$
10.47	$Fe(NH_3)_6Br_2$
10.47	$Mg(NH_3)_6Br_2$
10.48	K_2SnBr_6
10.51	$Co(NH_3)_6SO_4Br$
10.52	$Mn(NH_3)_6Br_2$
10.54	$Sr_2Ni(NO_2)_6$
10.55	$Pb_2Ni(NO_2)_6$
10.57	$(NH_4)_2SnBr_6$
10.58	Rb_2SnBr_6
10.62	$Co(NH_3)_6H_2OSO_4I$
10.63	$Co(NH_3)_6SeO_4Br$

a, Å.	Substance
	J 1_1 (*Contd.*)
10.67	$Ba_2Ni(NO_2)_6$
10.71	$Ca(NH_3)_6Br_2$
10.71	$Co(NH_3)_6SO_4I$
10.77	Cs_2SnBr_6
10.79	$Co(NH_3)_6SeO_4I$
10.9	$Ni(NH_3)_6I_2$
10.91	$Co(NH_3)_6I_2$
10.96	$Zn(NH_3)_6I_2$
10.97	$Fe(NH_3)_6I_2$
10.98	$Mg(NH_3)_6I_2$
11.04	$Mn(NH_3)_6I_2$
11.04	$Cd(NH_3)_6I_2$
11.24	$Ca(NH_3)_6I_2$
11.27	$Ni(NH_3)_6(BF_4)_2$
11.3	$Co(NH_3)_6(BF_4)_2$
(11.3)	$Zn(NH_3)_6(ClO_4)_2$
11.34	$Mg(NH_3)_6(BF_4)_2$
11.34	$Fe(NH_3)_6(BF_4)_2$
11.37	$Mn(NH_3)_6(BF_4)_2$
11.38	$Cd(NH_3)_6(BF_4)_2$
11.41	$Ni(NH_3)_6(ClO_4)_2$
11.43	$Co(NH_3)_6(ClO_4)_2$
11.46	$Ni(NH_3)_6(SO_3F)_2$
11.49	$Co(NH_3)_6(SO_3F)_2$
11.52	$Fe(NH_3)_6(ClO_4)_2$
11.53	$Mg(NH_3)_6(ClO_4)_2$
11.54	$Cd(NH_3)_6Br_2$
11.54	$Fe(NH_3)_6(SO_3F)_2$
11.58	$Mn(NH_3)_6(ClO_4)_2$
11.59	$Cd(NH_3)_6(ClO_4)_2$
11.59	$Mn(NH_3)_6(SO_3F)_2$
11.62	$Cd(NH_3)_6(SO_3F)_2$
11.91	$Ni(NH_3)_6(PF_6)_2$
11.94	$Co(NH_3)_6(PF_6)_2$
12.03	$Ni(NH_2.CH_3)_6I_2$
12.05	$Co(NH_2.CH_3)_6I_2$
12.19	$[NH(CH_3)_3]_2SnCl_6$
12.41	$[S(CH_3)_3]_2SnCl_6$
12.65	$[N(CH_3)_4]_2PtCl_6$
12.80	$[S(CH_3)_2C_2H_6]_2SnCl_6$
12.87	$[N(CH_3)_4]_2SnCl_6$
13.17	$[N(CH_3)_3C_2H_5]_2SnCl_6$
13.51	$[N(CH_3)(C_2H_5)_3]_2SnCl_6$
13.93	$[P(CH_3)(C_2H_5)_3]_2SnCl_6$
	J 2_1 and Related Structures
8.88	Li_3FeF_6
8.90	$(NH_4)_3AlF_6$
9.01	$(NH_4)_3CrF_6$
9.04	$(NH_4)_3VF_6$
9.10	$(NH_4)_3FeF_6$
9.10	$(NH_4)_3MoO_3F_3$
9.26	Na_3FeF_6
9.93	K_3FeF_6
9.96	$CuLi_2Fe(CN)_6$
10.0	$CuR_2Fe(CN)_6$ R = Na, K, Rb, NH_4, Tl
10.15	$K_2CdFe(NO_2)_6$
10.17	$K_2CaCo(NO_2)_6$
10.19	$K_2CaFe(NO_2)_6$
10.2	$Fe^{III}RFe^{II}(CN)_6$ R = Na, K, Rb, NH_4
10.22	$K_2HgFe(NO_2)_6$
10.23	$K_2SrCo(NO_2)_6$
10.25	$(NH_4)_2CaFe(NO_2)_6$
10.25	$NaTl_2Co(NO_2)_6$
10.28	$K_2CdNi(NO_2)_6$
10.28	$(NH_4)_2CdFe(NO_2)_6$
10.29	$K_2HgNi(NO_2)_6$
10.30	$K_2SrFe(NO_2)_6$
10.30	$Tl_2CaFe(NO_2)_6$
10.31	$K_2PbFe(NO_2)_6$
10.32	$K_2CaNi(NO_2)_6$
10.34	$(NH_4)_2SrFe(NO_2)_6$
10.37	$(NH_4)_2PbFe(NO_2)_6$
10.37	$Tl_2CdNi(NO_2)_6$
10.39	$Tl_2PbFe(NO_2)_6$
10.39	$NaRb_2Co(NO_2)_6$
10.40	$Tl_2SrFe(NO_2)_6$
10.4	$K_2PbCo(NO_2)_6$
10.41	$(NH_4)_2CdNi(NO_2)_6$
10.42	$Tl_2HgNi(NO_2)_6$
10.43	$K_2BaFe(NO_2)_6$
10.45	$K_2BaCo(NO_2)_6$
10.45	$K_3Co(NO_2)_6$
10.46	$(NH_4)_2HgNi(NO_2)_6$
10.47	$Rb_2HgNi(NO_2)_6$
10.49	$K_2SrNi(NO_2)_6$
10.49	$K_4Ni(NO_2)_6$
10.50	$(NH_4)_2BaFe(NO_2)_6$
10.54	$K_2LiBi(NO_2)_6$
10.55	$K_2PbNi(NO_2)_6$
10.55	$Tl_2BaFe(NO_2)_6$
10.58	$Rb_2CdNi, Cd(NO_2)_6$
10.58	$K_3Ir(NO_2)_6$
10.59	$Rb_2LiBi(NO_2)_6$
10.6	$K_2PbCu(NO_2)_6$
10.63	$K_3Rh(NO_2)_6$
10.63	$(NH_4)_2LiBi(NO_2)_6$
10.64	$Tl_2LiBi(NO_2)_6$
10.67	$K_2BaNi(NO_2)_6$
10.70	$NaCs_2Co(NO_2)_6$
10.70	$Ba_3[Rh(NO_2)_6]_2$
10.72	$Tl_3Co(NO_2)_6$
10.73	$Rb_3Co(NO_2)_6$
10.73	$(NH_4)_3Ir(NO_2)_6$

a, Å.	Substance
	J 2_1 and Related Structures (*Contd.*)
10.73	$Tl_3Ir(NO_2)_6$
10.77	$Rb_3Ir(NO_2)_6$
10.8	$(NH_4)_3Co(NO_2)_6$
10.81	$Cs_2Cd[Ni, Cd(NO_2)_6]$
10.82	$Co(NH_3)_6H_2OI_3$
10.83	$Rb_3Rh(NO_2)_6$
10.88	$K_2NaBi(NO_2)_6$
10.89	$Co(NH_3)_6I_3$
10.91	$Tl_3Rh(NO_2)_6$
10.91	$(NH_4)_3Rh(NO_2)_6$
10.94	$Cs_2LiBi(NO_2)_6$
10.95	$K_2AgBi(NO_2)_6$
10.98	$Rb_2NaBi(NO_2)_6$
10.99	$(NH_4)_2NaBi(NO_2)_6$
11.01	$Tl_2NaBi(NO_2)_6$
11.05	$Rb_2AgBi(NO_2)_6$
11.06	$Tl_2AgBi(NO_2)_6$
11.10	$(NH_4)_2AgBi(NO_2)_6$
11.15	$Cs_2NaBi(NO_2)_6$
11.15	$Cs_3Co(NO_2)_6$
11.17	$Cs_3Ir(NO_2)_6$
11.19	$Cs_3Bi(NO_2)_6$
11.19	$Cs_2AgBi(NO_2)_6$
11.21	$Co(NH_3)_6(BF_4)_3$
11.30	$Cs_3Rh(NO_2)_6$
11.32	$[Co(NH_3)_5.H_2O](ClO_4)_3$
11.39	$Co(NH_3)_6(ClO_4)_3$
11.67	$Co(NH_3)_6(PF_6)$
	K 6_1
7.46	SiP_2O_7
7.80	TiP_2O_7
7.98	SnP_2O_7
8.18	HfP_2O_7
8.20	ZrP_2O_7
8.61	UP_2O_7
	S 1_4
11.51	$Al_2(Mg, Fe)_3(SiO_4)_3$, pyrope
11.51	$Al_2Fe_3(SiO_4)_3$, almandite
11.60	$Al_2Mn_3(SiO_4)_3$, spessartite
11.87	$Al_2Ca_3(SiO_4)_3$, grossularite
11.89	$(Al, Fe)_2Ca_3(SiO_4)_3$, hessonite
11.95	$Cr_2Ca_3(SiO_4)_3$, uvarovite
12.03	$Fe_2Ca_3(SiO_4)_3$, andradite
12.10	$(Na, Li)_3AlF_6$, cryolithionite
12.35–12.46	$(Mg, Mn)_2(Ca, Na)_3(AsO_4)_3$, berzeliite
	S 6_1
13.68	$NaAlSi_2O_6H_2O$
	S 0_8
13.82	$Al_{13}Si_6O_{20}(OH, F)_{18}Cl$, zunyite
	S 6_2
8.87	$Na_4(AlSiO_4)_3Cl$, sodalite
	Tetrahedrite
10.19	$(Cu, Fe)_{12}As_4S_{13}$, binnite
10.2–10.6	$(Cu, Ag)_{10}(Zn, Fe)_2(Sb, As)_4S_{13}$

firmed the supposition that phase A is a solid solution of potassium iodide and phase B a solid solution of potassium bromide, one calculates the approximate composition of the two phases as 80 KI.20 KBr and 90 KBr.10 KI. A mixture of the two synthesized phases in a 1 to 1 mole ratio gives a diffraction pattern matching within experimental error that of the original unknown.

Scope of the Method

The identification of crystalline solids by the method of isomorphous comparison depends for its utility on the availability of systematically arranged diffraction patterns of the various known crystal structures. The cubic structures were considered first in view of the simplicity with which cubic patterns can be identified and indexed. (Data on the tetragonal structures and hexagonal structures are being classified to extend the usefulness of the method.) While the primary application of isomorphous comparison is the identification of compounds by diffraction methods, one can use the tabulated patterns to check

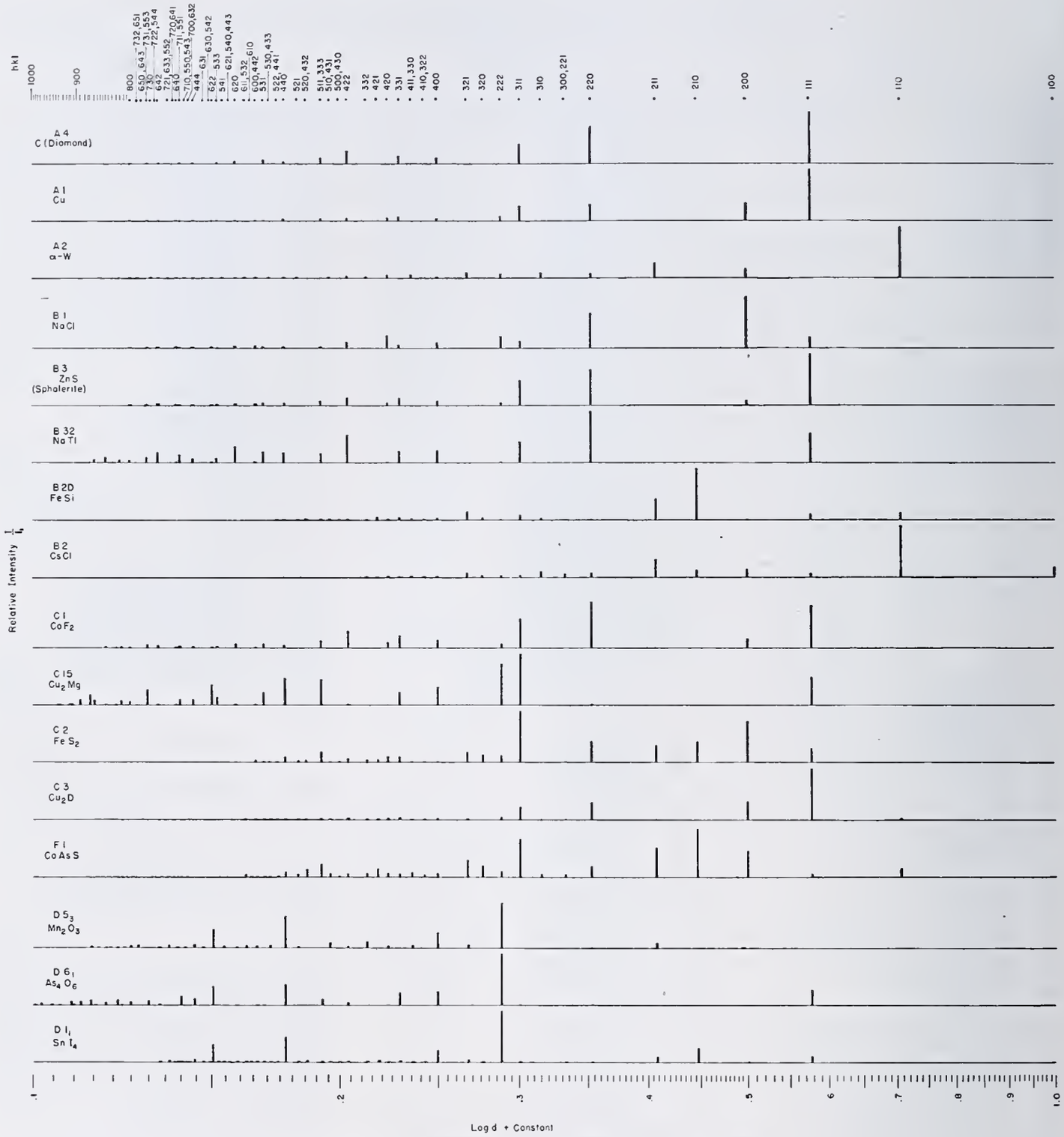

FIGURE 1. REPRESENTATIVE DIFFRACTION PATTERNS

The log d values of the various structures (Figures 1 and 2) are matched according to the indices hkl. The relative intensities of the powder pattern pertaining to a particular structure represent the arithmetically averaged relative intensities (for MoK_α radiation) of representative members of the isomorphous group.

questionable reflections of a published powder pattern. In general, the tabulated data should prove useful to an analyst in pointing out possible ambiguities of a chemical analysis by diffraction methods. In a case of that type some simple physical or chemical test will usually suffice to resolve the difficulty. Spectroscopic analysis should always supplement any chemical analysis by the powder method.

The crystal structure investigator may find the classified diffraction patterns helpful in checking trial structures or in establishing some structural similarity between a known structure and a new structure.

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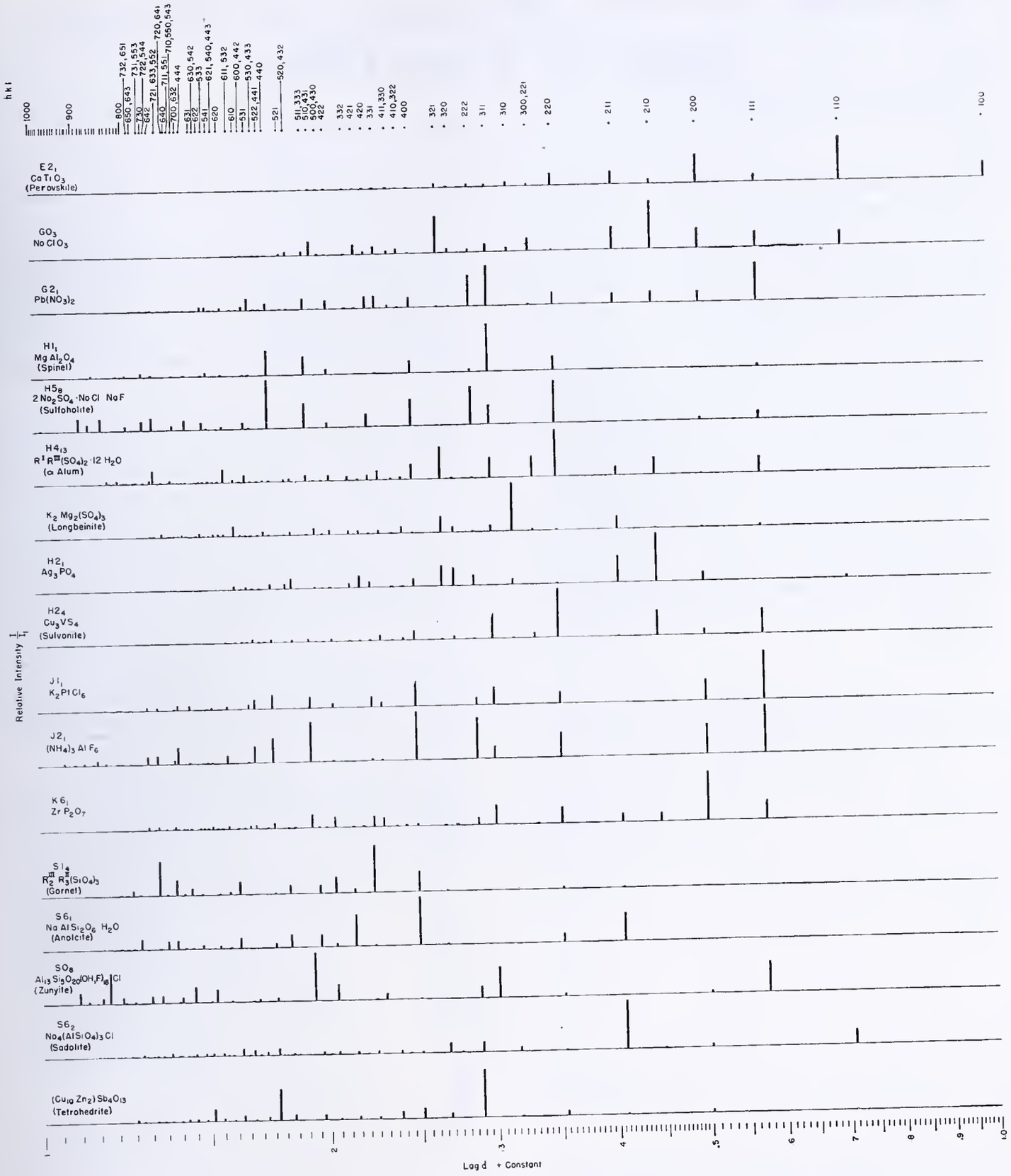

FIGURE 2. REPRESENTATIVE DIFFRACTION PATTERNS

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Isolation and Identification of the Resinous Binder in Water Paints

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An analytical procedure for the isolation and identification of the resinous binders used in water-emulsion paints is outlined and discussed. Three general methods for the separation of the binder are recommended for use either separately or together. The determination of characteristic physical and chemical properties is outlined. Particular emphasis is placed on the analysis of alkyd- and rosin-type resins. A procedure is given for the separation of an alkyd resin into its dibasic acids, polyhydric alcohols, and modifiers, and methods are suggested for identifying most of the probable members of each group. Rosin resins are included in some detail. A suggested method for the detection of rosin adducts by ultraviolet spectroscopy is outlined.

THE modern water paint, which is essentially a pigmented, stabilized oil-in-water emulsion, is the logical extension of the old-type, relatively simple aqueous dispersion such as casein pigmented with whiting. Water-wettable pigments of greater opacity have been introduced, resins and varnishes have been incorporated as water-resistant binders, and various protective colloids have been added as emulsifying agents and stabilizers. The modern water paint differs from the oil paint, not because it contains water, but because it contains water as the continuous phase.

Water paints are economical and nonflammable, and are characterized by outstanding brushing ease and spreading rate. They permit a wide variation of the solids-viscosity ratio, and are characterized by a degree of penetration which may be readily varied as desired. They dry rapidly, generally setting-to-touch in less than 2 hours after application, and unlike ordinary oil paints may be applied to moist or wet surfaces without detriment to the eventual dried film. It is usually true that the water resistance of the dried film from the water paint is less than that of a dried film of the same resin from an oil paint, owing to the retention of the emulsifying agent in the water-paint film. This disadvantage may be at least partially overcome by the selection of an emulsifying agent which is irreversibly dehydrated, during the drying of the film.

Water paints have been used extensively for interior coatings, and have been approved for this purpose by Federal Specification TT-P-88 (December 27, 1940). In addition, they have found considerable application for exterior coatings, particularly masonry. In the latter instance, the reaction of the casein usually present in the paint with the calcium in the surface coated produces increased water-impenetrability. Additional remarks on the versatility of water paints may be found in (*13*).

The typical water-emulsion paint is usually composed of the following phases:

Continuous Aqueous Phase, including the protective colloids or emulsifying agents, "dissolved" by the application of suitable solubility aids (such as alkalies). Buffers and preservatives are also included in this phase.

Emulsified Oil Phase, including the water-insoluble resinous binder, the driers, and usually small proportions of high-boiling volatile solvents for the driers.

Suspended Pigment Phase, treated to produce water wettability.

The present discussion is limited to the isolation and identification of the resinous binder, the "oil" of the oil-in-water emulsion. The resin may be any one, or a combination of available resins, selected to give optimum results in the specific application desired. Although a wide variety of resins, both natural and synthetic, may conceivably be present in water paints, those most commonly encountered by the author have been resins of the alkyd type—i. e., reaction products of polyhydric alcohols and organic polybasic acids and their anhydrides. These resins possess good adhesion, toughness, and pigment-binding qualities, and represent materials which generally have been developed especially for water paint formulation. Hitherto less common, but of increasing importance, are the rosin-type resins. Methods for the identification of these two types will be considered in particular. Modifying possibilities will be discussed briefly in passing.

Isolation of the Resinous Binder

Before the resin or mixture of resins present as the paint binder can be analyzed, separation from the other constituents is essential. This is often a rather difficult procedure, because of the complexity of the mixtures and wide variations of the ingredients, so that no general procedure is applicable to all water paints. However, the use of the following general procedures, either singly or in combination, usually results in the separation of the various constituents in a state sufficiently pure to permit identification of the resin. The first two methods discussed were devised during the analyses of emulsions over a period of 7 years, with constant modification due to the introduction of new constituents. Method C is a modification of the procedure introduced by Bradley (*3*) for the analysis of oil paints and lacquers. These methods have been devised for the majority of cases. There have been, and probably always will be, instances where further modifications are in order. Some of these are suggested in their proper places. It cannot be emphasized too strongly, however, that each sample presents an individual challenge to the ingenuity of the analyst.

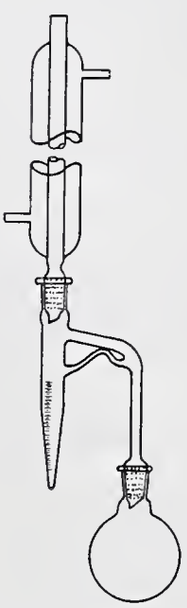

Figure 1. Modified Dean - Stark Moisture Apparatus

A. Benzene Distillation. Approximately 10 grams of paint emulsion as

received are weighed into a 500-cc. flask equipped with a graduated water trap and a reflux condenser (Dean-Stark moisture determination apparatus, Figure 1). Roughly 250 cc. of anhydrous benzene are added and the mixture is heated until the water level in the trap is constant. The following fractions result:

1. Volatile benzene-immiscible liquids, retained in the trap. This fraction will consist of water, together with any water-soluble volatile solvents present.
2. Benzene-soluble material, consisting of the resinous binder and benzene-soluble water-insoluble solvents.
3. Nonvolatile benzene-insoluble material, including the protective colloids, soaps, and other dispersing agents, bulk of the driers, inorganic salts, benzene-insoluble resins (such as cellulose ethers and esters, unalkylated urea resins, etc.), pigments, and dyes.

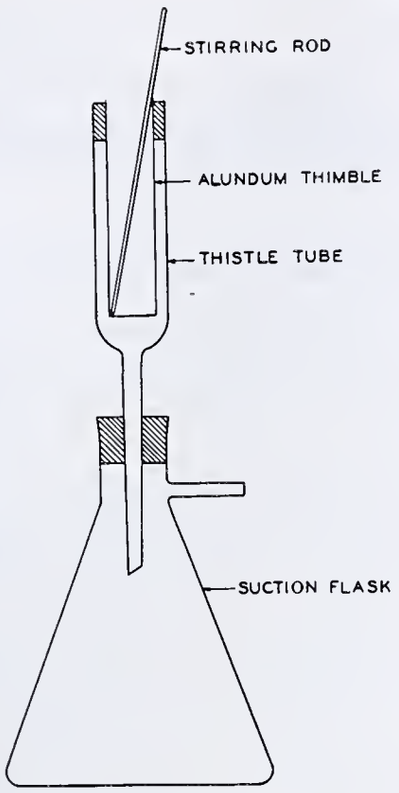

FIGURE 2. COLD SEPARATION FILTRATION APPARATUS

Benzene is substituted for the xylene or toluene usually employed (*29*), because it also is an excellent solvent for most resins generally found in the average water paint, and because, being more volatile than toluene, it is easier to separate from the resin. An additional advantage is the relative insolubility of most alkali metal soaps in anhydrous benzene, preventing contamination of the resin fraction by such possible ingredients. Toluene has been found to dissolve some soaps under the conditions described. Nitrogenous base, alkaline earth, and heavy metal soaps are generally soluble in benzene, but these would be present only in small amounts, if at all.

If subsequent analytical procedures indicate the presence of benzene-insoluble resinous constituents of the binder, these may be isolated from the benzene-insoluble fraction by extraction with suitable solvents, such as alcohol for cellulose ethers, acetone for cellulose esters, and water for unalkylated urea resins. The major proportions of any shellac present will also be found in this fraction, and may be isolated by extraction with alcohol.

B. COLD SEPARATION WITH ALCOHOL AND BENZENE. Occasionally, a water paint will contain ingredients which react with each other in the boiling benzene solution, or become occluded. As an example of the latter instance, the resin may be so coated with benzene-insoluble protective colloid that portions of it remain in the benzene-insoluble fraction even after prolonged extraction. In such cases, quantitative separation is seriously affected. When this situation occurs, separation may be expedited by the following procedure:

A small sample of paint is weighed into a tared Alundum thimble of medium porosity (Norton Alundum 5811 R A 360). A glass rod (for stirring) is included in the tare. After dilution with an equal volume of water, the mixture is stirred thoroughly, and the emulsion is broken by the addition of glacial acetic acid, or, if necessary, concentrated hydrochloric acid. The thimble containing the broken emulsion is then placed in the apparatus diagrammed in Figure 2, and the aqueous liquid is drawn off under suction. The material remaining in the thimble is thoroughly washed with water and the washings added to the original filtrate. This procedure yields (1) aqueous filtrate containing water-soluble salts (and possibly a small amount of protective colloid), and (2) precipitate in thimble, consisting of water-insoluble materials, such as the binder, protective colloids, etc.

The thimble is transferred to a second suction funnel and washed with 2B alcohol, to remove remaining water droplets, and finally with warm benzene until all the resin has been dissolved. Evaporation of the combined alcohol and benzene wash liquors leaves the resinous binder as residue. The protective colloid remains in the thimble, available for further examination.

The use of an Alundum thimble has been found advantageous because of its greater filtering surface. It may also be used in a Soxhlet extractor if such a step is thought necessary, and can be easily cleaned for re-use, either with chromic acid or by ignition. In addition, the insoluble residue remaining in the thimble can be dried and reweighed with good accuracy.

C. COLD SEPARATION WITH ACETONE, BENZENE, AND PETROLEUM ETHER. In other instances, it has been found advisable to use the method of Bradley (*3*), in which the binder is isolated from the other constituents by dilution with acetone, followed by concentration and then dilution of the acetone solution with benzene to precipitate cellulose esters. The benzene solution is then evaporated, and the residue thoroughly extracted with petroleum ether to separate natural resins and plasticizers (soluble) from synthetic resins (insoluble).

Since the above method was devised for the analysis of oil paints and lacquers, a few changes are required before it can be applied to water-emulsion paints. Addition of acetone to the original emulsion causes the precipitation of hydrophilic bodies, including methyl cellulose. Filtration of the broken emulsion yields a filtrate that not only contains the acetone-soluble binder, but also water-soluble constituents dissolved in the water associated with the acetone. The filtrate must therefore be evaporated to dryness and extracted with acetone. The resulting solution is treated as described. In carrying out the remaining procedure, it must be borne in mind that not all natural resins are soluble in petroleum ether—e. g., shellac—nor are all synthetic resins insoluble—e. g., rosin-esters, etc.

Particularly resistant mixtures may necessitate supplementing the methods discussed above with those described by Biffen and Snell (*2*).

Whatever the method used to separate the vehicle dissolved in a suitable solvent, care should be taken in the evaporation of the solvent. Carrying out this step on a steam bath, in a vessel through which is passed an inert gas, such as nitrogen, has been found both rapid and free of objectionable oxidation of the resin. Standard-joint flasks, equipped with a 45° side-arm tube, are suitable vessels available for this purpose (Figure 3).

Identification of the Separated Resin or Resins

After isolation in a reasonably pure, unoxidized condition, the resin or resins may be considered an organic unknown, and analyzed according to the usual procedures of organic analysis. These procedures include: physical examination, determination of physical and chemical constants, elementary analysis, solubility tests, classification tests, and preparation of a derivative.

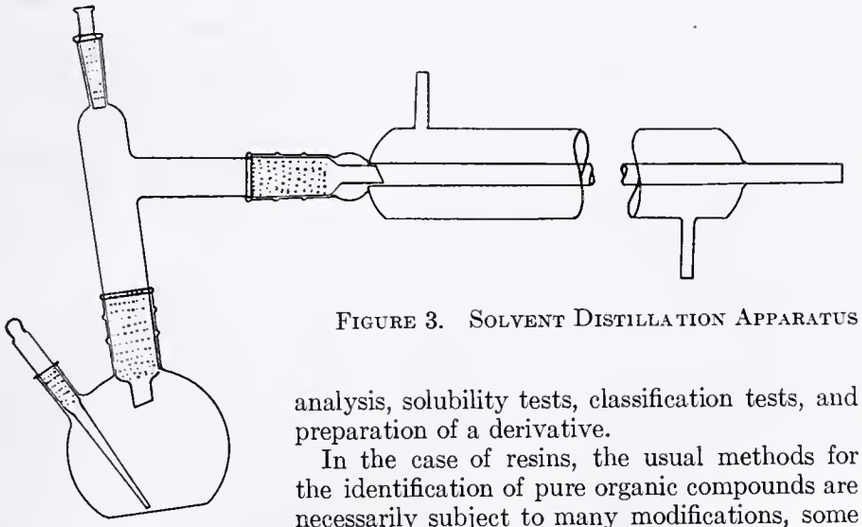

FIGURE 3. SOLVENT DISTILLATION APPARATUS

In the case of resins, the usual methods for the identification of pure organic compounds are necessarily subject to many modifications, some of which are discussed in the following paragraphs.

PHYSICAL EXAMINATION. The physical state, the relative degree of hardness, the homogeneity, color, and odor, are noted. Microscopical examination for possible dispersed phases, crystals, etc., should be included. The determination of the behavior

of the resin during its ignition in a platinum spoon may yield valuable clues. Odors evolved during this procedure may include:

Odor	Resin Indicated
Acrolein	Alkyd resins (glycerols and glycols)
Formaldehyde	Urea and phenolic resins
Acetaldehyde	Glycols, vinyls, or acetaldehyde resins
Phenols	Phenolic resins
Amines	Urea resins

Many other characteristic odors evolved during the ignition are indicative to the experienced analyst.

It is advisable to supplement this procedure with destructive distillation of the resin, both alone and intimately mixed with crushed sodium hydroxide. This determination may be readily carried out in a Pyrex test tube, with some arrangement for absorbing the volatile decomposition products in water. Determinative tests applicable to this solution are discussed under "Classification Reactions". The appearance of a white sublimate on the walls of the test tube during the destructive distillation of the resin alone is generally indicative of a phthalate resin.

Determination of Physical and Chemical Constants. The most common physical and chemical constants include softening range, refractive index, density, acid numbers, and saponification values.

Softening Range. Since none of the resins have sharply defined melting points, the softening range is determined instead. The two principal methods are the mercury bath method (*11*) and the ball and ring method (*31*). Softening points are not particularly determinative for synthetic resins, because of overlapping among various classes and intentional variations within each class. They may be valuable, however, when used in conjunction with other data.

Refractive Index. The Abbe refractometer is most commonly used with liquid resins, but is also applicable to solid resins (*1*, *32*). Immersion methods, however, are more generally used with solid resins. In the latter instance, a small fragment of the resin is immersed in aqueous standards of known refractive index, and this constant of the resin is determined by the Becke line method (*7*). Aqueous solutions of potassium mercuric iodide, containing a small proportion of a suitable wetting agent, and varying in 0.01 step, are recommended standards. Aqueous standards are used to prevent dissolution of the resin. The presence of the wetting agent facilitates the wetting of the resin fragments by the aqueous media. Refractive indices have been found particularly useful in differentiating natural and synthetic resins, and in establishing their identity (*3*). Table I includes the refractive indices of a number of alkyd- and rosin-type resins, the types most common in water paints.

Density. The density may be determined by water-displacement, sink-or-float, or pycnometer methods. When sufficient resin is available, a 4- to 5-gram fragment may be suspended on a fine wire and the density measured on the analytical balance by displacement of water or other nonsolvent liquid of known density. With small amounts of resin, a less accurate method consists of density determination by sink-or-float methods in salt solutions of known density. A more accurate method with relatively small amounts of material consists of determination of the density by use of a small (1.5 to 2 cc.) pycnometer. The pycnometer is weighed empty, and weighed again filled with water at room temperature. A 20- to 40-mesh fraction of resin (consisting of particles not too large for the stem of the pycnometer, but too large to float out through the overflow capillary) weighing 0.2 to 0.5 gram, is weighed into the dry pycnometer and the vessel is then filled with water containing a small proportion (0.1 to 0.5 per cent) of Aerosol OT (to facilitate wetting the resin particles) and weighed. The density is calculated as follows:

If a = weight of pycnometer
b = weight of pycnometer filled with water
c = weight of pycnometer plus sample
d = weight of pycnometer plus sample plus OT solution
D = density of water at $t°$

then $$D_t = \frac{c - a}{(b-a) - (d-c)} \times D$$

The density of the OT solution is practically equal to that of water alone.

The density of less solid resins is determined directly by the use of the pycnometer. Great care must be exercised in all cases to exclude air bubbles.

Table I includes refractive indices and densities determined on a number of resins of the alkyd and rosin types. Unless otherwise noted, the refractive indices were determined by the immersion method, and the densities by the pycnometric methods. All these resins have not been encountered by the author in the analysis of water paints, but they represent some of the possible variations which may be present.

Acid and Saponification Values. These values are generally best determined by treatment of an alcohol-benzene solution of the resin with 0.5 N alcoholic potassium hydroxide. Acid numbers are obtained by cold, direct titration to a phenolphthalein end point. Saponification is effected by refluxing the solution with excess potassium hydroxide and back-titrating to a phenolphthalein end point. Difficultly saponifiable materials may be refluxed with a 1 N solution of potassium hydroxide in diethylene glycol (*22*, *27*). However, the solvent used will probably have an effect on the saponification value (*28*). In general, if the saponification value exceeds 200, it is probable that the resinous binder contains an alkyd resin. If a sublimate of phthalic anhydride has been detected during fusion, and the saponification value of the resin is less than 200, the presence of unsaponifiable modifying materials (such as phenolic resins) is indicated.

Elementary Analysis (*27*). Ultimate analysis by fusion with metallic sodium permits the detection of sulfur, nitrogen, chlorine, and phosphorus, and therefore the inclusion or exclusion of resins known to contain these elements. Mark (*20*) in discussing the physical-chemical examination of high polymers, includes several useful tables permitting classification of resins according to nitrogen, sulfur, chlorine, and phosphorus content (and also saponification values).

Solubility Tests. The solubility of the resin in benzene, and perhaps in acetone and petroleum ether, has already been observed during isolation of the resin. Other solvents which may add pertinent information include ethanol, ethyl acetate, butyl acetate, Cellosolve, turpentine, and naphtha.

Table I. Densities and Refractive Indices of Resins

Alkyd-type resins	$D^{25}/_4$	$N^{25°}$
Rezyl 10 (glycerol phthalate)	1.389[a]	1.575[a]
Rezyl 337-1 (ethylene glycol phthalate)	1.352[a]	1.570[a]
Pentaerythritol phthalate	1.390[a]	1.584[a]
Rezyl 11 (nondrying alkyd)	1.210[a]	1.573[a]
Rezyl 7905 (drying alkyd)	1.014	1.5005[b]
Rezyl 775-1 (phenolic modified drying alkyd)	1.112	1.535
Beetle 592-8 (amine modified nondrying alkyd)	1.160	1.526
Rezyl 7019 (unmodified alkyd)	1.179	1.4744[b]
Rezyl 7020 (unmodified alkyd)	1.197	1.4840[b]
Melmac 599-8 (amine modified drying alkyd)	1.160	1.550
Rosin-type resins		
Rosin (Grade N wood)	1.082	1.548
Rosin (G gum)	1.077	1.546
Ester gum No. 6 (glycerol ester)	1.103	1.549
Abalyn (methyl abietate)	1.038	1.5288[b]
Hercolyn (methyl dihydroabietate)	1.030	1.5179[b]
Flexalyn (diethylene glycol diabietate)	1.071–1.074[c]	1.5361–1.5363[c]
Pentalyn (pentaerythritol abietate)	1.099	1.546
Poly pale resin (polymerized abietic acid)	1.069	1.546
Vinsol resin (oxidized rosin fractions)	1.222	1.614
Galex (principally dehydroabietic acid)	1.112	1.544
Staybellite (tetrahydroabietic acid)	1.063	1.529
Phenac 622N (modified phenolic)	1.113	1.555
Teglac Z152 (dibasic acid–rosin ester type)	1.145	1.541
Teglac 161 (dibasic acid–rosin ester type)	1.124	1.542
Teglac 127 (dibasic acid–rosin ester type)	1.162	1.532

[a] Reported by Bradley (*3*). Rezyl 775-1, Beetle 592-8, and Melmac 599-8 are available commercially as 50% solutions. Above results were determined on films that had been dried to constant weight *in vacuo*.
[b] Abbe refractometer.
[c] Hercules bulletin, refractive index determined at 20° C.

TABLE II. CLASSIFICATION COLOR REACTIONS

Test	Procedure	Observations: Coloration	Observations: Indicated Resin
Liebermann-Storch (Storch-Morawski in Europe) for rosin	Dissolve a small fragment of resin in hot acetic anhydride; cool. Add 1 drop of H_2SO_4 (sp. gr. 1.53) to soln. in spot plate or small porcelain crucible	Transient blue-violet Red Rose red Bluish green	Rosin, ester gum, etc. Coumarone (*3*), rosin adducts Cyclohexanone and cyclohexanone-CH_2O (*3*) Vinyl resins (*3*)
Halphen-Hicks for rosin (confirmatory)	Two reagents: A. 1 volume of phenol in 2 volumes of CCl_4; B. 1 volume of bromine in 4 volumes of CCl_4. Add 1 to 2 cc. of soln. A to particle of resin in depression in porcelain spot plate. Stir. Fill adjacent cavity with soln. B. Cover with inverted watch glass and note color development in A.	Deep purple or deep indigo blue	Rosin (*10*)
Resorcinol test for phthalates. Bradley (*3*) modification of Holde, Bleyberg, and Aziz test (*14*)	Heat 0.25 to 1 gram of resin with 2 to 3 times this amount of pure resorcinol to boiling point of latter. Extract with boiling water, dilute, and render alkaline.	Greenish-yellow fluorescence of fluorescein	Phthalates. Also applicable to phthalate plasticizers. Other dibasic acids may interfere.
Phenol test (*3*) for phthalates (phthalate confirmatory test)	Heat 1 gram of resin with 2 to 3 grams of pure phenol and 10 drops of concd. H_2SO_4 until formation of orange or brownish-orange melt. Cool, extract with boiling water, dilute, render alkaline.	Red coloration of phenolphthalein in alkaline solutions	Phthalates
Gibb's indophenol for phenols (*12*) (applicable to NaOH fusion residue or to destructive distillation products in aqueous solution)	Add 2 to 3 drops of 1% aqueous suspension of dibromoquinone chlorimide to 10 cc. of aqueous extracts of resin. Carefully neutralize by adding 0.1 *N* NaOH dropwise (to pH ca. 9.4)	Blue to wine Purple blue Faded purple	Phenol, cresols, and xylenols (*21*) *p-tert*-Butylphenol and *p-tert*-amylphenol (*21*) Reagent *p*-phenylphenol gives no color (*21*)
Millon's reagent for phenols (metallic mercury dissolved in concd. HNO_3, diluted with equal volume of water)	Add 2 drops of reagent to 5 to 10 cc. of aqueous soln. of products of destructive distillation. Shake, heat just to boiling.	Deep red to brown Distinct lavender to purple; sediment in tube is edged with blue or purple on standing. Rose	Phenol, cresols, and xylenols (*21*) *p*-Phenylphenol (*21*) *p-tert*-Butylphenol and *p-tert*-amylphenol (*21*)
Coumarone test for coumarone-indene resins	Dilute 1 cc. of 10% soln. of resin in $CHCl_3$ to 6 cc. with $CHCl_3$ and add 1 cc. of glacial acetic acid. Shake. Add 1 cc. of 10% soln. of bromine in $CHCl_3$, shake, allow to stand.	Permanent red color	Coumarone resin (*10*)

Miscibility is generally determined at 60 per cent concentration. Viscosity determinations of some solutions may also be helpful. The solubility and viscosity data of Van Heuckeroth (*30*) should be consulted. Comparison with known materials is advisable in all cases.

CLASSIFICATION TESTS. Most of the resin classification tests are color reactions applicable directly to the solid resin. Others may be applied to the water solution from destructive distillation. Table II includes some of these tests.

Other tests include testing the pH of the water solution of the decomposition products. If alkaline, condensation products of aldehydes with urea, melamine, aniline, or proteins are indicated. If acid, the acidic anions (and likewise the resins from which they are derived) may be detected by the addition of silver nitrate, barium chloride, nitron sulfate, etc. The author has found that identification of the various anions is facilitated by carrying out the tests by microscopical methods (*8*) which permit the use of very small samples (of necessity or for rapidity). Microscopical methods also permit more absolute identification of the precipitates obtained during the tests.

The combination of the information derived from the various procedures discussed above gives an excellent idea of the physical-chemical nature of the resin or resins isolated from the paint.

PREPARATION OF A DERIVATIVE. The preparation of derivatives is limited to those of individual constituents of a resin—i. e., dipotassium phthalates, etc. Their use may be employed wherever the analyst decides that additional information is required for absolute identification of a particular resin constituent.

Additional Analytical Methods

As has been stated above, the most common resins that the author has encountered in water paints have been: alkyd resins, usually oil-modified, and rosin esters or rosin adducts.

MODIFIED ALKYD RESINS. Alkyd resins may be modified by oils and/or other resins. The presence of modifying oils will have been indicated by the softening point, the refractive index, and the density of the resin. These determinations, as well as the other general procedures discussed, will also have supplied information as to the presence and identity of modifying resins.

In either case, additional information concerning the composition of the alkyd resin, including the percentages of dibasic acids, polyhydroxy alcohols, and modifying agents may be readily determined by a suitable saponification procedure.

Two common methods for the determination of phthalic anhydride in alkyd resins are available: (1) Kappelmeier method, and (2) Navy Department method.

Sanderson (*25*) described both these methods in detail in a report on the results of a cooperative test program, and recommended the adoption of the Kappelmeier procedure as a tentative A. S. T. M. method.

Weigh a quantity of the vehicle equivalent to 2 to 3 grams of solid content into a 500-ml. soil-digestion flask, fitted with a ground-glass air-cooled reflux condenser 32 inches in length. Add 10 ml. of benzol and warm until a homogeneous mixture is obtained, and then add 150 ml. of 0.5 *N* potassium hydroxide in absolute alcohol. The alcohol may be denatured 2B grade, but must be absolute.

Warm on the steam bath for 1 hour at 60° C. and then gently reflux for 3 hours. Cool, allow to stand for 1 hour, and wash down the sides of the flask with 50 ml. of absolute ether.

Filter at room temperature, using a Gooch crucible with asbestos mat, and wash the precipitate with 50 ml. of a mixture of equal parts of absolute alcohol and ether, using five 10-ml. portions. Do not allow air to suck through the crystals, as they are strongly hygroscopic and may absorb water from the air. Dry for 10 minutes in an oven heated to 60° C. and then to constant weight over sulfuric acid in an evacuated desiccator.

One gram of the precipitate, the potassium alcohol salt of phthalic acid, is equivalent to 0.513 gram of phthalic anhydride.

Application of this procedure results in the breakdown of the alkyd resin into its original constituents, and permits the

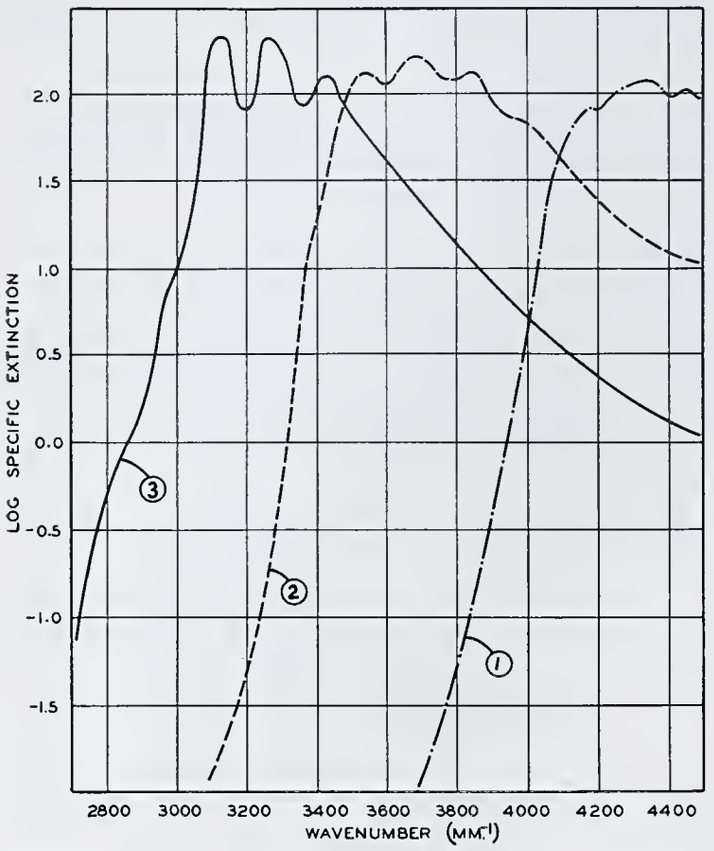

FIGURE 4. STANDARDS FOR ULTRAVIOLET CONJUGATION ASSAYS

1. Double conjugation, 10,12-linoleic acid, $CH_3(CH_2)_4(CH{=}CH)_2(CH_2)_8COOH$
2. Triple conjugation, α-eleostearic acid (*9*), $CH_3(CH_2)_3(CH{=}CH)_3(CH_2)_7COOH$
3. Quadruple conjugation, parinaric acid (*17*), $CH_3CH_2(CH{=}CH)_4(CH_2)_7COOH$

TABLE III. REFRACTIVE INDICES

Salt	N_1	N_2
Dipotassium alcohol phthalate	1.557	1.466
Dipotassium maleate	1.610	1.513
Dipotassium fumarate	1.530	1.420

separation of the insoluble potassium dibasic acid salts from the soluble potassium soaps of the oil fatty acids and the soluble unsaponifiable materials.

Potassium Dibasic Acid Salts. If phthalic acid is the only dibasic acid present, the precipitate will be composed of dipotassium phthalate, containing one molecule of alcohol of crystallization. Because of the increasing shortages of phthalic anhydride, however, the presence of other dibasic acids is also possible. Maleic and fumaric acids are usually the most common of these possibilities. Both acids form dipotassium salts which are insoluble in absolute alcohol. The author's attempts to detect the presence of these salts mixed with the phthalate salt have thus far been unsuccessful. Refractive indices, determined in two extinction directions on the individual salts, are data which permit identification of the various acids when present alone (Table III).

This method apparently is not applicable to the coprecipitated salts since the results obtained on mixtures precipitated from solutions of 3 to 1 and 1 to 1 molar ratios of phthalic anhydride, and maleic and fumaric acids were not determinative. In fact, they closely resembled the results obtained on dipotassium alcohol phthalate alone. X-ray diffraction methods also proved unsuccessful. The application of ultraviolet absorption methods has shown some promise, but has not been developed sufficiently to warrant publication at this time. Other possible methods are also being investigated.

Unsaponifiables. After removal of the precipitate of potassium dibasic acid salts, the alcohol-benzene solution is concentrated on the steam bath, diluted with several volumes of distilled water, and extracted with ether. The ether extraction may be carried out with a set of separatory funnels (avoid violent shading to prevent emulsions) or by use of the Knapp (*18*) continuous extractor. In either case, the ether extract must be thoroughly washed with dilute alkali to catch any fatty acids that might have passed into the ether layer by hydrolysis. The ether-extract-residue represents the unsaponifiable material and should not exceed 1 per cent if the fatty oil is the only modifying agent. Much higher proportions suggest the presence of ether-soluble unsaponifiable resins, which should be identifiable by the procedures previously described.

Fatty Acids. The aqueous layer from the ether extraction contains the potassium soaps of the oil-acids and also the polyhydric alcohols. Acidification with 6 *N* hydrochloric acid causes the precipitation of the free oil acids, which are removed by ether-extraction and dried to constant weight in an inert atmosphere. The water layer containing the polyhydric alcohols is reserved.

The most commonly reported physical constants for oil fatty acids include melting point, acid value, and iodine value (*19*). Refractive index, usually determined on the oil, is also generally included. In a great many instances, however, these constants are not sufficient to identify the source of the acids, particularly when the oil has been treated to alter its natural properties, or when the acids are derived from a mixture of two or more oils.

Ultraviolet absorption analysis, first applied to the esters of the acids (*5*), has also been found to give excellent results with acids separated from resins modified with known oils and mixtures of oils. In some instances, a quantitative estimation of the relative proportions of dehydrated castor, soy, and linseed oils has been found possible by the ultraviolet absorption analysis of mixed acids from these sources.

The identification depends on the presence of conjugated unsaturation. In general, the presence of more than 20 per cent conjugated unsaturation strongly indicates either dehydrated castor oils or alkali-isomerized oils (*6*). Ten to 20 per cent conjugation indicates the presence of dehydrated castor oil or alkali-isomerized oil diluted with a less conjugated oil such as soybean oil. With less than 5 per cent conjugated unsaturation, it is safe to assume that dehydrated castor oils are absent. The conjugation mentioned above refers to two conjugated double bonds. Varying proportions of three (or more) conjugated double bonds may be equally characteristic for other oils such as tung, oiticica, or isomerized linseed oils. It must be borne in mind, however, that the conjugation of all oils is changed (generally increased), owing to the effect of elevated temperatures during resinification (*4*). (The most highly conjugated oils show a decrease.)

Figures 4 and 5 serve to illustrate the ultraviolet absorption spectra of the basic conjugated acids (Figure 4) and some common oils illustrating their occurrence (Figure 5). Soy, perilla, and oiticica oils were not plotted because of their resemblance to linseed, linseed, and tung, respectively (Table IV).

Polyhydric Alcohols. The polyhydric alcohols originally present in the alkyd resin may be isolated and identified by neutralization of the acidified aqueous layer from the fatty acid extraction, followed by evaporation to dryness. It is advisable to divide the solution into two fractions of known volume before evaporation. Fraction 1 is evaporated to dryness and the residue extracted with *tert*-butyl alcohol. The extract is filtered, and the filtrate evaporated to remove the solvent. The high-boiling alcohols remaining (usually glycerol or ethylene glycol) may be identified by refractive index, specific gravity, and the

TABLE IV. TYPICAL CONJUGATION ASSAYS FOR SOME OILS AND FATTY ACIDS[a]

Oils	% Acids with Conjugated Double Bonds: Two	Three	Four
Castor	0.23	0.024	0.0025
Cottonseed	0.41	0.06	0.0
Linseed	0.69	0.12	0.04
Oiticica	0.0	75.8	0.36
Perilla	0.50	0.23	0.06
Soybean	0.79	0.12	0.02
Tung	0.0	84.5	0.38
Fatty Acids			
Dehydrated castor	28.4	0.19	0.06
Isomerized linseed	32.6	6.7	0.16
Isomerized soybean	37.8	1.9	0.07

[a] Figures are based upon the assumption that all observed absorption is due to the presence of conjugated acids.

Note the marked increase in the triple conjugation of isomerized linseed oil, as compared to that of isomerized soybean oil. In general, oils known to contain linolenic acid (which has three nonconjugated double bonds) will show substantial amounts of triple conjugated acids after isomerization. Soybean oil contains little linolenic acid.

application of color tests such as those of Hovey and Hodgins (*16*).

After qualitative identification of the polyhydric alcohol present, its proportion may be determined quantitatively on fraction 2 by application of several procedures. General methods include the acetin process (*15*) and the bichromate process (*15*). A suggested improvement of these methods is that of Shaefer (*26*), which is applicable to a dilute solution from which oxidizable impurities and chlorides need not be removed.

Rosin Esters or Rosin Adducts. The presence of a rosin-containing binder will have been detected during the general analysis. Rosin resins found in water paints may be divided into rosin esters, present as abietic or modified abietic acid esters of mono- and polyhydric alcohols, and as polyhydric alcohol esters of rosin-dibasic acid (usually maleic or fumaric acid) adducts. Both types are esters, but application of the Kappelmeier procedure does not yield the usual separation, because both the potassium salts of rosin and of rosin adducts are alcohol-soluble, and therefore are not readily isolated as a precipitate (as are the potassium salts of the dibasic acids alone).

In addition, rosin esters are generally rather difficult to saponify, particularly monohydric alcohol esters; so the Kappelmeier procedure often results in partial saponification at best, necessitating more rigorous conditions.

If the classification reactions have indicated the presence of rosin, the resin may be either of the above types. Rosin esters are not too difficult to identify by chemical methods (saponification and titration of the liberated rosin acids) but chemical procedures for the analysis of rosin adducts are very few (*24*). Ultraviolet absorption analysis, however, offers a method which appears to be both rapid and reasonably accurate, although it does not permit identification of the dibasic acid.

Rosin gives a characteristic ultraviolet absorption pattern, due to the conjugated unsaturation present. An unknown resin containing rosin which gives the usual pattern is either rosin or an untreated rosin ester. A decrease or abnormal change in the characteristic rosin absorption, however, indicates that the rosin has been hydrogenated, an adduct has been formed, or a diluent transparent to ultraviolet light is present. Hydrogenated rosins usually contain disproportionation products with their own characteristic ultraviolet absorption (*23*). If this particular absorption is absent, the resin is either an adduct or diluted rosin. Diluents are detectable by the general methods of analysis.

There is another possibility—i. e., an adduct plus an oil containing sufficient conjugated unsaturation to give a normal rosin absorption in the usual range. Since such oils show characteristic absorption over a wide range of wave lengths, their presence may be readily detected.

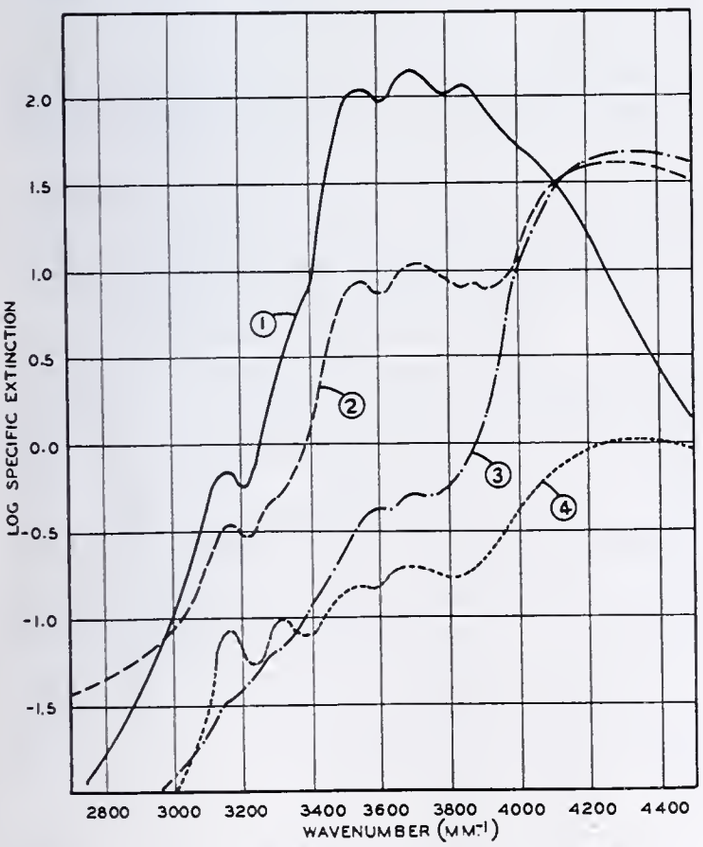

Figure 5. Ultraviolet Absorption Curves for Some Typical Oils

1. Tung oil (largely triple conjugation)
2. Isomerized linseed oil (mixed triple and double conjugation)
3. Dehydrated castor oil (double conjugation)
4. Linseed oil (little conjugation)

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Influence of Solvent on the Ultraviolet Absorption Maximum of Vitamin A

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PRESENT general acceptance of the spectrophotometric technique for the estimation of vitamin A seems well expressed by Morton (*8*), who states: "Provided that the absorption spectrum of a natural product shows the normal vitamin A band with good persistence, it affords a better estimate of the potency than almost any biological assay which is normally feasible."

In practice, however, certain limitations to the general application are also well recognized. Much has been written concerning the various factors influencing the exact measurement of the extinction of vitamin A solutions. One such factor is the effect which the solvent itself may exert on the observed value for $E^{1\%}_{1\,cm.}$ at a chosen wave length, say, 328 mμ. Solvents in common use include absolute ethanol, isopropanol, ether, hexane, cyclohexane, and occasionally chloroform, as well as alcohol-hydrocarbon mixtures.

Table I. Effect of Solvent on the Position of the Ultraviolet Absorption Peak of Vitamin A Materials

Sample No.	Type of Material	Potency Range (Approximate)[a] $\times 10^{-3}$ units g.	Isopropanol mμ	Cyclohexane mμ
	Ester Vitamin A (Oils)			
1	U. S. P. reference cod liver oil	1.7	323	323
2	Fortified sardine	1.3	316[b]	320[b]
3	0.42% A acetate in corn oil	13	326	328
4	Fish liver oil	15	326	326
5	Fish liver oil	50	325	326
6	Halibut liver oil	76	328	329
7	Distilled ester	230	327	328
11	Ester concentrate	930	327	328
12	Ester concentrate	260	327	328
13	Halibut liver oil	101	327	328
14	Fish liver oil	32	328	330[c]
		Av.	326.4	327.4
	Alcohol Vitamin A (Unsaps)			
1	Unsap	1.56	326	328
2	Unsap	0.88	326	326
3	Unsap	13	326	328
4	Unsap	14	325	328
5	Unsap	48	324	326
6	Unsap	75	327	328
7	Unsap	224	324	328
8	Crystalline A alcohol	3500	325	327
9	Unsap concentrate	2400	325	328
10	Unsap concentrate	1500	325	328
		Av.	325.3	327.5

[a] Calculated from $E^{1\%}_{1\,cm.}$ at 328 mμ using the commercial standard conversion factor, 2000.
[b] Point of inflection in absorption curve. No peak present. Values not included in averages.
[c] Sample contained a trace of "spurious" vitamin A.

Smith, Stern, and Young (*10*) reported that of the several solvents studied, chloroform gave the most pronounced shift of the absorption maximum, but found that not all vitamin A materials were equally affected by change of solvent. Ewing, Vandenbelt, Emmett, and Bird (*6*) determined $E^{1\%}_{1cm.}$ at 328 mμ in isopropanol and absolute ethanol and found no significant difference between the two alcohols. McFarlan, Bates, and Merrill (*7*) stated that little difference was found in curve shapes or peak positions of the vitamin A maximum using cyclohexane or isopropanol as solvents.

Adamson and Evers (*1*) reported last year, however, that isopropanol and cyclohexane gave comparable results for estimation of natural oils but that when the oils were saponified and the determination was made on their unsaponifiable fractions, readings in isopropanol were always appreciably higher than those in cyclohexane. Unfortunately these two investigators gave no other data than the $E^{1\%}_{1\,cm.}$ value at 328 mμ, which is the accepted position of the vitamin A absorption peak. Since it is a well-recognized fact that the absorption peak of vitamin A–bearing materials is often shifted from 328 mμ, a somewhat more critical examination of this solvent phenomenon seemed desirable.

After the work reported here was completed, Zscheile and Henry (*12*) published their studies on the ultraviolet absorption of vitamin A in various solvents. For the two solvents with which the author worked—that is, isopropanol and cyclohexane—they found crystalline vitamin A had peak positions of 324 and 327 mμ, respectively, with $E^{1\%}_{1\,cm.}$ at the peak approximately 6 per cent lower in cyclohexane than in isopropanol. This is in good agreement with the author's data on another preparation of crystalline vitamin A from the same source.

Experimental

In planning these studies, the practical application of results was given primary attention. Since the distinction between polar and nonpolar solvents appears a reasonable consideration, isopropanol and cyclohexane were chosen as typical representatives of the two classes, although it is undoubtedly true that some difference will be found between individual members of either class. The data chosen for presentation were selected as representative of materials commonly encountered in the routine spectrographic examination of commercial vitamin A samples.

The instrument used in this work was the Beckman quartz photoelectric spectrophotometer (*4*) equipped with a 6-volt, 50-c. p. automobile headlight bulb as light source and 1-cm. absorp-

Table II. Values for $E^{1\%}_{1\,cm.}$

(Of oils and their unsaponifiable fractions in the two solvents at the absorption peak and at 328 and 330 millimicrons)

Sample	Isopropanol Peak	Isopropanol 328	Isopropanol 330	Cyclohexane Peak	Cyclohexane 328	Cyclohexane 330
No. 1						
Oil	0.892	0.856	0.845	0.856	0.834	0.820
Unsap	0.787	0.778	0.758	0.745	0.745	0.738
Sap loss, %	11.7	8.9	9.8	12.5	10.0	9.3
No. 2						
Oil	0.815[a]	0.645	0.610	0.770[a]	0.600	0.560
Unsap	0.450	0.442	0.431	0.411	0.407	0.404
Sap loss, %	...	31.4	27.7	...	29.9	24.3
No. 3						
Oil	6.40	6.39	6.25	6.38	6.38	6.34
Unsap	6.41	6.38	6.20	6.15	6.15	6.06
Sap loss, %	0	0.2	0.8	3.6	3.6	4.2
No. 4						
Oil	7.57	7.49	7.40	7.40	7.34	7.28
Unsap	7.28	7.18	7.00	6.92	6.92	6.88
Sap loss, %	3.9	4.0	5.2	6.3	5.5	5.2
No. 5						
Oil	25.1	24.8	24.2	24.8	24.7	24.4
Unsap	24.7	24.1	23.2	23.4	23.2	22.8
Sap loss, %	1.4	3.0	4.0	5.5	6.8	5.9
No. 6						
Oil	37.9	37.9	37.6	37.6	37.6	37.5
Unsap	37.6	37.5	36.8	36.3	36.3	36.0
Sap loss, %	0.7	1.0	2.2	3.5	3.5	4.0
No. 7						
Oil	114.0	113.8	112.0	112.0	112.0	110.5
Unsap	113.5	112.0	109.0	109.0	109.0	108.0
Sap loss, %	0.4	1.5	2.6	2.6	2.6	2.2

[a] Values taken at point of inflection in absorption curve. No true peak was present.

TABLE III. EFFECT OF SOLVENT ON $E^{1\%}_{1\ cm.}$ VALUES

(Of vitamin A alcohol and ester preparations at the absorption peak and at 328 and 330 millimicrons)

Sample	Isopropanol			Cyclohexane		
	Peak	328	330	Peak	328	330
No. 8 Crystals	1783	1745	1680	1620	1615	1600
No. 9 Unsap concentrate	1215	1197	1160	1158	1155	1140
No. 10 Unsap concentrate	770	760	755	736	736	721
No. 11 Ester concentrate	468	466	452	463	463	460
No. 12 Ester concentrate	131	130	128	130	130	129
No. 13 Halibut	50.9	50.7	50.0	51.1	51.1	50.7
No. 14 Blend	16.1	16.1	16.0	16.1	16.1	16.0

TABLE IV. PERCENTAGE DIFFERENCES

[Showing the effect of solvent at different wave lengths. The isopropanol value is higher than that in cyclohexane except where shown (−)]

Sample No.	Ester Vitamin A (Oils)			Alcohol Vitamin A (Unsaps)		
	Peak	328	330	Peak	328	330
1	4.0	2.5	2.8	4.8	3.6	2.3
2	...	7.0	7.7	6.1	5.5	4.3
3	0.3	0.2	−1.3	4.0	3.6	2.1
4	2.2	1.9	−1.7	4.6	3.4	1.6
5	1.2	0.7	−0.5	5.3	3.5	1.4
6	0.7	0.7	−0.3	3.5	3.2	2.1
7	1.7	0.5	1.4	3.9	2.6	1.0
8	...	...	...	9.1	7.3	5.5
9	...	...	...	4.7	3.5	0.7
10	...	...	...	4.5	3.2	4.4
11	1.0	1.0	−1.8	...	...	...
12	0.8	0.0	−0.8	...	...	...
13	−0.1	−0.5	−1.3	...	...	...
14	0.0	0.0	0.0	...	...	...
Av.	1.2	1.3	0.4	5.1	3.9	2.5

tion cells supplied by the manufacturer. The calibration point chosen for setting the wave-length scale was the 3131.6 Å. doublet of mercury. In the author's experience, the reproducibility of setting the density scale shows an average deviation of ±0.25 per cent with a maximum error of twice this value. Combined with simple weighing and dilution errors, repeated determinations on the same sample may be expected to yield an over-all accuracy of the order of 1.0 per cent.

The two solvents used were carefully selected for purity. Ninety-nine per cent isopropanol was redistilled through a 15-plate all-glass bubble cap column, only that fraction coming over at 82° being used. The cyclohexane was a special spectrographic grade purchased from the Barrett Company, Edgewater, N. J., and in 3-cm. thickness showed no trace of benzene when examined with the medium quartz spectrograph (Bausch & Lomb) equipped with a hydrogen discharge tube. Both solvents were transparent down to the vicinity of 2250 Å., which is the limit of useful sensitivity of the Eastman 33 plates used.

All final dilutions were such that the range of the density scale of the Beckman instrument was limited to that between 0.300 and 0.500. Readings were made at twelve wave lengths over the region from 310 to 340 mμ and the location of the peak of absorption was determined from the plot of the smooth curve drawn through the points.

The saponification technique employed consisted in a 20-minute refluxing period of 0.5- to 1.0-gram samples in 35 ml. of 95 per cent ethanol with the addition of 3 ml. of 50 per cent aqueous potassium hydroxide. After extraction with peroxide-free ethyl ether and washing of the extract, the ether solution was dried with anhydrous sodium sulfate and divided into two equal portions, to each of which were added 20 ml. of the desired final solvent. After removal of the last traces of ether under reduced pressure, the unsaponifiable residue was left in a small volume of the solvent to be used for the spectrophotometric examination. This was transferred to an appropriate volumetric flask and diluted to volume. Amber glassware, as recommended by Embree (*5*), was used throughout the saponification and dilution procedures.

Results

For convenience in presentation, the experimental data on fourteen typical samples are assembled in Tables I to V. Samples 1 to 7 were examined both as oils and as their unsaponifiable fractions. In Table I, the effect of the solvent on the location of the peak absorption can be seen. The materials are rather arbitrarily grouped into "oils", in which the vitamin A may be presumed to exist in the esterified form, and "unsaps", in which no vitamin A esters should be present.

For the "oils" the solvent effect shows more inconsistencies than for the "unsaps". This is to be expected, for, with the single exception of sample 3, we have no real evidence as to the type of vitamin present, either as to state of esterification or molecular species. This particular material consisted of crystalline vitamin A acetate (Distillation Products, Inc., Rochester, N. Y.) dissolved at a concentration of 0.42 per cent in corn oil. It may therefore be taken to represent typical solvent effects for a single molecular species of vitamin A.

Among the "unsap" results, the case of sample 8 is significant. This is the crystalline vitamin A alcohol dissolved without treatment directly in the solvent. The same difference of 2 mμ exists between the positions of the peak, the values being 325 and 327, respectively, for the polar and nonpolar solvent.

The case of the U. S. P. reference cod liver oil is of particular interest, since its peak absorption occurs at 323 mμ, at a shorter wave length than any other material studied with the exception of sample 2. The latter, however, contained large amounts of interfering substances and exhibited no true vitamin A peak.

In Tables II and III are compiled the actual values for $E^{1\%}_{1\ cm.}$ for all the samples. These data require no special comment other than to mention the per cent saponification loss shown in Table II. The data are given for three wave-length values, at the peak, at 328, and at 330 mμ. The latter was included for reasons discussed below. Throughout, the apparent loss of vitamin A is greater when the solvent is cyclohexane than when isopropanol is used. It is evident that when E values are read at the peak in isopropanol, saponification of certain samples may be carried out with no apparent loss in potency (samples 3, 6, and 7). This is not true in cyclohexane. This difference is emphasized when the solvent effect on the magnitude of the E value for such materials as samples 8, 9, and 10 is compared with that for samples 12, 13, and 14. The former, pure vitamin or high potency concentrates of the alcohol form, yield appreciably lower values in the nonpolar solvent, while the latter show no appreciable difference and may be presumed to contain mostly esterified vitamin A.

TABLE V. PERCENTAGE DIFFERENCES

(Between peak values and those read at 328 and 330 millimicrons and between values at the latter two wave lengths)

Sample No.	Isopropanol			Cyclohexane		
	P-328	P-330	328-330	P-328	P-330	328-330
	Ester Vitamin A (Oils)					
1	4.0	5.2	1.2	2.5	4.0	1.5
2	..	..	5.4	..	..	6.1
3	0.1	2.3	2.2	0.0	0.7	0.7
4	1.1	2.2	1.1	0.8	1.7	0.9
5	1.0	3.5	2.5	0.5	1.8	1.3
6	0.0	0.7	0.7	0.0	1.0	1.0
7	0.2	1.7	1.5	0.0	1.4	1.4
11	2.5	3.4	0.9	0.0	0.6	0.6
12	0.8	2.3	1.5	0.0	0.7	0.7
13	0.4	1.7	1.3	0.0	0.5	0.5
14	0.0	0.6	0.6	0.0	0.6	0.6
Av.	1.0	2.4	1.7	0.4	1.3	1.4
	Alcohol Vitamin A (Unsaps)					
1	1.2	3.3	2.1	0.0	0.8	0.8
2	1.2	2.9	1.7	0.6	1.1	0.5
3	0.4	3.2	2.8	0.0	1.3	1.3
4	1.2	3.6	2.4	0.0	0.6	0.6
5	2.6	6.1	3.5	0.8	2.1	1.3
6	0.3	2.1	1.7	0.0	0.8	0.8
7	1.3	3.9	2.6	0.0	1.0	1.0
8	2.1	3.7	1.6	0.3	1.2	0.9
9	1.5	4.5	3.0	0.2	1.4	1.2
10	1.3	2.0	0.7	0.0	1.9	1.9
Av.	1.3	3.5	2.2	0.2	1.2	1.0

Table IV conveniently summarizes the foregoing on a percentage basis. The values in the body of the table are expressed in terms of per cent difference between the isopropanol and cyclohexane figures. Except where indicated by the minus sign, the polar solvent shows an increase over the value given by the nonpolar solvent.

Table V emphasizes the effects to be expected on the apparent vitamin A values when $E^{1\%}_{1\ cm.}$ is read at different wave lengths. All the values in the table represent decreases expressed as percentage difference between the value at the peak and 328, between the peak and 330, and between the 328 and 330 mμ readings.

The practical value of uniform and accurately determined effective wave bands for vitamin A work becomes apparent from such data, especially where close agreement between several laboratories is necessary. The use of various abridged spectrophotometers which employ a single fixed wave band is especially subject to error introduced by use of a polar solvent in observations on vitamin A alcohol, since many such instruments read at wave lengths longer than 328 mμ.

Discussion

These data offer confirmation of the report of Adamson and Evers (*1*) with the added information regarding the shift of the absorption peak. Thus the substitution of a nonpolar for a polar solvent has two effects: (1) The vitamin A absorption peak is shifted to a longer wave length which corresponds more closely to the accepted position of 328 mμ, and (2) the substitution results in actually lowering the observed E value read at any wave length near or at the peak, especially in unsaponifiable residues or samples where the vitamin is present essentially in the alcohol form.

The most usual theoretical explanation given for this solvent effect is somewhat vaguely expressed by saying that the polar solvent forms an association complex with the solute in question, which complex has an enhanced light-absorbing power (*3*). If this be accepted as true for vitamin A, it constitutes a valid objection to the use of a polar solvent for exact spectrophotometric determination of this vitamin. This is especially valid where it is customary practice to deal with ester and alcohol forms interchangeably, and particularly where it is standard practice to apply a single uniform conversion factor for expressing E values in terms of biological units. The data just presented are in line with the suggestion that the presence of the free hydroxyl group in the vitamin A molecule tends to promote the formation of a complex, whereas a fatty acid residue attached thereto tends to suppress such a process.

Finally, mention may appropriately be made of the significance of the conversion factor. Originally, the purpose of this factor was to produce agreement between the spectrophotometric and biological assay for vitamin A. In theory, if the true biological potency, in units, and the E value for pure vitamin A were known with exactness, the conversion factor would automatically follow. For obvious reasons, this is not yet possible. Furthermore, since the biological potency of the U. S. P. reference cod liver oil is the fixed standard in this country, it has become customary for each laboratory to determine its own conversion factor in order to correct for instrumental and technical variations (*11*). This practice led to considerable confusion, and a year ago a commercial standard conversion factor (*9*) was suggested and the figure 2000 has been in rather general use since.

To understand the rationale behind the adoption of this factor, it is necessary to realize that the growing use of the spectrophotometric technique has, for certain comparative purposes, made it an independent method, just as the bioassay is an independent method. Therefore, in instances where a direct comparison between the two types of method is not essential, and there are many such, the application of the conversion factor to the E value becomes merely a matter of convenience. No assumption need be implied that the resulting vitamin A value is the true biological potency of the material in question.

If, however, the spectrophotometric technique is to provide values closely approximating those based on biological assay, attention must again be focused on the meaning of the conversion factor. Its value must obviously depend on how it is determined. Referring to Table II, four possible conversion factors may be calculated from the E values on the U. S. P. reference oil under different conditions, even though no variation in wave-length setting is allowed. (Conversion factors calculated from E values at 328 mμ: in isopropanol, on the oil, 1985; on the unsap, 2190; in cyclohexane, on the oil, 2040; on the unsap, 2280.) Again, examination of the behavior of the reference oil with respect to solvent effect on its peak absorption, the shape of its curve, and the loss of extinction at 328 mμ which occurs on saponification, indicates that from the spectrographic point of view, it is a poor material for use in establishing a standard. A natural material of proved stability and more nearly approaching the behavior of pure vitamin A would appear to offer advantages.

Other considerations, such as the recent report by Baxter, Harris, Hickman, and Robeson (*2*) of the separation from cod liver oil of at least two forms of vitamin A of widely varying biological potency but nearly identical ultraviolet absorption, would indicate that the physiologically significant conversion factor must become a plurality of factors. At present, therefore, the most practical suggestion appears to consist in recommending better standardization of the E value determination, or at least, a precise definition of the conditions under which it is determined.

Summary

With the aid of the Beckman quartz photoelectric spectrophotometer, the question of the influence of solvent on the ultraviolet absorption maximum of vitamin A has been reexamined. Special reference is made to differences in behavior between ester and alcohol forms of the vitamin. A polar solvent results in high values and a shift of peak to shorter wave lengths as compared to data obtained in a nonpolar solvent. This effect is most pronounced for samples of unsaponifiable fractions of oils or vitamin A alcohol. The anomalous behavior of the U. S. P. reference cod liver oil is pointed out and the significance of the commercial standard conversion factor is discussed.

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Determination of Ammonia by a Diffusion Method

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RECENTLY it became necessary to run a large number of ammonia determinations in this laboratory. Of the methods available, the diffusion cell technique originally described by Conway (*5*, *6*) seemed the most satisfactory. Since there were no standard Conway diffusion units available, an analytical technique was developed, using apparatus and supplies which were readily obtainable. With a minimum of effort and expense, a large number of units were prepared for multiple determinations. While several modifications of the diffusion cell technique have already appeared in the literature (*3*, *6*), it is believed that the one described in this paper is the simplest and most economical. To demonstrate its reliability representative data are presented on the analysis of ammonium salt solutions, a meat extract, and a meat extract plus ammonium chloride solution.

Outline of Methods

The diffusion unit consists of either the top or bottom of a Petri dish with a circular glass plate cover. The ammonia or other volatile base is absorbed in drops of a saturated solution of boric acid in glycerol suspended from the cover. After absorption is complete the suspended drops are washed into a flask or beaker and titrated, either electrometrically or by the use of suitable indicators. The use of boric acid as an absorbent for ammonia was first recommended by Winkler (*17*) and has since been advocated by many authors (*1*, *2*, *4*, *8*, *10–16*). It has the advantage of requiring only one standard solution for the complete determination. Moreover, in the present method, the viscous boric acid–glycerol solution need not be weighed or accurately measured volumetrically. The electrometric titrations were carried out with a Beckman glass electrode pH-meter using micro extension electrodes. The colorimetric determinations were carried out using the mixed indicator, methyl red-bromocresol green, recommended specifically for ammonia titrations by Hähnel (*9*). The data reported here were obtained by the electrometric method and checked carefully by the indicator method. The latter was found satisfactory and has the advantage of being faster but may be affected by personal error.

Apparatus and Reagents

The covers and bottoms of Petri dishes of two sizes were used, 10 × 50 mm. and 15 × 100 mm., depending upon the size of the sample. The rims of the dishes were ground to a plane surface with coarse emery. With the machine-blown Pyrex Petri dishes this operation requires only a few minutes per dish. Resistance-glass Petri dishes with ground rims are available from some supply houses.

The circular glass plates were cut from window glass, about 1 cm. in diameter larger than the dishes used. Similar circular glass plates may be purchased.

The saturated solution of boric acid in glycerol was made up in advance at room temperature and decanted from the excess boric acid. This solution contains about 28 grams of boric acid per 100 grams of glycerol. To eliminate the necessity of running blank determinations the boric acid–glycerol solution was carefully acidified, so that when mixed with the wash water in the approximate proportions prevailing at the end of the titration, the mixture remained at a pH of 5.0 to 5.1, the end point of the titration. The final volume may be varied in accordance with the amounts of wash water and titration acid used. In most of this work a final volume of 60 ml. was used. Because of the effect of dilution and the fact that the pH of the color change of the indicator in glycerol solution is not the same as in an aqueous solution, the indicator could not be added directly to the glycerol solution. Instead it was necessary to follow the course of the acidification by dropping the boric acid–glycerol solution into the wash water already adjusted to the correct pH and observing any change in pH. At a pH of 5.0 to 5.1 the indicator has just changed through a gray color to a color with a distinct reddish cast. The use of approximately correct proportions of reagent and wash water was necessary to compensate for the change in pH that accompanies the dilution of the glycerol–boric acid solutions. This change in pH is enhanced by the presence of glycerol, which forms a relatively strong acid complex with the boric acid. For the acidification the use of 6 *N* hydrochloric acid is recommended in order to avoid unnecessary dilution of the glycerol. The boric acid–glycerol solution was prepared in 0.5-liter quantities and was conveniently dispensed from a dropping bottle with a glass pipet stopper.

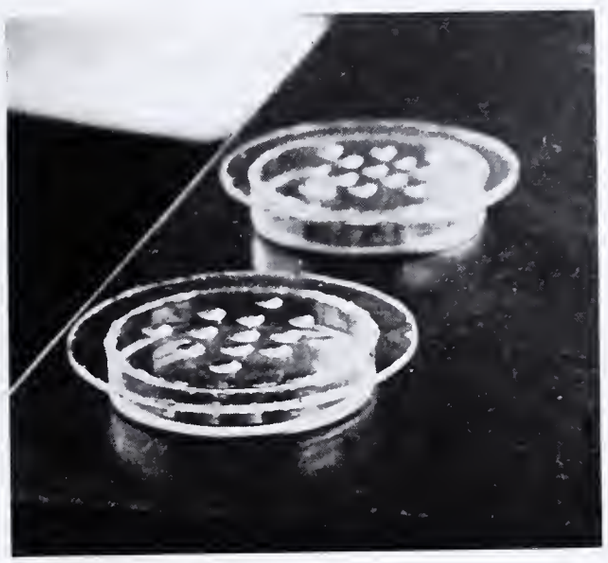

FIGURE 1. ARRANGEMENT OF APPARATUS DURING AN ANALYSIS (100-MM. PETRI DISHES)

The wash water, which was made up in a large Pyrex bottle, was acidified to pH 5.0 to 5.1 with hydrochloric acid. If the determination was to be carried out with indicators, the methyl red–bromocresol green indicators were added to the wash water in equal proportions. Enough indicator was added to produce a distinct color change under the conditions of the experiment.

Using the glycerol solution and the wash water prepared as described, the method had a zero blank value. Occasionally it was necessary to readjust the pH of the solutions after standing.

Standardized 0.01 *N* hydrochloric acid was used for the titration.

For alkalizing agents to liberate the volatile bases, both a solution saturated with sodium metaborate and potassium chloride and a 4 per cent sodium hydroxide solution have been used. The choice of alkalizing agents is governed by the character of the material being analyzed. Strong caustic should be avoided when working with most products of biological origin.

A Waring Blendor was used to prepare the meat extracts. This useful household mixer was first recommended for laboratory use by Davis (*7*). A 25-gram muscle sample and 100 grams of water were weighed by means of a large torsion balance directly into the glass mixing vessel. The vessel was placed on the mixer for a few minutes until the muscle tissue had been reduced to a creamy suspension. A portion of this was used for the analysis directly, while another portion was clarified by centrifuging before analysis. If the sample was used without clarification, care had to be exercised to prevent sampling errors due to air bubbles.

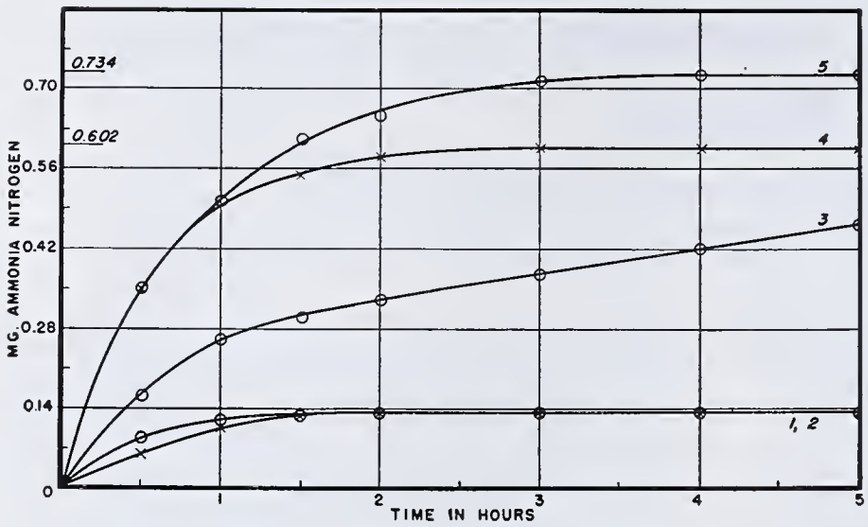

FIGURE 2. ABSORPTION OF AMMONIA NITROGEN AT VARYING TIME INTERVALS

1. Clarified meat extract plus sodium metaborate plus potassium chloride
2. Same as 1 except that meat extract was not clarified by centrifugation
3. Clarified meat extract plus sodium hydroxide solution
4. Standard ammonium chloride solution plus sodium metaborate plus potassium chloride
5. Clarified meat extract plus standard ammonium chloride solution plus sodium metaborate plus potassium chloride

Analytical Procedure

The rims of the Petri dishes were coated with vaseline. Aliquot samples of the solution to be analyzed were pipetted into the dishes. The size of the sample and the size of the dish were so chosen that the final liquid volume in the dish just covered the bottom. Ordinarily in a 100-mm. dish, a total volume of 10 ml. was used. Larger volumes required longer absorption time. The glycerol–boric acid solution was then carefully dropped on to a cover plate, each drop spaced so that no two drops would coalesce. With the 100-mm. Petri dishes 13 drops were used, while with the 50-mm. dishes 5 drops were used. The cover plate was then quickly inverted, the alkalizing agent added, and the cover placed over the dish. If the solution under analysis and the alkalizing agent were added carefully to the dish, it was possible to avoid mixing the two solutions until after the cover was in place; then the unit was rocked gently to ensure thorough mixing. Figure 1 shows the arrangement of the glycerol–boric acid drops during an analysis in which a 100-mm. dish was used.

No difficulty was experienced in inverting the plate carrying the glycerol drops. On long standing the drops absorb water and become more fluid, but even after 48 hours it was possible to invert the plate into a funnel without any loss.

The units were allowed to stand for 3 hours at room temperature. For the effect of temperature, rocking the absorption cell, etc., the reader is referred to a recent summary (*6*) on the subject.

After the absorption was complete the cover plate was carefully removed and held vertically over a glass funnel in a 125-ml. flask or beaker. The plate and funnel were thoroughly washed with the prepared wash water and the solution was titrated with the standard acid. It was not found necessary to boil the wash water or the final titration mixture.

Experimental Results

Representative data are given in Table I and are presented graphically in Figure 2 to demonstrate the precision and accuracy of the method. Table I contains the results of replicate analyses of varying amounts of an ammonium sulfate solution. In each case, 5 ml. of a saturated solution of sodium metaborate and potassium chloride were used as the alkalizing agent; the final volume in the cell was 10 ml.; and the dishes were allowed to stand for 3 hours at 25° C.

In Figure 2 each curve represents the results of an experiment in which seven dishes were allowed to stand at room temperature (25° C.) for varying time intervals before titration of the evolved volatile bases.

Curves 1 and 2 are practically identical and represent the volatile base evolved from 5 ml. of a meat extract by the use of 5 ml. of a solution saturated with sodium metaborate and potassium chloride as the alkalizing agent. After 3 hours the absorption was complete. Curve 1 represents a clear meat extract, while curve 2 represents the same extract containing suspended meat particles before clarification. These two curves show that all of the volatile base was quickly evolved and that no further change occurred on longer standing. Samples which were allowed to stand 24 hours showed no change in the titration value. Curve 3 represents 5 ml. of the same clear meat extract plus 5 ml. of 4 per cent sodium hydroxide solution, and indicates the effects of strong caustic in causing a continual slow evolution of volatile base due to reactions with the meat extract.

Curve 4 represents 5 ml. of a standard ammonium chloride solution plus 5 ml. of the borate–potassium chloride solution. Actual recovery was 0.598 mg. of ammonia nitrogen, whereas the calculated requirement for the solution used was 0.602 mg.

Curve 5 represents 5 ml. of the standard ammonium chloride solution plus 5 ml. of the clarified meat extract, plus 5 ml. of the borate mixture. Actual recovery was 0.728 mg. of ammonia nitrogen, whereas addition of the titers of the solutions gives 0.734 mg. In this case the absorption process was slowed down because the volume in the absorption cell was 15 ml. Curves 4 and 5 demonstrate the reliability of the method, since both a pure ammonium salt solution and an ammonium salt added to a meat extract were recovered quantitatively. For reference the calculated values of 0.602 mg. and 0.734 mg. have been indicated in Figure 2 along the scale on the left side.

TABLE I. ANALYSES OF A STANDARD AMMONIUM SULFATE SOLUTION

Ammonia Nitrogen Taken	0.01 *N* Acid Found	Ammonia Nitrogen Found
Mg.	*Ml.*	*Mg.*
0.246	1.76	0.247
	1.78	0.249
	1.76	0.247
	1.78	0.249
	1.77	0.248
		Av. 0.248
0.740	5.28	0.740
	5.24	0.734
	5.26	0.737
	5.26	0.737
	5.26	0.737
	5.27	0.738
		Av. 0.737
1.64	11.71	1.640
	11.68	1.636
	11.76	1.647
	11.70	1.639
	11.73	1.643
	11.65	1.632
		Av. 1.64
3.29	23.47	3.288
	23.42	3.281
	23.45	3.285
	23.36	3.272
	23.39	3.276
		Av. 3.28
8.21	58.36	8.175
	58.44	8.186
	58.14	8.144
	58.30	8.167
	58.50	8.195
		Av. 8.17

The method has been used successfully for amounts of ammonia nitrogen varying from 0.1 to 9 mg. By using 150-mm. Petri dishes, still larger amounts of ammonia can be accommodated.

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Kjeldahl Distillation without Absorbing Acid

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IN THE Kjeldahl method for the determination of nitrogen two procedures for running the distillation are in common use. In the older method the ammonia is distilled over into a known quantity of absorbing acid and is measured by titrating the excess acid with a standard base. In 1913 Winkler (*7*) suggested that the ammonia be absorbed in a solution of boric acid and titrated directly with standard hydrochloric or sulfuric acid. This modification of the method has been studied extensively in recent years (*1*, *2*, *6*) and widely used. It eliminates the need for a standard solution of a base and simplifies the calculation of the analysis, but requires an additional solution in the form of a standard indicator solution by which the end point is matched and adjusted. Furthermore, the attainment of the end point is slow. If this additional solution can be eliminated and the time of the titration decreased without affecting the accuracy of the procedure, the Kjeldahl distillation will be further improved.

In the method described in this paper all absorbing acids are dispensed with. The ammonia is distilled directly into pure water and titrated with a standard acid, using methyl red or methyl orange as an indicator, and taking for the end point the first tinge of pink color. In order to prevent the loss of ammonia from the distillate during the analysis, the operation is run in a closed still equipped with a safety balloon to relieve the pressure. The method has been compared with the usual Kjeldahl procedure in which an excess of absorbing acid is used and seems to give on the average a slightly higher degree of accuracy. This would be expected, since, as has been pointed out by Miller (*4*), ammonia is lost during the first few minutes of distillation. This loss would be prevented by running the distillation in a closed system.

The proposed method decreases the time required for an analysis and calls for only one standard solution, an acid. The end point is attained easily and quickly, and the calculation is simplified. From the point of view of time and motion study the efficiency of the three procedures may be compared as follows:

	Solutions Needed Including Indicators	Solutions Measured	End-Point Adjustment	Calculation, % N
Old method	3	2	Rapid	$\frac{1.401\,(N_a V_a - N_b V_b)}{w}$
Boric acid method	4	2	Slow	$\frac{1.401\,N_a V_a}{w}$
Proposed method	2	1	Rapid	$\frac{1.401\,N_a V_a}{w}$

where N_a and N_b are normalities of acid and base, V_a and V_b are volumes of acid and base in cc., and w is the weight of the sample. This comparison shows that the proposed method is more efficient than either of the two older methods in time, materials, and operation.

Validity of the Procedure

It may be asked whether, from a theoretical point of view, one might expect the proposed method to give reliable results. It is reasonable to assume that in a closed system all the ammonia can be distilled over into the receiver. But what, if any, of the distilled ammonia is lost from the absorbing water by passing into the gas phase in the receiver?

For more concentrated solutions there are data on vapor pressure which would give this information. For the dilute solutions handled in the Kjeldahl analysis, however, it was not possible to find reliable data concerning the partial pressure of the ammonia in the gas phase. It was possible, nevertheless, by the analysis of the work of Meldrum, Melampy, and Myers (*3*) on the aeration of ammonium hydroxide to get a fair idea as to what error, if any, might be expected from the loss of ammonia from a dilute solution to an inert gas in contact with it. The authors showed in this paper that the removal of ammonia from a dilute solution depended on the aeration rate, the temperature, and the concentration of the solution. They found that the rate was not affected by the size of the air bubble, a conclusion confirmed by the work of Shabilin, Krylov, and Obarin (*5*). Such an aeration would result in the partition of ammonia between the liquid and gas phases comparable to the equilibrium attained in a closed system containing gas in contact with a dilute ammonia solution.

It can be shown mathematically that in such an aeration the fraction of ammonia remaining in solution is given by the equation

$$2.303 \log \frac{c - x}{c} = - Kv$$

where c = the original ammonia concentration in moles per liter
$c - x$ = the final concentration
v = the volume of the air used in aeration
K = a constant

Using the data of Meldrum, Melampy, and Myers for 25° C., the values for K were found to be 0.0057, 0.0043, 0.0034,

and 0.0040. This shows a fair agreement and gives an average value of 0.0043.

In the work described in the present paper the average distillation resulted in about 300 cc. of solution in a closed receiver in contact with about 400 cc. of air. If this system were in equilibrium, it could be shown, using the constant obtained above, that only 0.5 per cent of the ammonia would be found in the gas phase. This would be, of course, an extreme, limiting case, impossible under the conditions of the distillation. The actual results show that the deviation from 100 per cent ammonia recovery is considerably less than this (as would be expected, since equilibrium is not attained) and that the process is essentially equal in reliability, and perhaps slightly better than the usual Kjeldahl method in which an absorbing acid is used.

From the experimental point of view, furthermore, the validity of the process is confirmed not only by the analytical results, but also by the observation, made repeatedly, that the distillate gave no odor of ammonia.

Procedure

In this work the interest was centered entirely in the distillation, not in the Kjeldahl method as a whole. The problem was to determine how much of the ammonia in the distilling flask was recovered in the receiver and measured in the titration. In order to simplify the work and to avoid possible errors of weighing and calibration, the same buret was used both for introducing into the distilling flask a known quantity of ammonia and for making the final titration. Throughout the work one standard solution only was used, a sulfuric acid solution of normality 0.1996.

The distillation was carried out in a closed system. This was made by modifying the usual Kjeldahl still by attaching the receiving flask by a one-hole rubber stopper to the lower end of the condenser. The receiving flask was a side-arm suction flask. To the side arm of this flask was fitted a small rubber balloon to relieve the pressure set up in the receiver as the distillation proceeds. The receiver was arranged so that it could be lowered during the last few minutes of distillation to permit drainage.

A representative run might be described as follows: A definite volume of standard acid was delivered by buret to a beaker, 100 cc. of distilled water and 2 drops of methyl orange solution were added, and the resulting solution was titrated carefully to the end point with freshly distilled ammonium hydroxide solution having a concentration of about 0.2 *N*. This prepared solution which contained an accurately known quantity of ammonium sulfate was quantitatively transferred to the distillation flask. One hundred cubic centimeters of distilled water were then added to the receiving flask, enough to cover the lower end of the condenser's inner tube when the still was assembled. Enough water was added to the distilling flask to make a total volume of about 400 cc., 25 cc. of 10 per cent potassium hydroxide were also introduced, the assembly was closed, and distillation was begun. To ensure smooth boiling, fine capillary tubes, sealed at one end, were used in the distilling flask with their open ends down. After about 250 cc. of distillate were collected, the receiving flask was disconnected and lowered to the desk and the distillation was continued for a few minutes longer. This was to allow for drainage, but it is doubtful whether this part of the procedure is necessary. The receiving flask was then taken to the titration table, a few drops of methyl orange were added, and the contents were titrated with the original standard acid, using the same buret that was used initially in making up the ammonium sulfate solution for the distillation. The experimental results are given below.

Four series of distillations were run. In Series A the usual Kjeldahl procedure was followed, the receiver being open to the air, and the ammonia being absorbed in an excess of standard acid. In Series B absorbing acid was also used, but the distillation was carried out in the closed still with the safety balloon. In Series C no absorbing acid was used. The ammonia was absorbed in pure water. For the sake of completeness, another series was run, Series D, in which an open still was used, but the ammonia was absorbed in pure water. The amounts of ammonia used in these distillations ran from about 70 to about 200 mg.

Series	A	B	C	D
Still used	Open	Closed	Closed	Open
Absorbent used	Acid	Acid	Water	Water
No. of runs	21	4	12	7
Ammonia recovered, %				
Maximum	100.0	100.0	100.0	99.4
Minimum	99.4	99.9	99.4	98.3
Average	99.7	99.9	99.8	98.7

The proposed method (Series C) gives slightly better results than the usual Kjeldahl procedure (Series A). Series D, as might be expected gave no useful results. When the closed still is used with the regular Kjeldahl procedure (Series B), the results are definitely improved. This can be explained by reference to the work of Miller (*4*) who found that a loss of ammonia occurred at the beginning of the distillation when it comes over so rapidly that some of it escapes absorption by the acid.

An incidental study was made of the effect of the excess of the absorbing acid on the accuracy of the results. In Series A some runs were made using 100 per cent excess acid; others using 4 per cent excess; others using no excess. As will be seen from the following table, the excess of acid seemed to have no effect on the results of the distillation:

Composite Series A, Open Still, Absorbent Acid Used

Excess acid used, %	100	4	0
No. of runs	7	3	11
Ammonia recovered, %			
Maximum	100.0	99.9	100.0
Minimum	99.4	99.9	99.2
Average	99.7	99.9	99.7

Certain objections to the method naturally present themselves. First, will the pressure developed in the receiver blow out the balloon or a stopper? In none of the distillations did this happen. The pressure seemed never to be greater than 20 to 25 cm. (8 or 10 inches) of water. Second, will water condense in the balloon and absorb ammonia? The inside of the balloon was never observed to be wet. If such a condition were found, however, it would be a simple matter to discard the balloon and fit the apparatus with a new one.

Summary

The Kjeldahl distillation has been modified in such a way as to dispense with the absorbing acid, thus decreasing the time and materials required for the process and simplifying the manipulations and calculations. The distillation is carried out in a closed still, the pressure being relieved by a small rubber balloon attached to the receiver. The ammonia is determined by titration directly with a standard acid.

The results of this modified method have been found to be slightly better than those obtained in the usual Kjeldahl distillation in which an absorbing acid was used.

In the regular Kjeldahl distillation, using absorbing acid, different excesses of acid were found to have no effect on the accuracy of the process.

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Activation of Dicalcium Phosphate for the Chromatographic Determination of Carotene

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A PREVIOUS publication (*1*) demonstrated that in methods of carotene determination that utilize the Willstätter-Stoll principle of separating xanthophyll from the carotene portion of the extract some noncarotene pigments are not removed and are determined as carotene. Dicalcium phosphate was proposed as an adsorbent for the chromatographic removal of noncarotene pigments in the determination of carotene from plant materials. It was shown that this compound removed those noncarotene pigments which usually develop in stored hays, silages, and fecal matter.

Shortly after publication of the above paper, however, dicalcium phosphates of the brand specified were encountered which were not good adsorbents, and it was thought desirable to find some simple method of activating an inactive phosphate. Such a method is presented herein, together with some of the data collected in the study.

Table I. Effect of Treating Dicalcium Phosphate with Disodium Phosphate

Lot No.	Na_2HPO_4	Chlorophyll per Gram of Adsorbent	Filtration Rate		Height of 8.0-Gram Column: Before vacuum	Height of 8.0-Gram Column: After vacuum
	Grams	*Mg.*	*Min.*	*Sec.*	*Cm.*	*Cm.*
21,940[a]	0	2.4	7	20	8.0	5.0
67[b]	5	9.8	17	00	9.5	6.0
68	10	13.8	11	52	15.5	9.0
69	15	16.2	9	40	17.0	10.5
70	20	17.8	9	30	16.5	10.0
10,440	0	1.4	2	28		
53	5	6.2	8	6	10.0	6.0
54	10	9.8	12	49	14.0	
55	15	14.0	4	27	17.3	10.6
56	20	17.4	5	37	24.0	15.2

[a] Manufacturer's number.
[b] Smaller numbers are author's laboratory lot numbers.

Procedure and Results

In order to arrive at some conclusion as to the effect of various procedures on the activation process, the ability of the treated phosphate to adsorb chlorophyll was studied.

For this purpose a petroleum ether solution (Skellysolve B, used throughout this investigation), containing 1 mg. of crystalline chlorophyll per ml., was prepared. The same lot of petroleum ether was used in the adsorption studies throughout this investigation. One-half gram of the adsorbent was placed in a 30-ml. test tube graduated at the 15-ml. mark, 1 to 10 ml. of the chlorophyll solution were added to the test tube, depending upon the expected activity of the adsorbent, and petroleum ether was added to the 15-ml. mark. The tube was then corked, shaken vigorously, and centrifuged. If all the chlorophyll was removed from the solution, an amount of petroleum ether was removed equal to the volume of chlorophyll solution to be added and the procedure was repeated. This procedure was repeated until the solution remained green, at which time 2 ml. were pipetted into a microcell of an Evelyn photoelectric colorimeter with an M-440 filter. An end-point reading of 60.0 for the galvanometer was arbitrarily chosen. If the galvanometer reading was above 60.0, more of the chlorophyll solution was added and the procedure was repeated until the reading fell below 60.0. From the amount of chlorophyll solution added, the milligrams of chlorophyll adsorbed per gram of adsorbent were calculated.

While it is not contended that the ability of the treated phosphates to adsorb chlorophyll is proportional to their ability to adsorb other pigments in extracts from plant material, it does indicate the extent of the activation.

For practical purposes the activity of the phosphate can be determined roughly by the use of a dehydrated alfalfa leaf meal known to contain the noncarotene pigments. Meal which has been exposed to the air at laboratory temperature for 2 or 3 weeks, so that the noncarotene pigments will have developed, is suitable for this purpose. The meal is extracted by placing about 10 grams in a 200-ml. Erlenmeyer flask, adding about 50 ml. of petroleum ether, and shaking for a few minutes. About 20 ml. of the extract are then decanted onto a column of the phosphate being studied.

If the phosphate is properly activated the carotene will pass through, while the chlorophyll will be held to within 3 to 7 mm. from the top of the column and a lower yellow band will form 12 to 25 mm. below, with a shade of red above. The lower yellow band should be so held that it does not pass into the filtrate after considerable washing with petroleum ether.

The rate of filtration through the adsorbent with a vacuum of 65 cm. of mercury was determined by noting the time in seconds required for 100 ml. of petroleum ether to pass through a column 2.2 cm. in diameter containing 8 grams of the adsorbent packed with vacuum and slight tamping. While there was some variation in the results obtained by the procedure, an average of several trials gave a fairly reliable figure.

The height of an 8-gram column 2.2 cm. in diameter before and after application of vacuum was also recorded, in order to gain some information of the effect of the activation procedure on the weight-volume relationship of the adsorbent.

It was found, after trying many procedures which might produce activation, such as heat applied under various conditions, that treatment with disodium phosphate, trisodium phosphate, sodium hydroxide, or potassium hydroxide increased the activity of an inactive dicalcium phosphate.

The effects of adding different quantities of disodium phosphate to two different lots of Baker's C. P. dicalcium phosphate were first studied (lots 21,940 and 10,440). One hundred-gram portions of each lot were placed in 2.0-liter flasks and different quantities of disodium phosphate, dissolved in 1 liter of water, were added. The mixtures were boiled on a hot plate for 0.5 hour and then decanted into a 20-cm. (8-inch) Büchner funnel, washed with 1 liter of water, and dried in an evaporating dish in an oven for 24 hours at 100° C. The phosphate was then broken up in a mortar, after which various data were collected on its properties (Table I).

The effects of treating one lot of Merck's reagent dicalcium phosphate with disodium phosphate, potassium hydroxide, and trisodium phosphate were next studied. The results are shown in Table II

Table II. Effect of Treating Dicalcium Phosphate with Disodium Phosphate, Potassium Hydroxide, and Trisodium Phosphate

Lot No.		Chlorophyll per Gram of Adsorbent	Filtration Rate		Height of 8.0-Gram Column: Before vacuum	Height of 8.0-Gram Column: After vacuum
	Grams	*Mg.*	*Min.*	*Sec.*	*Cm.*	*Cm.*
32,689	0	1.6	1	10	11.0	6.5
71	5	2.4	4	30	7.5	4.5
72	10	6.8	4	0	10.5	5.7
73	15	9.8	7	25	12.0	7.5
74	20	12.2	6	3	13.2	8.0
	KOH					
80	1	3.6	4	30	5.0	3.5
81	3	6.6	4	45	6.3	4.5
82	5	18.0	4	10	8.0	
83	10	21.4	3	0	11.5	9.0
	Na_3PO_4					
76	5	8.6	4	20	6.5	4.0

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The effect of boiling the mixture for varying lengths of time was next studied (Table III). Ten grams of disodium phosphate were used throughout for the activation. One lot was just brought to a boil, one was boiled for one-half hour, and another was boiled for one hour.

The effect of adding various quantities of water to the mixture was next studied, using 10 grams of disodium phosphate as the activating agent and boiling for 15 minutes. The results are shown in Table IV.

Table III. Effect of Time of Boiling

Lot No.	Treatment	Na_2HPO_4	Chlorophyll per Gram of Adsorbent	Filtration Rate		Height of 8-Gram Column Before vacuum	Height of 8-Gram Column After vacuum
		Grams	*Mg.*	*Min.*	*Sec.*	*Cm.*	*Cm.*
57	Heated to boiling	10	10.4	7	19	18.0	10.1
58	Boiled 0.5 hour	10	11.4	7	35	15.4	8.8
59	Boiled 1 hour	10	11.6	6	36	15.5	9.1

Discussion of Results

Table I indicates that increasing amounts of disodium phosphate markedly increased the activity of both lots of phosphates, as shown by the increasing amounts of chlorophyll adsorbed. This observation was further confirmed by the fact that both original lots only partially active now held the chlorophyll, xanthophyll, and the lower yellow band in the upper portion of the column. Lot 21,940 was originally slightly more active than lot 10,440, and equal amounts of disodium phosphate produced more activity in lot 21,940 as shown by the milligrams of chlorophyll adsorbed.

The filtration rate was decreased by the activation procedure. For instance, lot 10,440 had an original filtration rate of 2 minutes, 28 seconds, but after activation with 20 grams of disodium phosphate the filtration rate was 5 minutes and 37 seconds, or half as rapid. In both experiments the activation produced with 5 and 10 grams of disodium phosphate decreased the filtration rate more than the larger amounts. The cause of this alteration is not apparent and was not investigated further. It apparently does not lie in the weight-volume relationship, since the height of an 8-gram column increased almost progressively with increasing quantities of disodium phosphate.

The results in Table II show that potassium hydroxide and trisodium phosphate are much more effective in the activation procedure than disodium phosphate. This would indicate that alkalinity was the primary factor of the activation process. Although no data are given in this report, sodium hydroxide was also very effective. Experiments should have been performed under controlled pH conditions with the various alkaline salts and hydroxides, but were not deemed necessary to the scope and purpose of this report. Other adsorbents may be more highly activated in respect to their ability to adsorb chlorophyll by treatment with alkali—for instance, the activity of barium hydroxide and aluminum oxide can be increased as much as ten times by treatment with alkali.

In comparing the rate of filtration between the phosphates used in Tables I and II, it will be noted that the one used in Table II gave a much more rapid rate of filtration for equal activity than the phosphates used in Table I. The phosphate in Table II was Merck's reagent with a coarse granular crystalline texture. Because this particular brand can be highly activated and yet retain a fairly rapid rate of filtration, it is used almost exclusively in this laboratory for carotene determination work. Some grades of phosphates have been ground, which makes them especially unsuitable for carotene determinations because of the slow rate of filtration.

A rapid rate of filtration is desirable not only from the standpoint of saving time, but also because slow filtration promotes some slight destruction of carotene. A column 7.5 to 10 cm. (3 to 4 inches) deep should permit 100 ml. of petroleum ether to pass through it with application of vacuum in 4 to 5 minutes. The time should not exceed 6 minutes at the most.

If a filter aid is needed to speed up the filtration rate, Dyno, a commercial dextrose, has been found effective. (Dyno is made by the Corn Products Refining Company, 17 Battery Place, New York, N. Y., and can be obtained at some grocery and drugstores.) It is as effective as a siliceous earth commonly used for this purpose and does not cause destruction of carotene when used in conjunction with the phosphate.

Table III shows that the length of the time of boiling apparently had little effect on the activity of this particular phosphate. However, owing to the variability of various phosphates, boiling for 15 to 30 minutes has been adopted as a general procedure. Apparently little is gained by boiling for longer than 30 minutes.

Table IV indicates that water used in amounts of 500 to 1500 ml. had no noticeable effect on the activation process.

The following procedure was finally adopted:

One hundred grams of the inactive dicalcium phosphate (preferably Merck's reagent) are weighed out and placed in a 2-liter Erlenmeyer flask. A quantity greater than 100 grams usually causes considerable bumping. Five grams of potassium hydroxide are added in 1 liter of water. The contents of the flask are boiled on a hot plate for 15 to 30 minutes, transferred to a Büchner funnel, and washed with 0.5 to 1 liter of water. The phosphate is placed in an evaporating dish and dried for 24 hours at 100° C., and is then broken up in a mortar, after which it is ready for use.

The activated phosphate will sometimes hold up the carotene on the column when first removed from the oven, especially where more than 5 grams of potassium hydroxide are used. This property has been utilized in making chromatographic separations of extracts of yellow corn. However, after a few days' exposure to room temperature this particular property is lost.

The use of dicalcium phosphate as an adsorbent for the chromatographic determination of carotene (*1*) along with a rapid extraction procedure previously reported (*2*) offers an efficient and accurate method for the determination of carotene in plant materials.

Table IV. Effect of Various Quantities of Water

Lot No.	Water Added to Mixture	Na_2HPO_4	Chlorophyll per Gram of Adsorbent	Filtration Rate		Height of 8-Gram Column Before vacuum	Height of 8-Gram Column After vacuum
	Ml.	*Grams*	*Mg.*	*Min.*	*Sec.*	*Cm.*	*Cm.*
64	500	10	10.2	14	11	14.2	8.7
65	1000	10	11.0	13	50	14.7	8.7
66	1500	10	11.0	14	35	14.2	8.0

Summary

Inactive dicalcium phosphate for the chromatographic determination of carotene in plant material can be activated by proper treatment with various strong alkalies. A procedure using potassium hydroxide is described. A commercial dextrose is suggested as a filter aid.

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Solubility of Strontium Chromate and the Detection of Strontium

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THE removal of strontium chromate in the qualitative analysis of the alkaline earth group is a very slow process. The salt is generally precipitated by adding alcohol to a hot alkaline solution containing strontium chromate and then the mixture is thoroughly cooled before it is filtered. Noyes' original directions for this process are very satisfactory, in the sense that they allow the detection of rather small amounts of strontium in the presence of much calcium, but the filtering and the washing of the precipitate are very time-consuming. If the filtration and washing could be carried out with hot solutions, without loss of sensitivity or reliability, the procedure would be far more convenient.

The cooling of the alcoholic mixture containing strontium chromate seems to be advised for two reasons: (1) Calcium chromate precipitated with the strontium compound should redissolve on cooling, for its solubility increases at lower temperatures, and (2) the precipitation of the strontium chromate itself should be more complete at lower temperatures than at higher. One thinks in this connection of the next step in the procedure, which consists of dissolving the strontium chromate in hot water. Both reasons for filtering the mixture cold, however, are spurious. In the first place, if one follows the original Noyes directions, which prescribe minimal excess amounts of both chromate and alcohol, no calcium will precipitate unless the amount present in the solution exceeds 500 mg., yet as little as 1 mg. of strontium will precipitate under the same conditions. These claims have been confirmed by the author's direct observations. If one uses a modification of Noyes' procedure, such as that of Miller (*9*), as little as 80 mg. of calcium in the original sample will lead to a precipitate during the strontium removal. But if calcium chromate precipitates at all, it cannot be redissolved in the course of several hours' shaking at room temperatures. The extraordinary slowness with which calcium chromate dissolves has been noted by others. Kohlrausch (*6*) reported, for example, that a solution was still undersaturated after more than 15 months of contact with anhydrous calcium salt.

The cooling of the strontium chromate mixture neither eliminates possible precipitation of calcium chromate nor promotes a more complete removal of strontium chromate itself. Contrary to earlier figures appearing in the literature (*12*), the solubility of strontium chromate in water diminishes at higher temperatures and one should heat rather than cool a solution to promote more thorough precipitation. The solubility trends may be different in another solvent, but the probabilities are that strontium chromate is less soluble in hot aqueous alcohol than in cold. Because there are no data in the literature on this point, the solubilities of strontium chromate in alcoholic solutions were investigated before attempting to modify the well-established Noyes procedure (*11*).

Determination of Solubilities

The preparation of strontium chromate and the procedure followed in determining the solubility by iodometric titration of chromate have already been described (*2*). The concentration of alcohol in the mixed solvents was established by density measurements. The best strontium chromate which could be prepared from ammonium chromate and strontium chloride was 99.6 per cent pure as determined by titration of the chromate, and this was used for the solubility experiments. Numerous modifications in the preparative procedure were made without any improvement in the product.

The dissolving of the strontium salt like the dissolving of the calcium compound is very slow, saturation not being attained after a year's rotation of a gram of solid with about 100 grams of water at 25°. The changes in the liquid after the first month, however, amount to a few per cent only.

The solubilities were all run from undersaturation except for two determinations in pure water at 50° and 75° that were made from supersaturation, which was checked by analysis before rotating with solid. Some of the water solutions were examined for strontium content as well as chromate, the strontium being precipitated as sulfate in presence of alcohol. The agreement in every case was within 2 or 3 per cent with no evident trends.

TABLE I. SOLUBILITY OF STRONTIUM CHROMATE

Medium	25° *G./100 g.*	50° *G./100 g.*	75° *G./100 g.*
Water	0.096	0.090	0.080
19.7% alcohol	0.011	0.012	0.008
41.8% alcohol	0.0004	0.0009	0.0003
92.5% alcohol	~0.00005	<0.00005	<0.00005

The solubility determinations seem to involve more than ordinary difficulty for three distinct reasons. (1) Saturation is approached extremely slowly; (2) the solid phase slowly takes up water, presumably to form a hydrate; and (3) the solid is gradually contaminated by glass from the solubility bottles and in the presence of alcohol by chromic salt formed by reduction of the chromate. The slow contamination by glass, however, appeared not to affect appreciably the concentrations in the liquid phase. The very sluggish hydration of the solid is suggestive of the behavior of anhydrous calcium chromate which Mellor records as being unhydrated after a year's contact with solution (*7*). One sample of strontium chromate, which was allowed to stand without agitation for 16 months in contact with mother liquor, showed no appreciable change in analysis during that time. The water content of the strontium chromate residues left after rotating with solvent for periods ranging from a few months to more than a year was variable and pointed to the formation of a monohydrate or more likely a dihydrate. Calcium chromate, it may be noted in analogy, forms a hemi-, a mono- and a dihydrate (*14*). The hydration of the strontium salt seemed to make little difference in the solubilities, however, inasmuch as the concentrations of the water solutions increased slowly for periods in excess of a year. Probably hydrate and anhydrous salt have nearly equal solubilities. In some alcohol solutions, however, the chromate concentrations passed through a maximum, which is probably due to partial destruction of the chromate ion and appearance of an excess of strontium in solution.

The solubilities determined in this investigation are reported in Table I, in terms of grams of anhydrous salt dissolved in 100 grams of solution. The densities of all the saturated solutions are close to the densities of the original solvents. The data are not precise and accordingly are given to one or two significant figures only. The few determinations in the

Table II. Solubilities of Strontium Chromate According to the Literature

Medium	Temperature °C.	Solubility G./100 g.	Reference
H_2O	10	0.465	(12)
H_2O	15	0.120	(4)
H_2O	15	0.088	(2)
H_2O	16	0.119	(8)
H_2O	20	1.000	(12)
H_2O	25	0.091	(2)
H_2O	50	2.417	(12)
H_2O	75	0.0615	(2)
H_2O	100	3.000	(12)
H_2O	100	0.043	(2)
29% alcohol (by volume)	Room	0.0132	(5)
43% alcohol (by volume)	Room	0.002	(5)

literature with which to compare these results are assembled in Table II. The alcohol solutions whose concentrations are noted in weight percentages in Table I are approximately 25, 50, and 95 per cent by volume. The Ricci and Davis figure for solubility in water at 25° is a little lower than the average reported in Table I but their figure for 75°, which is about 30 per cent lower, probably represents undersaturation.

It will be observed that whatever the solvent the solubility is less at 75° than at 25°. The increase noted for 25 and 50 per cent alcohol solutions in the range 25° to 50° is unexpected on the basis of any commonly accepted theory, but the change in the whole range 25° to 75° is not large and the figures in Table I can be used confidently as a basis for judging analytical procedures and the like.

While the determinations turned out to have rather poor precision, the values are reported here as giving a basis for an improvement in the Noyes analysis of the alkaline earth group. Two equations (*3*, *13*) for solubilities, one based on Born's relation for the energy of charging spheres in a uniform medium, and the other on the assumed constancy of activity coefficient in saturated solutions, predict such widely different solubilities in the alcohol-water mixtures that experimental determinations seemed to be very desirable.

Detection of Strontium as Chromate

The diminished solubility of strontium chromate at higher temperatures recorded in Table I indicates the possibility of removing strontium chromate in the analysis of the alkaline earth group at temperatures around 75°.

To test this conclusion, the salt was precipitated from solutions containing strontium ion, under various conditions. The precipitate was removed and the amount of strontium remaining in solution as well as the amount precipitated was determined by conversion into the sulfate which was subsequently filtered, washed, ignited, and weighed. (It was observed during this work that even in the absence of carbon or organic matter the sulfate loses oxygen when it is heated in the full Bunsen flame. Sulfide is formed. This source of error is not mentioned in the standard reference works, and it is possible that traces of chromate or chromic compounds catalyze the decomposition. If the final heating of the strontium sulfate is carried out in a sand bath, the decomposition is avoided.)

In each of the experiments recorded in Table II, unless otherwise noted, 25 ml. of a solution containing 25 mg. of strontium ion were treated with 1 ml. of dilute acetic acid, 2 ml. of dilute ammonium hydroxide, 2 ml. of 3 *N* potassium chromate, and the amount of 95 per cent alcohol indicated. The volumes are roughly those dealt with in the procedure of Miller. The column headings indicate the nature of the observations.

The amount of strontium precipitated in the several trials was about independent of the temperature of precipitation and of alcohol in excess of 40 per cent by volume. It was found by separate experiments that strontium chromate was precipitated in every case when as little as 1 mg. of strontium was used in the original solution instead of 25 mg. This observation means that the Noyes procedure will reveal the presence of as little as 1 mg. of strontium, regardless of whether the precipitation is effected at 25° or 75°. The high-temperature precipitate is coarse, while the low-temperature product is finely divided and much more difficult to filter. It is obviously advantageous to carry out the precipitation and filtration of strontium chromate at higher temperatures. These procedures should be of interest also in the quantitative separation of strontium from calcium.

The familiar separation of strontium from calcium based on the leaching of a mixture of the dried nitrates with organic solvents (*1*) is very slow, often gives low results according to Noll (*10*), or involves application of fairly large empirical correction factors (*15*). Separation by means of the chromates avoids the use of expensive anhydrous reagents and should be faster than and about as reliable as the nitrate process, although direct tests should be made with the idea of applying the data to quantitative separations.

Summary

The solubility of strontium chromate in alcoholic as well as in water solutions is found to diminish somewhat in the temperature range 25° to 75° C. Since the removal of strontium as chromate will be more complete at the higher temperatures, a modification of the classical A. A. Noyes procedure for the qualitative analysis of the alkaline earth group is suggested, providing for precipitation and filtration of strontium chromate from hot solutions. The new procedure effects a considerable saving in time. Solid strontium chromate in contact with aqueous solutions undergoes a very slow hydration which has not been mentioned previously in the literature. The hydration at 25°, like the formation of a saturated solution at the same temperature, would probably not be complete in less than several years' time.

Table III. Qualitative Removal of Strontium as Chromate

Expt.	Temp. °C.	Alcohol Added Ml.	Time of Shaking Min.	Time for Filtering Min.	Strontium in Precipitate Mg.	Strontium in Solution Mg.
7	..	..	..	..	24.8[a]	...
17	..	..	..	..	24.4[a]	...
16	..	..	..	..	25.8[b]	...
26	25	16	6	[c]	22.0	3.1
21	25	20	6	[c]	24.2	0.5
10	25	20	15	22	23.9	0.5
11	25	30	15	22	23.9	1.3
14	50	20	6	6	24.2	0.6
20	50	30	6	[c]	23.7	0.5
27	75	16	6	[c]	21.1	2.7
12	75	20	2	5	24.1	0.4
19	75	20	2	[c]	22.4[d]	1.8
18	75	20	6	5	23.2	0.7
13	75	30	0	6	24.4	0.5

[a] Strontium sulfate precipitated directly from original solution after adding potassium chromate but not alcohol.
[b] Strontium sulfate precipitated directly from alkaline chromate solution in presence of alcohol.
[c] Time not recorded.
[d] Precipitation effected at 75°, sample cooled and shaken at 25° for 2 minutes before filtering.

Acknowledgment

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Volumetric Determination of Iron and Titanium

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A METHOD for the determination of iron and titanium has been described by Lundell and Knowles (*6*, *7*). The iron is determined by the stannous chloride reduction method or precipitated as the sulfide from an ammoniacal tartrate solution and both iron and titanium are determined by means of the Jones reductor, the titanium being found by difference.

Nakazono (*8*) recommends the use of liquid zinc amalgam for the reduction of an aliquot portion of the solution and titration with standard permanganate to give the total iron and titanium. A second portion of the solution is reduced, and added to the first solution, and the combined solutions are titrated for excess iron necessarily present in using this method. According to Gooch and Newton (*5*) the use of bismuth trioxide to oxidize only the titanium, after the titanium and iron have been reduced by heating with zinc and acid, gives excellent results for the iron. The method requires the filtration of the bismuth trioxide preceding the titration of the ferrous iron. Thornton and Roseman (*12*) reduced titanium and iron by passing the solution through a Jones reductor (a zinc-amalgam column), using air as the differential oxidizing agent for estimating iron in the presence of titanium. They suggest that "it may be well to introduce the proviso that the procedure is most apt to succeed when the iron is equal to, or preponderates over, the titanium." Axt and LeRoy (*1*) state that they were able to air-oxidize reduced titanium within 3 to 8 minutes. They worked with 0.04485 and 0.116 gram of titanium but gave no direct results of their experiments other than a curve. According to this method the titanium was reduced with liquid zinc amalgam and finally air or oxygen was bubbled through the reduced titanium solution by means of an apparatus so constructed that a porous plate divided the air or oxygen into a multitude of small bubbles. Brandt (*3*) used titanium trichloride as the reducing agent for the iron, the excess titanium trichloride being destroyed with copper sulfate. In this procedure, too, it is necessary to carry out a filtration before the titration of the ferrous iron.

The object of this investigation was to find a more rapid, accurate, and convenient method for the determination of iron and titanium. Reduction of the iron and titanium and subsequent titration with permanganate would give the combined amounts of each. Air-oxidation of the titanium alone, following the reduction of both the iron and titanium, would make it possible to titrate the iron and determine the titanium by difference. This required a study of the air-oxidation of titanous and ferrous solutions.

Reagents

Titanium dioxide, purity 98.62 per cent as determined by the cupferron method (*10*). (Spectrographic analysis gave no positive test for other reducible materials such as chromium and vanadium but did show presence of some magnesium which does not interfere.)

The following materials were C. P. reagent grade: ferric ammonium sulfate, 100 grams per liter of 0.5 *M* sulfuric acid; ammonium sulfate; sulfuric acid, 36 *N;* mercuric chloride, saturated solution in distilled water; zinc, 30-mesh.

Zimmermann-Reinhardt solution: 70 grams of manganous sulfate, 125 ml. of concentrated sulfuric acid, and 125 ml. of 85 per cent phosphoric acid diluted to a liter.

Potassium permanganate, approximately 0.1 and 0.05 *N* solutions prepared in the usual way and standardized against sodium oxalate according to the National Bureau of Standards method (*4*).

Air-Oxidation of Titanium

A standard solution of titanium sulfate was prepared by dissolving titanium dioxide in concentrated sulfuric acid and ammonium sulfate. The solution was adjusted to an acidity of 3 to 4 *N* with respect to sulfuric acid, and analyzed by reducing the titanium, pouring through a zinc reductor, and receiving into a ferric alum solution (*10*). The reduction was repeated with solutions containing from 0.0203 to 0.2512 gram of titanium dioxide, the reductor was removed from the suction flask, and a rubber stopper containing a glass tube was fitted into the mouth of the flask with the end of the tube dipping into the solution. Air was bubbled through the solution by means of water suction for 25 to 30 minutes. The unoxidized titanium was determined by titration with potassium permanganate. The results are given in Table I.

A more rapid method for the oxidation of Ti^{III} to Ti^{IV} was investigated. Fifty milliliters of a saturated solution of mercuric chloride were added to a reduced titanium solution. After standing for one-half hour, with occasional shaking, the solution showed incomplete oxidation of titanium.

A solution of titanium sulfate in 4 *N* sulfuric acid was reduced, by running through a reductor, and received into a 4 *N* sulfuric acid solution. To this were added 50 ml. of a saturated solution of mercuric chloride, and air was bubbled through the solution until the violet color disappeared and then for 5 minutes longer in an attempt to ensure complete oxidation of the titanium. The unoxidized titanium was determined by permanganate titration.

TABLE I. AIR-OXIDATION OF TITANOUS SULFATE SOLUTION

TiO_2 Taken *Gram*	Air *Min.*	TiO_2 Oxidized *Gram*
0.2512	40[a]	0.1991
0.1252	40[b]	0.0946
0.0626	30	0.0526
0.0203	25	0.0200

[a] Solution had a pronounced violet color.
[b] Solution had a faint violet color.

TABLE II. AIR-OXIDATION OF TITANOUS SULFATE SOLUTION IN THE PRESENCE OF MERCURIC CHLORIDE

TiO_2 Taken *Gram*	$HgCl_2$ *Ml.*	Air *Min.*	TiO_2 Oxidized *Gram*	Number of Determinations
0.3425	50	15	0.3423	4
0.2512	50	15	0.2512	3
0.1557	50	10	0.1557	7[a]
0.1252	50	10	0.1250	3
0.0626	50	10	0.0625	2[b]
0.0203	50	5	0.0203	2

Individual values:
[a] 0.1557, 0.1557, 0.1555, 0.1557, 0.1556, 0.1556, 0.1558.
[b] 0.0624, 0.0627.

Table III. Air-Oxidation of Ferrous Sulfate Solution

Fe_2O_3 Taken	Air	Fe_2O_3 Found	Number of Determinations
Gram	*Min.*	*Gram*	
0.6231	15	0.6219	4
0.6231	20	0.6224	3
0.2963	15	0.2961	4
0.2963	20	0.2963	4[a]
0.1732	20	0.1732	2
0.1732	25	0.1732	2
0.1226	15	0.1226	2
0.1226	20	0.1226	2
0.0835	15	0.0835	3[b]
0.0754	20	0.0754	3

Individual values: [a] 0.2963, 0.2961, 0.2964, 0.2964.
[b] 0.0832, 0.0835, 0.0837.

Table IV. Air-Oxidation of Ferrous Sulfate Solution in the Presence of Mercuric Chloride

Fe_2O_3 Taken	$HgCl_2$	Air	Fe_2O_3 Found	Number of Determinations
Gram	*Ml.*	*Min.*	*Gram*	
0.3424	50	120	0.3418	2
0.3243	50	30	0.3243	4
0.1835	50	10	0.1835	3
0.1835	50	20	0.1836	2
0.1835	50	30	0.1836	4[a]
0.1698	50	10	0.1697	3
0.1698	50	20	0.1696	2
0.1698	50	30	0.1697	3
0.1698	50	40	0.1698	2
0.0971	50	30	0.0971	3[b]
0.0686	50	30	0.0686	4
0.0463	50	30	0.0463	4[c]
0.0174	50	30	0.0173	4

Individual values: [a] 0.1835, 0.1835, 0.1836, 0.1837.
[b] 0.0970, 0.0970, 0.0972.
[c] 0.0463, 0.0464, 0.0462, 0.0464.

The results are given in Table II. Thus, although air alone or mercuric chloride alone was not sufficient for the oxidation of trivalent titanium, the action of the two combined was very effective and resulted, in a short time, in a catalytic oxidation of the titanium. In Tables II to VI the final values are the averages of the results of the number of determinations indicated in the last column. The accuracy of the method may be judged further by examination of the individual values given in Tables II, III, IV, and V as typical.

Air-Oxidation of Ferrous Iron

It has been known for some time that ferrous salts are only slowly oxidized by bubbling air through their solutions.

Baskerville and Stevenson (*2*) observed no oxidation of ferrous to ferric iron upon passing air through neutral or acid solutions of ferrous sulfate for 3 hours. The results were shown to be influenced only negligibly by dust in the air, glass wool, pumice stone, and various salts. They also observed no oxidation on reducing a sulfuric acid solution of ferric sulfate with zinc, filtering off the zinc, and passing air through the solution for 3 hours. More recently (*9*) Pound concluded that the oxidation of ferrous salts was a minimum in the presence of a strong nonoxidizing acid such as sulfuric or hydrochloric. When the concentration exceeded 0.2 *N* for the hydrochloric and 6.0 *N* for the sulfuric acid, the tendency for the oxidation of ferrous iron by air became appreciable.

A solution of ferric alum was prepared in 4 *N* sulfuric acid, analyzed for iron (*11*), and calculated as iron oxide. Aliquot portions of this solution were poured through the reductor in the usual way, and air was bubbled through the solutions for 15 to 25 minutes. The results given in Table III show some oxidation of the ferrous sulfate when large quantities of iron were present.

In another series of experiments a saturated solution of mercuric chloride was added, after the reduction of iron, and air bubbled through the solutions for 10 to 120 minutes. The ferrous iron was titrated with standard permanganate and calculated as iron oxide. Results are shown in Table IV.

Air-Oxidation of Iron and Titanium Together

Accurately measured volumes of standard solutions of ferric sulfate and titanium sulfate were transferred to a beaker. The solution was adjusted to approximately 4 *N* in sulfuric acid, reduced with zinc, poured through the reductor with moderate suction, and received into a ferric alum solution. The ferrous iron was titrated with standard permanganate, and from this the number of milliequivalents of permanganate equal to the iron and titanium was obtained. The reduction was repeated, and the reduced iron and titanium were poured through the reductor, and received into a solution of 4 *N* sulfuric acid. To this solution were added 50 ml. of a saturated solution of mercuric chloride. Air was bubbled through the solution until the violet color disappeared, then for 5 minutes longer. The ferrous iron was titrated with standard permanganate and calculated as ferric oxide. The difference between the number of milliequivalents in this and the first titration represents the number of milliequivalents of titanium dioxide. The results obtained are shown in Table V. The procedure was repeated for the determination of iron in the presence of titanium by reducing and bubbling air through the solution in the absence of mercuric chloride (Table VI).

Table V. Air-Oxidation of Titanous Sulfate Solution in the Presence of Ferrous Sulfate and Mercuric Chloride

Fe_2O_3 Taken	TiO_2 Taken	Fe_2O_3 Found	TiO_2 Found	Number of Determinations
Gram	*Gram*	*Gram*	*Gram*	
0.7800	0.2782	0.7795	0.2776	2
0.7800	0.1391	0.7798	0.1385	2
0.3900	0.2782	0.3904	0.2780	5
0.3900	0.1391	0.3903	0.1392	3[a]
0.3900	0.0927	0.3902	0.0929	2
0.3900	0.0685	0.3902	0.0688	2
0.3900	0.0343	0.3898	0.0345	2
0.3900	0.0171	0.3899	0.0173	2
0.1950	0.1391	0.1948	0.1390	4
0.0975	0.1391	0.0975	0.1388	2[b]
0.0325	0.1391	0.0326	0.1392	3

Individual values: [a] Fe_2O_3 found: 0.3905, 0.3903, 0.3901
TiO_2 found: 0.1390, 0.1392, 0.1394.
[b] Fe_2O_3 found: 0.0977, 0.0973
TiO_2 found: 0.1389, 0.1387.

Table VI. Air-Oxidation of Titanous Sulfate in the Presence of Ferrous Sulfate

Fe_2O_3 Taken	TiO_2 Taken	Air	TiO_2 Unoxidized
Gram	*Gram*	*Min.*	*Gram*
0.3982	0.2512	40[a]	0.1672
0.2389	0.2512	40[a]	0.0786
0.0637	0.2512	40[a]	0.1954
0.2389	0.1256	25[b]	0.0428
0.2389	0.0628	15[c]	0.0081

[a] Solution had a pronounced violet color.
[b] Solution became colorless within 13 minutes.
[c] Solution became colorless within 2 minutes.

Discussion

The simplicity of construction of the zinc reductor prepared by sealing a glass tube on the end of a 2.5-cm. funnel and stocking the funnel with 30-mesh zinc makes it available at very little cost to any laboratory. Air alone is not sufficient for the complete oxidation of titanium by this method. However, when the reduced titanium solution is aerated in the presence of a large amount of mercuric chloride, catalytic oxidation takes place in a short time.

The results obtained by this method are not in agreement with those of Thornton and Roseman (*12*), and a part of their work was repeated using a Jones reductor (zinc-amalgam column). Although their results were reproducible, it was found that a considerably larger volume of solution remained to be aerated and no definite time could be set for complete oxidation of the titanium. According to the results and statement given in the summary of their paper, oxidation of the titanium is more likely to succeed if the amount of iron is equal to or greater than the titanium present. A definite procedure is given in this paper whereby the time factor for the quantitative oxidation of titanium is controlled irrespective of the amounts of iron present. It might appear at first that it is not necessary to add so much as 50 ml. of a saturated solution of mercuric chloride. It is true that smaller amounts of mercuric chloride can be used for the oxidation of small quantities of titanium, but such quantities cannot be assumed in a sample of unknown composition. A thorough investigation resulted in the use of 50-ml. of mer-

curic chloride in order to give the best results for any amount of titanium up to about 0.6 gram of titanium dioxide. Since such large amounts of titanium would not be used in a single analysis, these values are omitted from the tables.

The volumetric estimation of iron and titanium generally used in the titanium pigment industry involves reduction of solution (zinc-amalgam reductor), titration of the combined iron and titanium with standardized potassium permanganate, and separate titration of titanium with standardized ferric ammonium sulfate. The method described in this paper has the advantage of requiring the use of only one standardized solution.

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Analysis of Tin-Base Bearing Metal

Permanganate and Iodometric Methods for Antimony and Copper

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NUMEROUS combinations of standard methods can be used to obtain accurate and reliable values for antimony and copper in tin-base bearing metal. However, since these methods have been found to be too time-consuming for routine work, various methods have been investigated in this laboratory.

Experimental

When copper was separated from 10 to 20 times as much tin plus antimony by the nitric acid–hydrolysis method (*8*, *10*), some copper invariably remained with the tin and antimony acids. The quantity of copper retained by different precipitates varied so much that a constant correction could not be used, and each precipitate had to be treated to recover the occluded copper. Fusion of the precipitate with sodium carbonate and sulfur (*5*), leaching with sodium sulfide and alkaline tartrate solution, and also volatilization of the tin and antimony by heating with ammonium iodide at 500° to 550° C. were found to give quantitative recoveries of the copper.

Separation of copper from a dilute sulfuric acid solution as the thiocyanate (*11*) required much less time than any of the above procedures, while separation of the copper as the sulfide from an alkaline tartrate solution (*7*) was the fastest method found in the literature.

Attempts to determine copper in tin-base bearing metal by the iodometric method (*1–4*, *10*) led to low and variable results. The voluminous precipitate of tin and antimony apparently interfered, perhaps by retaining some copper from the solution and by adsorbing some of the iodine formed in the reduction of the cupric salt by potassium iodide.

Kolthoff and Sandell (*9*) discuss the iodometric determination of arsenic in a tartrate solution of Paris green. They add that it is possible to determine the copper iodometrically in the same solution after adding sulfuric acid to give the proper acidity. The proper acidity is not stated. Since antimony can be determined by permanganate titration of an acid solution containing tartaric acid (*6*), the iodometric titration of the copper in the resulting solution should permit the rapid determination of antimony and copper in tin-base bearing metal.

A series of tests was carried out along these lines. When the solution was acidified with acetic acid and the usual amount of potassium iodide added, 3 grams per 80 ml. of solution, the titration was not satisfactory because of drifting end points and incomplete reduction of cupric salts. The concentration of cupric ion evidently was too low, owing to the complex tartrate of copper.

In another series of tests the added potassium iodide was varied up to 10 grams per 80 ml. of solution. Increasing the potassium iodide concentration favored the reduction of cupric ions and permitted quantitative titrations when 8 to 10 grams were added to each 80 ml. of solution, but the reaction was slow and the drifting end points were troublesome.

Since satisfactory titrations were not obtained on weakly acidified tartrate solutions, another series of tests was carried out in which the pH was lowered by the addition of various amounts of sulfuric acid. At a pH of approximately 2.65 and a potassium iodide concentration of 6 grams per 80 ml. of solution, the reduction of cupric ion was both quantitative and relatively rapid. Antimony was not reduced even when twice the quantity of sulfuric acid was added, although the additional acid accelerated the air oxidation of the potassium iodide and caused an iodine blank equivalent to 0.15 to 0.20 ml. of 0.1 *N* thiosulfate. When still more acid was added the reduction of antimony became apparent. Evidently, the tartrate complex of antimony requires a relatively high acid concentration for reaction with potassium iodide.

Because of the large concentration of potassium iodide required, Griffin's procedure for antimony (*6*) was modified so as to reduce the volume of solution and thereby reduce the quantity of potassium iodide required for the reduction of cupric ion. Several difficulties were encountered in the determination of antimony by this modified method, and the four main difficulties are explained below.

1. When the sample was digested in a too rapidly heated sulfuric acid–potassium sulfate mixture, some sulfur sometimes collected high up on the sides of the flask. Because of this, it has been found advisable to heat the acid mixture moderately while solution of the sample is taking place.

2. Some difficulty due to fading of the end point was experienced in the permanganate titration, owing to the presence of

Table I. Analysis of Mixtures of 0.85 Gram of Tin and Varying Amounts of Antimony and Copper by the Permanganate and Iodometric Methods

Sample No.	Antimony			Copper		
	Present	Found	Error	Present	Found	Error
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
1	73.2	72.2	−1.0	37.5	36.9	−0.6
2	73.2	71.6	−1.6	76.0	76.3	+0.3
3	150	...	...	0.6[a]	0.6	0.0
4	149.9	148.9	−1.0	7.6	7.2	−0.4
5	150.0	149.9	−0.1	10.1	9.8	−0.3
6	150	...	...	19.3	19.9	+0.6
7	150.0	149.3	−0.7	25.2	25.6	+0.4
8	150.0	150.5	+0.5	50.4	50.8	+0.4
9	150	...	...	57.8	57.3	−0.5
10	150.0	150.5	+0.5	75.6	76.3	+0.7
11	149.4	148.6	−0.8	95.5	96.1	+0.6
12	150.0	149.9	−0.1	100.7	100.9	+0.2

[a] Method not applicable to so small an amount of copper.

Table II. Antimony and Copper Found in Tin-Base Bearing Metal by the Permanganate and Iodometric Methods

Sample No.	Antimony			Copper		
	Present	Found	Error	Present	Found	Error
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
Bureau of Standards No. 54	73.3	74.3	+1.0	37.5	38.6	+1.1
Bureau of Standards No. 54	73.3	73.3	0.0	37.5	37.7	+0.2
Bureau of Standards No. 54	73.3	73.8	+0.5	37.5	37.7	+0.2
Bureau of Standards No. 54	73.3	73.5	+0.2	37.5	36.6	−0.9
Bureau of Standards No. 54a	73.2	72.6	−0.6	37.5	37.6	+0.1
Bureau of Standards No. 54a	73.2	72.9	−0.3	37.5	38.0	+0.5
Bureau of Standards No. 54a	73.2	73.5	+0.3	37.5	37.3	−0.2
Bureau of Standards No. 63[a]	1.4	[d]	...	195.2	194.4	−0.8
Bureau of Standards No. 37b[b]	...	...	...	176.0	175.3	−0.7
Bureau of Standards No. 52[c]	0.4	[d]	...	220.9	220.2	−0.7
1	70.2	69.8	−0.4	30.0	30.2	+0.2
2	73.3	72.6	−0.7	44.0	44.5	+0.5

[a] Phosphor-bronze bearing metal.
[b] Sheet brass.
[c] Cast bronze.
[d] Method not applicable to so small an amount of antimony.

hydrochloric acid. By cooling the solution to 5° C. this difficulty has been eliminated.

3. When 10 per cent tartaric acid was added to the too warm sulfuric acid–potassium sulfate mixture following digestion of the sample, decomposition products formed which reduced permanganate in the subsequent titration of antimony. By previously cooling the hot acid mixture to 25° C. this decomposition has been eliminated.

4. The antimony factor for the 0.1 *N* permanganate varied from the calculated value. The factor was influenced principally by the quantity of antimony present and slightly by the technique peculiar to different analysts. When different chemists standardized the permanganate against tin-base bearing metals of various antimony contents, the factors found ranged from 0.955 to 0.975 times that obtained against Bureau of Standards sodium oxalate.

The copper factor for 0.1 *N* thiosulfate varied but little from the calculated value. When the thiosulfate was standardized against tin-base bearing metals of various copper contents, the factor found was 1.006 times that obtained iodometrically against 0.1 *N* permanganate and iodide.

Standards Used

Potassium permanganate, 0.1 *N*, standardized against a standard tin-base bearing metal of approximately the same antimony content as the metal to be analyzed.

Sodium thiosulfate, 0.1 *N*, standardized against a standard tin-base bearing metal of known copper content.

Metallic antimony, assaying 99.5 per cent.

Copper sulfate, crystals, reagent grade.

Metallic tin, C. P., practically free from antimony and copper.

Procedure

Antimony. In a 250-ml. wide-mouthed conical flask digest 1 gram of tin-base bearing metal in a mixture of 5 grams of potassium sulfate and 15 ml. of sulfuric acid. Gradually increase the heat until the sample has been completely attacked and no dark particles can be seen through the bottom of the flask. Cool the mass to 25° C.

Add 50 ml. of 10 per cent tartaric acid and 10 ml. of hydrochloric acid to the cooled mass. Bring to a slow boil and continue the boiling for several minutes, or until all soluble matter has been dissolved. Add 40 ml. of water to this solution, cool in ice water to below 5° C., and titrate with 0.1 *N* permanganate to an end point which persists for several seconds.

Copper. Add 3 drops excess of 0.1 *N* permanganate to the above solution and render it ammoniacal with ammonium hydroxide. About 50 ml. are required. Exactly neutralize the solution with (1 to 1) sulfuric acid and add 2 ml. in excess. Cool the solution to 14° to 16° C.

Add 10 ml. of 10 per cent potassium thiocyanate and 15 grams of solid potassium iodide, and immediately titrate the liberated iodine with 0.1 *N* thiosulfate, using starch indicator at the end point. Back-titrate with 0.1 *N* iodine to the reappearance of the blue starch-iodine coloration.

Discussion

The results for antimony and copper (Tables I and II) were obtained by using the procedure as outlined, and as nearly as possible under routine conditions. Where more than one set of values are given for the same sample, that sample was not run in duplicate, but was run singly at different times, or by different chemists.

These results indicate that the procedures for antimony and copper are sufficiently precise under routine conditions for use where errors of 0.2 to 1.0 mg. are acceptable.

If any changes are made in the procedure as outlined regarding temperatures, quantities of reagents, or volumes of resulting solutions, the pH of the solution and the activity of the antimony and copper tartrate complexes may be changed.

In this event, compensating changes will be required in the procedure.

Summary

A rapid method for the determination of antimony and copper in tin-base bearing metal is given in which both metals are determined on the same portion of sample.

The sample is digested in sulfuric acid and potassium sulfate and brought into solution with hydrochloric and tartaric acids, and the antimony is titrated with standard potassium permanganate. The resulting solution is neutralized with ammonia and acidified to pH 2.5 to 2.7 with sulfuric acid, the cupric salt is reduced with a large excess of potassium iodide, and the liberated iodine is titrated with standard thiosulfate.

Antimony and tin do not interfere with the copper determination under the prescribed conditions.

Arsenic, if present, will be titrated with the antimony.

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The views presented in this article are those of the writers and should not be construed as the official views of the Navy Department.

Determination of Germanium in Silicate Rocks

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THE method here described for the determination of germanium in silicate rocks and the rock-forming silicates involves decomposition of the sample with sulfuric-nitric-hydrofluoric acid, isolation of germanium by distillation of the tetrachloride, and its colorimetric estimation in the distillate with ferrous sulfate–ammonium molybdate. The latter reagent has been used by Poluektov (*3*) for the determination of germanium after isolation as the sulfide. The composition of the reagent has been modified for the present purpose.

In addition to germanium, phosphorus, silicon, and arsenic yield complex molybdic acids which are reduced to molybdenum blue by ferrous sulfate. Silicon and arsenic may give rise to error in the procedure. Even after double evaporation of the decomposed sample to fumes with sulfuric acid, traces of fluoride remain, which in the subsequent distillation pass over as fluosilicic acid and lead to high results. This difficulty can be overcome by distilling the sulfuric acid solution of the decomposed sample until all fluorine has been expelled, as shown by a negative reaction with ammonium molybdate–ferrous sulfate. Hydrochloric acid may then be added and the germanium distilled. The interference of arsenic is not serious in samples for which the method is intended. In order to keep the procedure as simple as possible no special method (*1*) for the separation of arsenic and germanium has been included here. The arsenic content of igneous rocks is so low (ordinarily less than 0.001 per cent) that the amount of the element finding its way into the distillate is insignificant if it is originally present in the quinquevalent state, as it will be after the decomposition. A rock sample containing 0.01 per cent of added arsenic showed an increased germanium content of 0.00004 per cent (No. 6, Table I), a deviation which is within the limit of error of the method. The possible effect of selenium was not investigated because it occurs only in vanishingly small amounts (of the order 10^{-6} per cent) in igneous rocks. There is no reason to believe that amounts considerably larger than this would interfere if selenium should find its way into the distillate.

The presence of small amounts of chloride in the sample does not lead to the loss of any significant amount of germanium in the decomposition (No. 5, Table I), as already shown (*1*).

Beer's law is obeyed by the blue product given by the ammonium molybdate–ferrous sulfate reagent up to a concentration of 1.5 micrograms of germanium per milliliter, but at higher concentrations the color intensity is less than that demanded by the concentration under our conditions.

Care must be taken to avoid contamination of the final solutions by silica. The sodium hydroxide solution in which the germanium distillate is caught must be contained in a paraffined vessel. It is important to use sodium hydroxide containing minimal amounts of silicate. A blank should show only a trace of blue color. Exactly the same amount of sodium hydroxide solution must be used for the unknown, standard, and blank solutions.

When a 1.0-gram sample is taken for analysis, less than 0.0001 per cent of germanium can be detected with certainty if a filter photometer with a 1-cm. cell is used. Since the average germanium content of igneous rocks is 0.0004 per cent according to Goldschmidt and co-workers (*2*), the proposed method appears to have sufficient sensitivity for the determination of the element in the samples for which it is intended.

Apparatus

The all-glass (Pyrex) distilling apparatus of Scherrer (*4*) may conveniently be used for the distillation of the germanium tetrachloride. The flask should preferably have a volume of 100 ml. The end of the condenser tube should be drawn to a narrow point. A test tube, drawn to a narrow taper at the lower end, is used as a receiver to hold the sodium hydroxide solution in which the germanium tetrachloride is absorbed; it should have a capacity of at least 12 ml. and should be well paraffined to prevent attack by the sodium hydroxide solution.

Special Solutions

AMMONIUM MOLYBDATE. Dissolve 6.00 grams of ammonium molybdate tetrahydrate in about 35 ml. of water, add a cooled mixture of 16.0 ml. of concentrated sulfuric acid (sp. gr. 1.84) with 35 ml. of water, dilute the solution to 100 ml. with water, and preserve in a paraffined bottle.

FERROUS AMMONIUM SULFATE. Dissolve 10.0 grams of the hydrated salt in water containing 1.5 ml. of 6 *N* sulfuric acid and dilute to 500 ml.

SODIUM ACETATE. Dissolve 67.5 grams of sodium acetate trihydrate in water and dilute to 200 ml. Keep the solution in a paraffin-lined bottle.

AMMONIUM MOLYBDATE–FERROUS AMMONIUM SULFATE REAGENT. This solution is unstable and must be prepared immediately before use. Add successively with shaking 10 ml. of ammonium molybdate solution, 10 ml. of ferrous ammonium sulfate solution, and 25 ml. of sodium acetate solution to 50 ml. of water and dilute to 100 ml. with water. Allow the reagent to stand 5 minutes before use.

SODIUM HYDROXIDE. Dissolve 25 grams of analytical quality reagent as low as possible in silica in 100 ml. of water and store the solution in a paraffined bottle.

STANDARD GERMANIUM SOLUTION, 0.01 PER CENT GERMANIUM OR GERMANIUM DIOXIDE. Dissolve a weighed amount of pure dry germanium dioxide in a few milliliters of water to which a drop or two of dilute sodium hydroxide has been added, neutralize with dilute sulfuric or hydrochloric acid, add a drop or two of acid in excess, and make up to volume.

Procedure

Weigh 1.0 gram of rock powder into a platinum dish and add a few milliliters of water, 6 ml. of 1 to 1 sulfuric acid, 0.5 to 1 ml. of concentrated nitric acid, and 10 ml. of hydrofluoric acid. Evaporate slowly on a hot plate until the sulfuric acid just starts to fume, cool, add a few milliliters of water, and again evaporate to fumes. Repeat the addition of water and evaporation at least twice, never letting the mixture fume strongly. Transfer the decomposed sample to the distilling flask with 1 to 1 sulfuric acid (chloride-free) and water. The total volume of the solution should be approximately 50 ml., of which 35 ml. should be 1 to 1 sulfuric acid.

REMOVAL OF RESIDUAL HYDROFLUORIC ACID. To remove traces of fluoride, bubble air slowly through the solution and raise the temperature to 140° C. Maintain the temperature at this figure during the distillation by the slow addition of water through the funnel. In this manner distill over and discard 150 ml. of liquid. Allow the distilling flask to cool, add 15 ml. of water and, while passing air through the solution, distill until a temperature of 120° is reached. Catch the distillate in a paraffined drawn-out test tube containing 1 ml. of 25 per cent sodium hydroxide solution and 1 ml. of water. Neutralize the distillate with 1 to 1 hydrochloric acid, using phenolphthalein as indicator, add a drop of sodium hydroxide, and then acidify with acetic acid and add the mixed ammonium molybdate–ferrous ammonium sulfate reagent as described below, but use the whole solution instead of an aliquot. Prepare the comparison solution by adding the reagent to a solution obtained by mixing sodium hydroxide and hydrochloric acid to give a mixture having the same composition as the neutralized distillate. If the distillate shows no color when compared against the reference solution, all hydrofluoric acid has been expelled from the solution in the distilling flask and germanium may be distilled over as described in the next section; if a color

is shown, distill again at 140° as already described until a negative result is obtained.

Distillation of Germanium Tetrachloride. Add 2 ml. of 1 to 1 hydrochloric acid and 2 ml. of water to the cool contents of the distilling flask, and distill while bubbling air through the solution until the temperature reaches 120°. Collect the distillate in the drawn-out test tube containing 2.00 ml. of 25 per cent sodium hydroxide solution. At the end of the distillation, neutralize the solution with 1 to 1 hydrochloric acid, transfer to a 25-ml. volumetric flask, and make up to volume with water.

Allow the distilling flask to cool, add 2 ml. of 1 to 1 hydrochloric acid and 2 ml. of water, and distill as before. Neutralize the solution, transfer to a 25-ml. volumetric flask, and make up to the mark with water. With small amounts of germanium practically all is found in the first distillate, but with quantities in the neighborhood of 25 micrograms a small amount is found in the second distillate.

Colorimetric Determination of Germanium. Prepare two standard germanium solutions as follows. Measure out two portions of germanium oxide solution containing, for example, 5 and 10 micrograms of germanium, and add 10 ml. of water, 1.5 ml. of 1 to 1 hydrochloric acid, and 2.00 ml. of 25 per cent sodium hydroxide solution. Neutralize the solutions carefully with 1 to 1 hydrochloric acid (phenolphthalein as indicator) and dilute to 25 ml. In a similar manner prepare a blank solution for the color comparison by neutralizing 2.00 ml. of sodium hydroxide and diluting to 25 ml.

Take a 10-ml. aliquot of each standard, of the two germanium distillates, and of the blank. Make each solution basic with a drop of 25 per cent sodium hydroxide and then acidify with 0.10 ml. of 1 to 1 acetic acid. Add 10.0 ml. of ammonium molybdate–ferrous ammonium sulfate reagent, mix, and dilute to 25 ml. with water. After 15 minutes compare the germanium distillates and the standards against the blank solution in a filter photometer, using a red filter. The standard series method of comparison may also be used. The color intensity of the solutions increases slowly on standing and the readings should, therefore, be made after the period of standing specified. Although germanium in appreciable amounts is unlikely to occur in the reagents, it is nevertheless advisable to run a blank through the procedure, especially since any contamination by silica will then be revealed.

Table I. Determination of Germanium in Silicate Rocks

	Sample	Germanium Present[a] %	Germanium Found %	Error %
1	Granite[b]	0.0005	0.0005	0.0000
2	Granite	0.0010	0.0009	−0.0001
3	Granite	0.0030	0.0029	−0.0001
4	Granite	0.0058	0.0050	−0.0008
5	Granite[c]	0.00026	0.00028	+0.00002
6	Granite[d]	0.00026	0.00030	+0.00004
7	Diabase[e]	0.0008	0.0007	−0.0001
8	Diabase	0.0014	0.0013	−0.0001
9	Diabase	0.0056	0.0052	−0.0004

[a] Sum of germanium originally present in samples as determined by method described (0.00026% in granite, 0.00014% in diabase) and that added.
[b] Percentage composition: SiO_2, 76.8; Al_2O_3, 13.2; Fe_2O_3, 0.3; FeO, 0.4; MgO, 0.2; CaO, 0.7; Na_2O, 3.8; K_2O, 4.5; TiO_2, 0.08; P_2O_5, 0.02; MnO, 0.05.
[c] Contained 0.05% Cl.
[d] Contained 0.01% As.
[e] Percentage composition: SiO_2, 52.7; Al_2O_3, 14.5; Fe_2O_3, 7.4; FeO, 5.6; MgO, 3.7; CaO, 8.0; Na_2O, 3.2; K_2O, 1.1; TiO_2, 1.8; P_2O_5, 0.25; MnO, 0.15; S, 0.02.

Acknowledgments

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A Volumetric Method for Determining Tin

Based on the Formation of a Dioxalatothiometastannate

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Wheeler (*1*) recognized that stannic tin in oxalic acid solution forms a fairly stable compound with sulfur when hydrogen sulfide is passed into such a solution. He made use of this fact in devising a volumetric method of determining tin in bronze by titrating the sulfur with iodine.

The tin was separated from other metals by addition of phosphoric acid, the precipitate was dissolved in concentrated sulfuric acid, and the solution was neutralized with ammonia, using methyl orange indicator. Six or 7 grams of oxalic acid were added per 0.2 gram of tin and the solution was boiled until clear. The hot solution, 100 ml. in volume, was saturated with hydrogen sulfide, diluted to 250 ml., and cooled to room temperature, and a stream of air was bubbled through it 15 to 20 minutes to remove the excess of hydrogen sulfide. A slight excess of standard iodine was added and back-titrated with thiosulfate.

Difficulties in the Use of the Existing Method

In attempting to verify Wheeler's work, it was found that conditions had to be adjusted so carefully in order to obtain the theoretical relationship between tin and sulfur that the method was not practical. It was necessary to pass hydrogen sulfide into the solution for at least 20 minutes at a rate of 1 liter per minute to ensure complete absorption. Using air, nitrogen, and carbon dioxide as agents to sweep the excess hydrogen sulfide out of the solution, the results obtained were dependent upon the gas used and the time of its passage. The passage of air at a rate of 1.5 to 2 liters per minute for more than 15 minutes always gave low results, whereas nitrogen and carbon dioxide at the same rate gave low results when passed for much over 20 minutes. At the end of the passage of the gas, there was still an odor of hydrogen sulfide.

Since it was difficult to regulate the passage of gas in the same manner for each determination, it was likewise difficult to compare the times of passage on a quantitative basis.

Table I. Effect of Passing Nitrogen on the Sulfur-Tin Ratio

Time *Min.*	S/Sn Ratio
0	No result, excess H_2S
10	1.000
30	0.990
50	0.987
80	0.973
110	0.969

For this reason, nitrogen gas at room temperature was passed continuously through a solution, and samples for analysis were removed at regular intervals. Table I shows the results obtained. If the nitrogen was replaced by air, lower results were obtained, owing to partial oxidation of the sulfur. The data show that the dioxalatothiometastannic acid formed according to Wheeler's directions is relatively unstable.

Solutions of oxalatostannic acid which were allowed to stand for some time in the cold were found to become somewhat opalescent because of hydrolysis of the tin. These solutions always gave low sulfur-tin ratios, indicating that the absorption of hydrogen sulfide might be a measure of the unhydrolyzed tin present. To verify this the following experiments were performed:

Some of the oxalatostannic acid solution was refluxed at boiling temperature for 3 hours, and its opalescent appearance indicated that rather extensive hydrolysis had taken place. The sulfur-tin ratio after the hydrogen sulfide treatment was found to be 0.104. Some of this same hydrolyzed solution was evaporated to fumes of sulfuric acid, and then the procedure as outlined by Wheeler (*1*) was followed. The sulfur-tin ratio of this solution was 1.000, indicating that the hydrolyzed tin had been rendered soluble. A second sample was treated in the same manner up through the addition of the oxalic acid. After the solution had been heated to dissolve the stannic hydroxide, it was allowed to cool down to room temperature and stand overnight before treatment with hydrogen sulfide. Although the solution appeared perfectly clear, the sulfur-tin ratio was 0.640, indicating that heating even for this short time caused appreciable hydrolysis.

Table II. Determination of Tin Using Potassium Sulfide

pH after Addition of $K_2C_2O_4$	Final pH after CO_2 Passage	Tin Taken *Gram*	Tin Found *Gram*
2.2	3.0	0.1301	0.1301
2.5	...	0.1416	0.1414
2.5	3.0	0.1328	0.1330
2.6	3.3	0.1372	0.1370
3.3	3.4	0.1312	0.1315

The New Method

In the first place, the tin present should be converted to a form in which it is less readily hydrolyzed than the oxalatostannic acid. It was shown in studies on the complex oxalatostannates (*2*) that the potassium salt was very stable and could be recrystallized from hot solutions. Moreover, dipotassium dioxalatothiometastannate is very much more stable (*3*) than the dioxalatothiometastannic acid formed according to Wheeler's directions. Instability of the potassium salt in solution is caused by hydrolysis and can be prevented by employing a large excess of potassium oxalate. Neither sodium oxalate nor ammonium oxalate is suitable for this purpose because they are not sufficiently soluble.

Accordingly, it was decided to convert the oxalatostannic acid to the potassium salt by the use of neutral potassium oxalate to increase the pH. The proper pH was obtained from a study of the oxalatostannates and the dioxalatothiometastannates. The upper limit is a pH of 5, since a titration of potassium oxalatostannate (*2*) showed that the hydrolysis of the tin takes place at this pH. The stable pH for dipotassium dioxalatothiometastannate is in the region 3.3 to 3.5. The reactions involved may be represented by the following equations:

$$K_6Sn_2(C_2O_4)_7 + 2H_2S \rightarrow 2K_2SnS(C_2O_4)_2 + 2H_2C_2O_4 + K_2C_2O_4$$

$$2K_2SnS(C_2O_4)_2 + 2I_2 + 2H_2C_2O_4 + K_2C_2O_4 \rightarrow K_6Sn_2(C_2O_4)_7 + 4HI + 2S$$

With these modifications, a sample of pure tin gave the following results:

Sn taken, 0.1630 gram
pH after addition of $K_2C_2O_4$, 3.37
Passage of H_2S at 2 liters per minute, 25 minutes
Passage of CO_2 at 2 liters per minute, 1 hour
Final pH, 3.23
Sn found, 0.1632 gram

The iodine was standardized against arsenious oxide.

Several other improvements in the process were made.

An appreciable length of time was required for the passage of hydrogen sulfide because the manner of introducing the gas through a tube did not afford very good contact with the solution. Furthermore, the use of the gas caused high localized concentrations at the gas-liquid interface, such that a very small amount of stannic sulfide was always deposited on the tube. In order to eliminate these difficulties, hydrogen sulfide was liberated internally by the addition of a solution of potassium sulfide. Carbon dioxide was bubbled through the solution to remove the excess hydrogen sulfide until a piece of lead acetate paper held in the escaping gases showed no blackening. The time of 1 hour used above was much greater than necessary, but it proved that dipotassium dioxalatothiometastannate in a large excess of potassium oxalate is very stable.

Since the potassium sulfide solution is strongly alkaline, the pH resulting from the addition of potassium oxalate was set at a lower value, such that the final pH after the addition of the sulfide solution was close to the desired value of 3.3. Addition of potassium sulfide in the cold caused too high local concentrations of sulfide ion, resulting in the precipitation of some stannic sulfide. This eventually went back into solution, but in some cases only after rather extensive boiling. The difficulty was eliminated by adding the potassium sulfide solution dropwise at a temperature of 60° with constant stirring. This temperature was found to be sufficiently high to give a satisfactory rate of reaction in forming the dioxalatothiometastannate.

Special Solutions

Potassium Sulfide. Dissolve 20 grams of potassium hydroxide in 100 ml. of water and saturate with hydrogen sulfide, keeping the solution cold. Keep this solution in an inert atmosphere, because thiosulfates and sulfites formed by oxidation are not removed in the procedure and will be oxidized by iodine. An inert atmosphere can be maintained by bubbling some carbon dioxide through the solution before stoppering the flask each time after use.

Wash Liquid. Dissolve 5 grams of oxalic acid in 1 liter of water and add potassium oxalate until a pH of 3 is attained.

Procedure

Dissolve about 0.15 gram of tin in 2 ml. of 18 *N* sulfuric acid and several milliliters of concentrated nitric acid in a 200-ml. electrolytic beaker. Evaporate the solution to fumes of sulfuric acid to expel the nitric acid. Too large a quantity of sulfuric acid cannot be used because too much potassium oxalate is required to raise the pH to the desired point. Cool, and add 2 to 3 grams of solid potassium oxalate to the concentrated acid solution. Wash the sides of the beaker and the cover glass with about 20 ml. of the wash liquid. At this point and during the subsequent addition of potassium oxalate, all the solid will not dissolve because much potassium bioxalate is formed. Continue adding solid potassium oxalate until a pH of 2.5 is attained (a range of 2.2 to 2.8 is permissible). A glass electrode was used in this work, but in later work the following indicators were found to be satisfactory: thymol blue, add potassium oxalate until all the red tint disappears; xylenol blue, add potassium oxalate until the pink tint disappears; metacresol purple, add potassium oxalate until the red tint disappears. After a little practice, no trouble was encountered in adjusting the pH to the proper range.

Heat the solution, diluted to about 60 ml., to 60°, and, while stirring mechanically, add dropwise 3.5 ml. of the potassium sulfide solution. The beaker should be covered with a split watch glass because considerable gas is evolved. Continue heating at this temperature for 5 minutes to ensure decomposition of the excess potassium sulfide and to ensure complete reaction with the tin. Add a few drops more of the potassium sulfide solution, continue heating for 1 minute, and cool the solution to room temperature under a stream of cold water. Neither the temperature nor the time of heating was found to be critical (temperatures from 55° to 80° C. and times ranging from 3 to 10 minutes were satisfactory). At this point the volume should be sufficiently great (about 120 ml.) to prevent the precipitation of any solid material which would occlude some of the sulfur complex. Using mechanical stirring, pass in carbon dioxide until the escaping gas gives no test for hydrogen sulfide with lead acetate paper. (The

dry tank carbon dioxide causes deposition of solid material by evaporation in the entry tube and may clog it. This difficulty is eliminated by bubbling the carbon dioxide through water before passing it into the solution.) This usually requires about 30 minutes but much longer if stirring is omitted. Titrate the solution with 0.1 *N* iodine solution to a faint yellow color (about 1 ml. past the end point), add starch solution, and back-titrate with standard thiosulfate. One milliliter of 0.1 *N* iodine is equivalent to 0.005935 gram of tin.

A moderate variation in pH is permissible. Because of the increased solubility of hydrogen sulfide, the addition of potassium sulfide at higher pH values gives local concentrations sufficient to precipitate stannic sulfide. This can be dissolved by long boiling, but such a procedure is troublesome. High pH values also increase the difficulty of removing the excess of hydrogen sulfide.

Application to Alloys Containing Tin

In the case of pure tin, it has been demonstrated that this method of analysis gives accurate results. In alloys the problem becomes one of a separation from those substances which would interfere with the titration. Table III shows the results obtained on National Bureau of Standards samples.

Table III. Determination of Tin in Alloys

Bur. of Standards Sample No.	Tin Present %	pH after Addition of $K_2C_2O_4$	Tin Found %
54a, tin-base bearing metal	88.61	2.50	88.68
		2.57	88.73
		2.61	88.48
53, lead-base bearing metal	10.91	2.43	10.87
		2.38	10.90
		2.43	10.93
52, cast bronze	7.88	2.80	7.87
		2.61	7.88
		2.65	7.88
37b, sheet brass	1.00	2.45	1.00
		2.45	1.00

Standrd Sample 54a. This is a high-tin bearing metal with small quantities of antimony and copper. A sample of about 0.15 gram was weighed into a 150-ml. beaker and dissolved with 2 ml. of 18 *N* sulfuric acid and 1 ml. of concentrated nitric acid. The solution was evaporated to fumes of sulfuric acid and treated according to the procedure given above. The addition of the potassium sulfide caused precipitation of the antimony and copper, and the solution was filtered hot into a 250-ml. wide-mouthed Erlenmeyer flask after the 5-minute heating period. It was found best to use suction and a small funnel fitted with a Witte plate on which was placed a filter paper covered with asbestos. The beaker was washed out with about 50 ml. of hot wash solution. With constant stirring, the filtered solution was heated to 60°, 0.5 ml. of potassium sulfide solution added, and the heating continued for another minute. The solution was cooled to room temperature, carbon dioxide was passed through, and it was titrated as already described.

For passing the carbon dioxide through the solution, it was found convenient to attach a 1-mm. capillary tube on the side (near the bottom) of the Erlenmeyer flask. Small quantities of antimony or copper sulfides which may have passed through the filter did not interfere with the titration.

Standard Sample 53. This sample contains about 79 per cent lead, 10 per cent antimony, and 10.91 per cent tin. Attempts to make all the separations in one operation or to separate the lead as sulfate, chloride, or oxalate gave low results. The alloy was dissolved in nitric acid, and the tin and antimony were precipitated as insoluble acids. The precipitate was filtered off, and washed with hot water, and the whole filter paper was decomposed by sulfuric and nitric acids to recover the oxides. When the antimony was allowed to remain in the pentavalent form, the subsequent treatment always left a somewhat turbid solution, and the addition of the potassium sulfide gave a very gelatinous antimony precipitate which retained appreciable quantities of tin. The antimony was, therefore, reduced to the trivalent state by adding some hydroxylamine sulfate to the concentrated sulfuric acid solution and boiling until the excess hydroxylamine sulfate was decomposed. Potassium chloride added with the potassium oxalate aided in keeping the antimony in solution.

A 1-gram sample was dissolved in 5 ml. of concentrated nitric acid in a 200-ml. electrolytic beaker. After the reaction had ceased, 30 ml. of water and some filter paper pulp were added and the solution was allowed to digest for 30 minutes, filtered hot through a small Whatman No. 42 filter, and washed several times with hot water. The filter paper was transferred back to the beaker, and 2 ml. of concentrated sulfuric acid and 10 ml. of concentrated nitric acid were added. The beaker was covered with a watch glass and heated to destroy the filter paper. More nitric acid was added as needed, but no more sulfuric acid. The solution was then evaporated to sulfuric fumes, 0.5 gram of hydroxylamine sulfate or hydrazine sulfate was added, and the solution was heated strongly until all the reducing agent was decomposed. This point can be recognized as that when all the evolution of gas ceases.

After the solution had cooled, a few grams of solid potassium oxalate were added, then 20 ml. of water and 5 grams of potassium chloride. The procedure from here was exactly the same as for the tin-base bearing metal.

Standard Samples 52 and 37b. Both samples contain large amounts of copper, and the same procedure applied to both. Attempts to remove copper as a sulfide were unsuccessful. The most satisfactory procedure was a preliminary separation of the tin as metastannic acid. (In samples which contain large quantities of iron, the precipitation of metastannic acid is incomplete. Preliminary experiments indicate that this difficulty may be eliminated by adding about 2 grams of ammonium sulfate to the diluted solution of the alloy followed by boiling and digesting, or by reducing all the iron to the ferrous state by adding some sodium bisulfite.) The metastannic acid was filtered off and treated as in the lead-base bearing metal except that no hydroxylamine sulfate or potassium chloride was added. For the bronze a 1.5 gram sample was used, and for the sheet brass a 5-gram sample was taken.

Discussion

The method described is rapid, and the interferences are few. It has an advantage over the volumetric method of reducing tin to stannous chloride and oxidizing it with iodine in that special precautions to maintain an inert atmosphere are unnecessary. In the method described here, the tin is at all times in the stannic state, its most stable condition. Hydrogen sulfide gas may, of course, be used instead of potassium sulfide, but the time required for the reaction will be greater and more potassium oxalate will be needed.

Summary

A solution of stannic tin is converted to potassium oxalatostannate, $K_6Sn_2(C_2O_4)_7$, by adding potassium oxalate to a definite pH (a range of 2.2 to 2.8 is satisfactory if potassium sulfide is subsequently used, or approximately 3.3 in case hydrogen sulfide gas is used). The salt is then converted to dipotassium dioxalatothiometastannate, $K_2SnS(C_2O_4)_2$, by the addition of potassium sulfide or hydrogen sulfide. The excess of hydrogen sulfide is removed by a current of carbon dioxide, and the sulfur in the complex is titrated by standard iodine.

In the presence of considerable amounts of other metals, the tin is first separated as metastannic acid.

An inert atmosphere is required only to remove the excess of hydrogen sulfide.

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Presented before the Division of Analytical and Micro Chemistry at the 103rd Meeting of the American Chemical Society, Memphis, Tenn. From a dissertation submitted by T. Y. Toribara (Florence Fenwick Memorial Fellow, 1939–42) in partial fulfillment of the requirements for the degree of doctor of philosophy at the University of Michigan, February, 1942.

Determination of Aluminum in Manganese and Aluminum Bronzes

Using the Mercury Cathode Cell

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BECAUSE of the increased use of manganese and aluminum bronzes, it was necessary to find a newer and more rapid and accurate method for the determination of aluminum in these alloys. This paper describes a procedure that fills the above requirements. After a thorough search of the literature, it was decided that the mercury cathode cell (*3*, *6*, *8*, *9*) would be best suited for the rapid and complete separation of iron, manganese, lead, zinc, nickel, and copper from aluminum.

The composition of typical manganese and aluminum bronzes is given in Table I.

TABLE I. COMPOSITION OF BRONZES

	Manganese Bronze, Bureau of Standards, No. 62a, %	Aluminum Bronze, Heat 3794, %
Cu	61.50	91.87
Zn	33.05	...
Mn	1.50	...
Fe	1.04	0.89
Al	0.92	6.41
Sn	0.84	0.40
Ni	0.61	...
Pb	0.50	0.27

Experimental

The procedures of Shubin (*11*) and Gerke and Lyubomirskaya (*5*) were tried but each had its limitations: mainly, that two steps were necessary for complete separation of iron from aluminum (*5*), the rather large error (*11*), and the use of sulfuric acid as the indifferent electrolyte. For alloys of high manganese content (1 per cent or higher), the mixed hydrous oxides (iron, manganese, and aluminum) which were formed on neutralization with ammonium hydroxide failed to redissolve on acidification with sulfuric acid.

Consequently, a new conducting medium was sought. Acetic acid gave very good results. The hydrous oxides readily dissolved. With an acetic acid medium the electrolytic separation of aluminum from iron, manganese, lead, zinc, nickel, and copper was found to be complete and quantitative. Acetic acid foams badly, but this can be overcome by adding a defoaming agent such as octyl alcohol (*10*) or Antifoam L F X (du Pont).

To test the accuracy of the procedure, samples of National Bureau of Standards Standard Sample 62a, manganese bronze (0.92 per cent aluminum), were run. The average accuracy was 0.005 per cent.

Apparatus and Reagents

APPARATUS. Figure 1 shows the complete setup, which consists of a Melaven mercury cathode cell (*9*) and a Fisher electroanalyzer to supply the current and stirring action. The anode, which is of the platinum stirring type, is attached to the motor of the electroanalyzer.

REAGENTS. Ammonium chloride solution (2 per cent) was prepared by mixing 15 ml. of hydrochloric acid (sp. gr. 1.19) with 250 ml. of water, adding 2 to 3 drops of methyl red, neutralizing with ammonium hydroxide (sp. gr. 0.90), and diluting to 500 ml. with water.

The mercury used was purified by shaking with a few milliliters of water and mercurous nitrate crystals (*4*).

Octyl alcohol (*10*) or Antifoam L F X worked exceedingly well as defoaming agents.

Procedures

I. Weigh out a 1-gram sample of bronze and transfer to a 250-ml. beaker, add 20 ml. of nitric acid (sp. gr. 1.42), and heat until the bronze has dissolved and the oxides of nitrogen are driven off. Add 100 ml. of hot water, bring to a boil, and allow to digest on a hot plate for 1 hour or longer, keeping the temperature just below the boiling point. Filter off the metastannic acid (double thickness of No. 42 Whatman paper) and wash thoroughly with boiling hot water. Discard the precipitate. To the filtrate add 10 ml. of sulfuric acid (sp. gr. 1.84) and evaporate until copious fumes of sulfuric acid are evolved. Complete removal of the nitric acid is essential. Allow to cool, add 50 ml. of water, heat to boiling to dissolve all soluble salts, and filter off the lead sulfate formed through a Gooch crucible. Wash thoroughly and transfer the filtrate to a 600-ml. beaker. Neutralize the acid present with sodium hydroxide solution (10 per cent) and add 5 ml. in excess. Bring the solution back to acidity with acetic acid (1 to 3), heat to boiling to complete the reaction, and add 5 ml. of glacial acetic acid in excess. Cool, dilute to 350 ml., and add 2 to 3 drops of defoaming agent.

Transfer to the Melaven mercury cathode cell and electrolyze at a current of 1 to 2 amperes for 3 hours. To test for the complete deposition of copper and iron, use potassium ferrocyanide and potassium ferricyanide, respectively, using fine-bore capillary tubes to take the spot tests (*7*). When the electrolysis is complete, lower the leveling bulb until the mercury reaches the upper end of the stopcock bore, turn the stopcock through 180 degrees, and allow the electrolyte to drain off into a 600-ml. beaker.

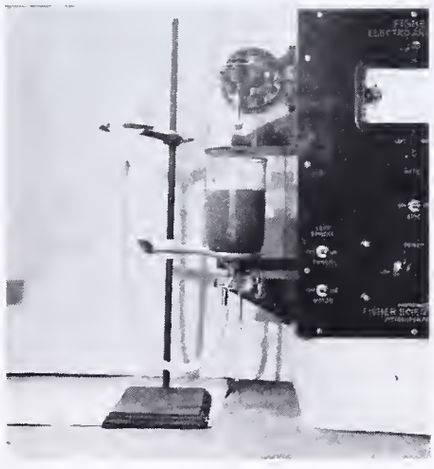

FIGURE 1. APPARATUS

Wash out the electrolytic cell with water. Filter off the precipitated manganese dioxide (No. 40 Whatman paper) and wash thoroughly with warm water. To the filtrate add 10 grams of ammonium chloride, 2 to 3 drops of methyl red, and ammonium hydroxide (1 to 2) until the solution is distinctly yellow. Heat the solution to boiling and boil gently for 1 to 2 minutes, filter off the aluminum hydroxide (No. 40 Whatman paper), and wash the precipitate 2 to 3 times with hot ammonium chloride solution (2 per cent). Discard the filtrate and dissolve the precipitate in 40 ml. of hot hydrochloric acid (1 to 1). Wash the filter thoroughly and dilute to 100 ml. Add methyl red and neutralize with ammonium hydroxide (1 to 2) as before. Heat to boiling, filter off the aluminum hydroxide, and wash with hot ammonium chloride solution. Place the paper and the precipitate in a platinum crucible, char the paper, and burn off the carbon. Then ignite the oxide in a furnace or over a blast lamp at a temperature of 1200° C. Cool and weigh as Al_2O_3.

II. Weigh out a 1-gram sample and determine the tin, lead, and copper stepwise by standard methods (*1*). To the filtrate from the electrolytic copper determination add sodium hydroxide and acetic acid as in procedure I. After neutralization and acidi-

fication with acetic acid, place the solution in the mercury cathode cell and proceed with the determination of aluminum as given in procedure I. If preferred, 8-hydroxyquinoline may be used instead of ammonium hydroxide as a reagent for the precipitation of aluminum.

Discussion

Aluminum in a Bureau of Standards Standard Sample No. 62a (manganese bronze) was determined by the above procedure. A few comparative results are given:

Provisional Analysis	Procedure of This Paper
%	%
0.92	0.928
	0.925
	0.922
	Av. 0.925

The use of an acetic acid medium eliminates the two-step procedure of Gerke and Lyubomirskaya (*5*) for the separation of iron and also increases the accuracy of the determination. The maximum accuracy that Shubin (*11*), using a sulfuric acid medium, could obtain was 0.07 per cent, whereas with the acetic acid medium the minimum accuracy is 0.008 per cent. The procedure is far superior to the old sodium hydroxide separation, whose main disadvantage was that the sodium hydroxide dissolved to a slight extent the iron and manganese hydroxides (*2*).

Acknowledgments

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Testing Writing Inks

With Rubber Fountain Pen Sacs

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Many commercial writing inks hasten deterioration of soft-rubber fountain pen parts. Soft rubber affects stability of some writing inks, particularly permanent inks. Methods of testing are proposed.

THE present necessity for conserving rubber and extending the life of existing rubber articles prompts the authors to report their observations of the action of writing inks on soft-rubber fountain pen parts, and to propose a comparative test.

FIGURE 1. SACS FROM SCRAP BOX

There is a dearth of published data on this subject, though Waters (*5*) stated, "The general use of the self-filling pen, which has almost supplanted the older kind with the medicine dropper, seems to be good evidence that the rubber bags are not damaged by ink. The failure of a bag in an old pen is no proof that the ink is to blame. Rubber does not last indefinitely."

These latter two statements are true. It is well known that oxygen acts upon soft rubber, causing loss of tensile strength and eventual hardening. However, many substances have a profound effect on the rate of such aging (*3*). Casual observations over limited periods of time may reveal no serious action of inks on rubber sacs, but examination of the sacs customarily dismantled from pens returned for repair always shows some hardened and broken from causes other than mere time in service. Figure 1 shows a representative handful of sacs taken from a scrap box, with some of the worst specimens moved into the foreground. The condition of these sacs is duplicated by sacs subjected to the authors' aging test. Incidentally, the effect of stress on aging is also illustrated by the sacs that became twisted in the pen barrel and hardened prematurely.

The authors subjected sacs, after immersion in inks, to oxygen or air at elevated temperatures, the recognized methods of accelerated testing (*4*), and found that many writing inks greatly hastened and some retarded the aging of the sacs.

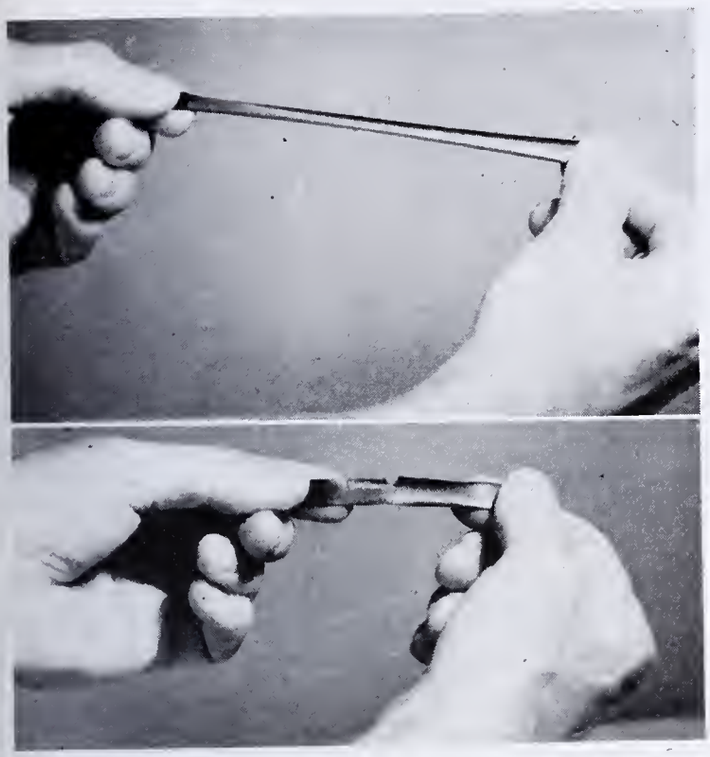

FIGURE 2. EFFECT OF WRITING INKS ON SACS, AFTER PARALLEL SOAKING AND ACCELERATED AGING

New sacs were cut in two lengthwise, keeping one half of each as a control. The test halves were immersed in the writing inks to be tested and the controls immersed in distilled water. To eliminate the effect of possible variation in sacs, two or three halves were put in each sample of ink.

To study simultaneously the effect of rubber on inks, particularly permanent inks, immersion was in 25 ml. of liquid for 2 weeks in 25 × 150 mm. test tubes loosely stoppered with nonabsorbent cotton, in accordance (except for addition of the sacs) with the stability test in the Federal Specification for Writing Inks (*1*).

After immersion all half-sacs were removed, blotted dry, clipped on racks, and placed in a Bierer-Davis oxygen bomb (*4*) under 21.1 kg. per sq. cm. (300 pounds per square inch) pressure at 70° C. The Geer oven test (*4*) has also been used, circulating air at 70° C.

Moderate flexing with the fingers revealed the progress of deterioration at periodic inspections. All sacs became soft and tore readily, finally becoming hard and brittle. There was a decided difference in the time required to reach each stage exhibited by sacs immersed in different inks. The bomb-aging was continued until the controls hardened, noting the condition of test sacs. Alternatively, the time required for each sac to reach the softened or hardened state may be taken as a measure of the relative service life of sacs when used with the different inks. As a variant of this test the authors are studying the action on sacs and inks of alternate immersion and bomb-aging; and are also studying sacs in combination with similar tests on pyroxylin plastic of which fountain pen holders and other parts are made.

Figures 2 and 3 show typical results on sacs aged together for the same length of time after soaking in different brands of writing inks. In Figure 2 (lower) the sac is tearing under very slight tension, while the upper sac shows no failure under vigorous flexing. Figure 3 shows the condition of sacs after testing with a group of commercial permanent blue-black writing inks.

It was found that contact with the rubber sacs affected the stability of some permanent writing inks, causing precipitation.

After sacs were removed, the 25-ml. portions of ink were shaken, 10 ml. of each were centrifuged, the precipitate was washed free of solubles and whirled again, and sediment volume was recorded.

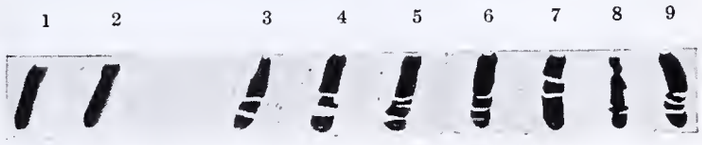

FIGURE 3. EFFECT OF WRITING INKS ON SACS

This is the method previously suggested (*2*) for determining the volume of precipitate formed by writing fluids in stability or other tests, instead of the qualitative comparative estimate called for in government specifications (*1*). A refinement is the use of "Constable protein" tubes shown in Figure 4. These tubes enable a much more precise evaluation of ink sediment; they hold 10 ml. and the lower portion is graduated in 0.005-ml. divisions to 0.1 ml.

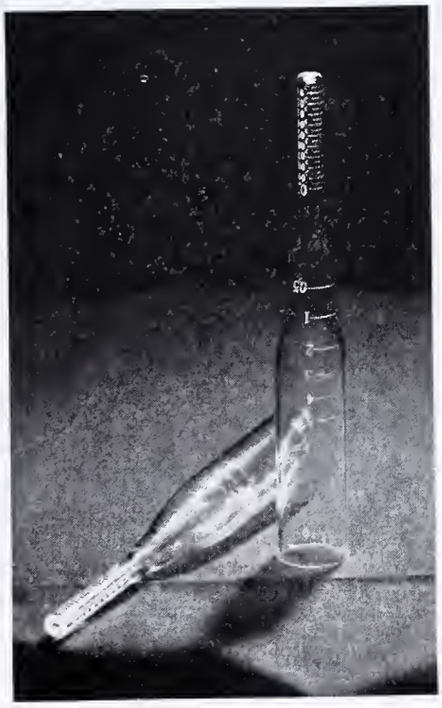

FIGURE 4. CONSTABLE PROTEIN TUBES

These test methods have been used in this laboratory over the past 6 years in connection with writing ink formulation and comparison.

Acknowledgments

The authors wish to express their thanks to the W. A. Sheaffer Pen Company for permission to publish these results and to John E. Hauck and David Jamerson for assistance in the experimental work.

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Colorimetric Determination of Small Amounts of Metaphosphate and Pyrophosphate

Determination of Iron with Thiocyanate in the Presence of Metaphosphate and Pyrophosphate

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SINCE small amounts of metaphosphate and pyrophosphate are added to water in order to control scale formation and corrosion it is desirable to have some method for the determination of 0.01 to 5 p. p. m. of these substances if suitable concentrations are to be maintained in all parts of treated systems. The method employed by the Hall Laboratories (*3*) depends on the determination of the increase in orthophosphate content on reversion of metaphosphate after boiling for 4 hours. This method is inaccurate in those cases where the initial orthophosphate content is abnormally high.

Pyrophosphate has been shown by Woods and Mellon (*5*) and others to interfere seriously with the determination of iron by the thiocyanate method, owing to the formation of complexes with the iron. At low pH metaphosphate has a greater tendency than pyrophosphate to form such complexes. These facts are made the basis of a colorimetric method for the determination of small amounts of pyrophosphate and metaphosphate in water. The decrease in the ferric thiocyanate color when a fixed amount of iron is present is a measure of the amount of metaphosphate and pyrophosphate present. This is the principle employed by Foster (*2*) for the colorimetric estimation of fluoride.

It was also found that in the presence of a large excess of aluminum ion the iron is released from its complexes with metaphosphate and pyrophosphate. Iron can therefore be accurately determined with thiocyanate in the presence of relatively high concentrations of metaphosphate and pyrophosphate if an aluminum nitrate–nitric acid reagent is employed.

Materials

Commercial glassy sodium metaphosphate ("unadjusted Calgon flakes") and samples prepared by fusing C. P. sodium dihydrogen phosphate were used with similar results. Sodium pyrophosphate was prepared by fusing C. P. disodium hydrogen phosphate.

The 20 per cent ammonium thiocyanate solution used was freed of iron by three extractions with 5 to 2 isoamyl alcohol–ethyl ether solution. The standard iron solution was prepared from ferrous ammonium sulfate.

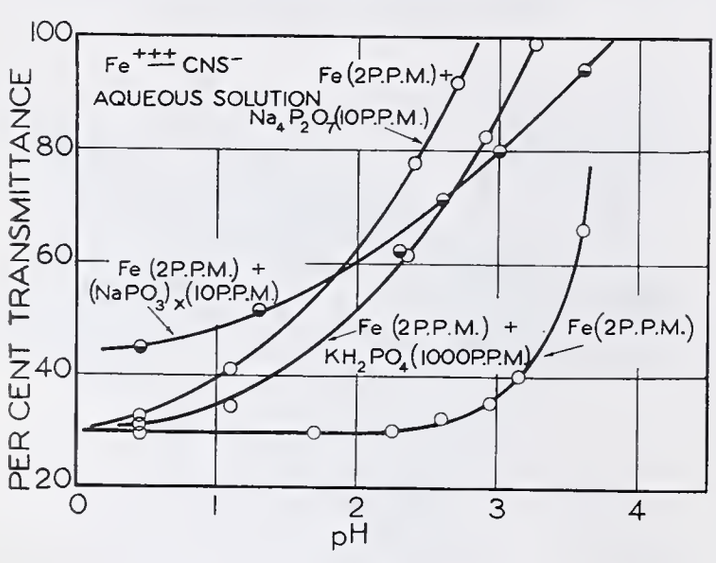

FIGURE 1. EFFECT OF METAPHOSPHATE, PYROPHOSPHATE, AND ORTHOPHOSPHATE ON FERRIC THIOCYANATE COLOR IN AQUEOUS SOLUTION AS A FUNCTION OF pH

Iron concentration 2.0 p. p. m.

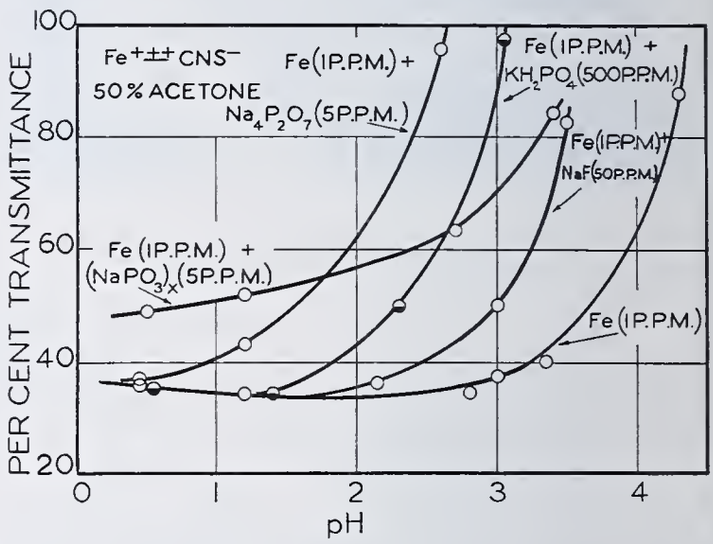

FIGURE 2. EFFECT OF METAPHOSPHATE, PYROPHOSPHATE, ORTHOPHOSPHATE, AND FLUORIDE ON FERRIC THIOCYANATE COLOR IN 50 PER CENT ACETONE SOLUTION AS A FUNCTION OF THE pH

Iron concentration 1.0 p. p. m.

Aluminum Nitrate Reagent. To 200 grams of aluminum nitrate nonahydrate dissolved in 200 ml. of water were added 15 ml. of 20 per cent ammonium thiocyanate. The solution was extracted three times with 75-ml. portions of 5 to 2 isoamyl alcohol–ether. Concentrated nitric acid (200 ml.) was added and the solution warmed until the ammonium thiocyanate was completely decomposed. The solution was cooled and diluted to 500 ml.

Apparatus

An industrial model glass electrode was used to determine the pH of the solutions. The probable accuracy was ±0.1 unit. The per cent transmittance was determined using a Coleman regional spectrophotometer with a 30-mμ slit. The wave length was set at 470 mμ. The values were reproducible to within ±0.5 per cent.

Procedure

Three variations of the thiocyanate method for the determination of iron were employed.

I. Iron standard plus whatever quantities of metaphosphate, pyrophosphate, orthophosphate, or fluoride were to be tested, and nitric acid required to give the desired pH were diluted to 90 ml., 5 ml. of 20 per cent ammonium thiocyanate were added, and the solution was diluted to exactly 100 ml. After 5 to 10 minutes the transmittance and pH were determined.

II. Iron standard, metaphosphate (or other added substance), and nitric acid were diluted with 50 ml. of acetone before addition of 5 ml. of ammonium thiocyanate. The solution was diluted to 100 ml. and the pH and transmittance were determined.

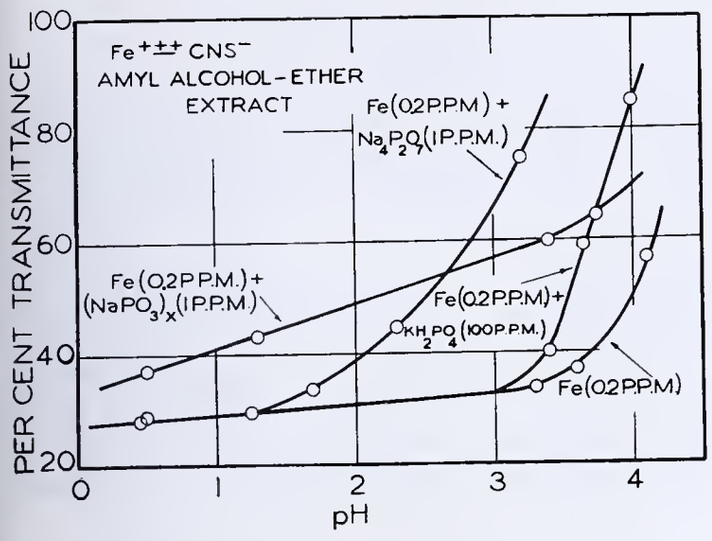

FIGURE 3. EFFECT OF METAPHOSPHATE, PYROPHOSPHATE, AND ORTHOPHOSPHATE ON THE FERRIC THIOCYANATE COLOR IN ISOAMYL ALCOHOL–ETHER SOLUTION AS A FUNCTION OF pH OF AQUEOUS LAYER
Iron concentration 0.20 p. p. m.

III. In this method, the aqueous solution prepared as in Method I was shaken with 15 ml. of 5 to 2 isoamyl alcohol–ether solution. Exactly 5 ml. of the isoamyl alcohol–ether layer were removed, 0.5 ml. of acetone was added to clear up any turbidity, and this solution was used for the transmittance measurement. The pH of the aqueous layer was determined.

The amount of iron chosen in each method was sufficient to give a transmittance of about 30 per cent in the absence of interfering ions. The amounts of iron, metaphosphate, pyrophosphate, etc., added were those required to give the concentrations indicated in the figures after dilution to 100 ml.

Results

In order to establish the conditions for maximum decrease in the ferric thiocyanate color by metaphosphate and pyrophosphate, the transmittance was determined for given amounts of iron and metaphosphate or pyrophosphate at different pH values. For comparison the effect of pH on the fading of the ferric thiocyanate color by orthophosphate and fluoride was also determined. The results are given in Figures 1, 2, and 3 for the three different modifications of the thiocyanate method. The curve for 100 p. p. m. of sodium fluoride was found to be identical within the experimental error with the curve for 10 p. p. m. of sodium pyrophosphate (Figure 1). The points for 10 p. p. m. of sodium fluoride are close to those for 100 p. p. m. of potassium dihydrogen phosphate in Figure 3.

The decrease in the ferric thiocyanate color by metaphosphate is influenced by the pH, but not to the same extent as the decrease due to pyrophosphate, fluoride, and orthophosphate. In all cases the amount of decrease is greater at higher pH values. If the decrease in color is due to the combination of ferric ion with some substance that is also in equilibrium with the hydrogen ion, then the reduction in concentration of the effective ion in solutions of low pH will be related to the ionization constant of the corresponding acid. The effect of pH on the decrease in ferric thiocyanate color should increase in the order of decreasing strength of the acids. This is clearly shown in Figure 3.

The steep slopes of the curves emphasize the necessity for close control of the pH in all methods based on the fading of the ferric thiocyanate color. An error of 0.3 pH unit at a pH of 3 can cause a 50 per cent error in the determination of pyrophosphate (Figures 3 and 4).

In comparing the results in Figures 1, 2, and 3 it should be kept in mind that although the $Fe/NaPO_3$, $Fe/Na_4P_2O_7$, etc., ratios are kept constant, the fading effect of different substances does not change in the same way with concentration; so that the different relative positions of the curves is a combined effect of the method and the concentration employed. [Calculations made on the results reported in Figure 6 indicate that at a pH of 3 there is one ferric ion and one metaphosphate ion (or one dimeric or trimeric metaphosphate ion) in the complex, and two ferric ions to each pyrophosphate ion in its corresponding complex.]

Other reagents for the determination of iron which depend on the formation of a complex with the ferric ion are also affected by metaphosphate and pyrophosphate. For example, the color of ferron-iron reagent used by Fahey (*1*) for the determination of fluoride is also faded by metaphosphate.

INTERFERING IONS. It is to be expected that the fading of the ferric thiocyanate color due to metaphosphate would be decreased by the presence of any ion which would form a complex with metaphosphate. Table I gives values for the percentage decrease in transmission produced by various concentrations of ions (commonly found in natural waters) in solutions containing 0, 1, and 5 p. p. m. of sodium metaphosphate. The results, calculated by the formula given in Table I and reported in the last two columns of the table, represent the decrease in sensitivity in the method caused by the interfering ion.

TABLE I. EFFECT OF FOREIGN IONS

[Relative decrease in per cent transmittance $= \frac{(I_1 - I_2)100}{(I_1 - I_3)}$, where I_1 is the transmittance reading in the presence of $NaPO_3$, I_2 is the transmittance reading in the presence of $NaPO_3$ and the foreign ion, and I_3 is the transmittance reading for 0.2 p. p. m. of Fe in the absence of $NaPO_3$. Extraction method, pH = 0.5]

Added Substance	Concentration, P. p. m.	Effect on Fe Determination: 0 p. p. m. of $NaPO_3$	1 p. p. m. of $NaPO_3$	5 p. p. m. of $NaPO_3$
		%	%	%
Ca^{++} (added as $CaCl_2$)	27	0	0	4.8
	136	0	5	20
	272	0	17	24
	1360	0	40	48
Mg^{++} (added as $MgCl_2$)	12	0	0	1
	60	0	0	6
	120	0	0	12
	600	0	12	23
NaCl	5000	0	..	−2
K_2SO_4	5000	0	..	−4
$Na_2B_4O_7.10H_2O$	500	0	..	6
Al^{+++} [added as $KAl(SO_4)_2$]	0.1	..	0	20
	0.25	..	15	69
	0.5	..	24	78
	1.0	..	37	84
	2.0	..	55	89

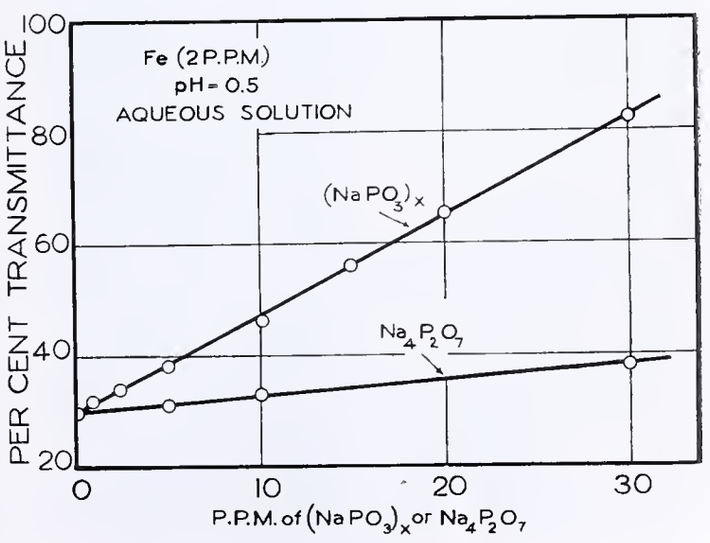

FIGURE 4. EFFECT OF METAPHOSPHATE AND PYROPHOSPHATE CONCENTRATION ON TRANSMITTANCE OF AQUEOUS SOLUTIONS CONTAINING 2.0 P. P. M. OF IRON
pH = 0.5

Monovalent positive ions have no effect on the fading of the iron color by metaphosphate, divalent positive ions have appreciable effects, and aluminum ion has a very pronounced effect. This is the order of affinity of the metaphosphate for the different types of ions.

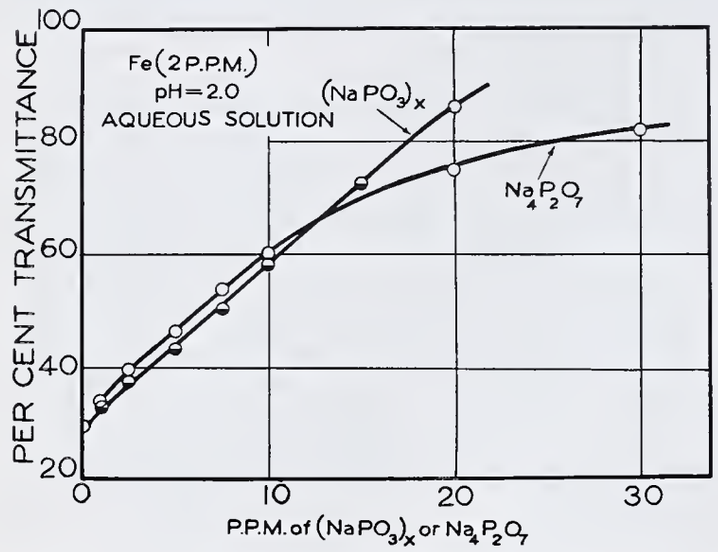

FIGURE 5. EFFECT OF METAPHOSPHATE AND PYROPHOSPHATE CONCENTRATION ON TRANSMITTANCE OF AQUEOUS SOLUTIONS CONTAINING 2.0 P. P. M. OF IRON

pH = 2.0

DETERMINATION OF IRON IN THE PRESENCE OF METAPHOSPHATE AND PYROPHOSPHATE. Since aluminum ion forms a stable complex with metaphosphate and pyrophosphate but does not interfere with the determination of iron it is possible to determine iron in the presence of these substances. If 5 ml. of aluminum nitrate reagent are used in place of an equal volume of 6 *N* nitric acid (Method I, pH = 0.5) the presence of 100 p. p. m. of metaphosphate does not introduce any error in the determination of 2 p. p. m. of iron. An error of but 7 per cent is caused by 500 p. p. m. of metaphosphate. With the extraction method 10 p. p. m. of metaphosphate do not interfere in the determination of 0.2 p. p. m. of iron when the aluminum nitrate reagent is used. Similar results are obtained in the presence of pyrophosphate.

Vanossi (*4*) has suggested the use of aluminum chloride or zirconium oxychloride to liberate iron from its complexes with metaphosphate, pyrophosphate, orthophosphate, and fluoride. Aluminum nitrate was found to be more convenient and effective than aluminum chloride.

CHOICE OF METHOD. The choice of method for the determination of metaphosphate or pyrophosphate is governed by the amounts present and possible interfering ions. For amounts of metaphosphate between 1 and 30 p. p. m. and in the absence of pyrophosphate, Method I using a pH of 0.5 (5 ml. of 6 *N* nitric acid per 100 ml.) is preferable. By using the low pH, difficulties of pH regulation are reduced. Figure 4 is a typical standard curve obtained in distilled water.

At a pH of 2 sodium metaphosphate and sodium pyrophosphate have about the same effect on the ferric thiocyanate color in the concentration range 1 to 15 p. p. m. (Figure 5). By making determinations at pH values of 0.5 and 2 it is possible to estimate metaphosphate and pyrophosphate separately when they are present together in the sample.

For metaphosphate contents between 0.05 and 2 p. p. m. and for pyrophosphate contents between 0.05 and 1 p. p. m. the extraction method is to be preferred. Figure 6 is a typical standard curve.

There is no advantage in using the acetone method in the analysis of water samples if the size of the sample is taken into consideration. While the acetone method is twice as sensitive as Method I for the determination of iron and metaphosphate, a smaller original sample must be used (40 ml. as compared to 90 ml.), so there is no over-all gain in sensitivity.

Since divalent and trivalent positive ions affect the sensitivity of the method it is not possible to use standards prepared from distilled water in the analysis of samples of appreciable mineral content. Standards should be prepared from metaphosphate- and pyrophosphate-free water of the same composition as that to be tested. Such water is usually available (water previous to treatment with the conditioning agent) or can be prepared by boiling an acid solution of treated water for several hours. (As shown by Figures 1, 2, and 3, orthophosphate does not seriously interfere.)

The aluminum-ion concentration should not be greater than 0.1 p. p. m.

Suggested Method for 0.05 to 2 P. P. M. of Sodium Metaphosphate or 0.05 to 1 P. P. M. of Sodium Pyrophosphate

The iron in portions of the sample and standard is determined by adding 5 ml. of aluminum nitrate reagent and 5 ml. of 20 per cent ammonium thiocyanate solution to 90-ml. samples and comparing the color with that developed in distilled water standards containing known amounts of iron treated with the same reagents. The amount of dilute (0.5 *N*) nitric acid required to give a pH of 3.0 is determined on separate 90-ml. samples to which 5 ml. of ammonium thiocyanate and the amount of iron standard required to give an iron concentration of 0.2 p. p. m. have been added. The glass electrode is best employed for the determination of pH, although suitable indicators may be used.

To 90-ml. portions of the metaphosphate and pyrophosphate-free water are added 0, 0.2, 0.4, 0.6, 1.0, 1.5, and 2.0 ml. of standard sodium metaphosphate (0.100 gram per liter) or sodium pyrophosphate (0.100 gram per liter), sufficient iron standard to give a total ferric ion concentration of 0.2 p. p. m., nitric acid to give a pH of 3.0 to the final solution, and 5 ml. of 20 per cent ammonium thiocyanate. The solutions are diluted to 100 ml. Ninety-milliliter samples are treated with iron standard to give 0.2 p. p. m. of iron, nitric acid to give a pH of 3.0, and 5 ml. of ammonium thiocyanate. After 5 minutes the colors are compared with the standards. One hundred-milliliter Nessler tubes may be used for the comparison. Increased sensitivity is obtained by extracting the solutions with 15.0 ml. of 5 to 2 isoamyl alcohol–ether, removing 5.0 ml. of the extract, adding 0.5 ml. of acetone, and determining the per cent transmittance with a photoelectric colorimeter.

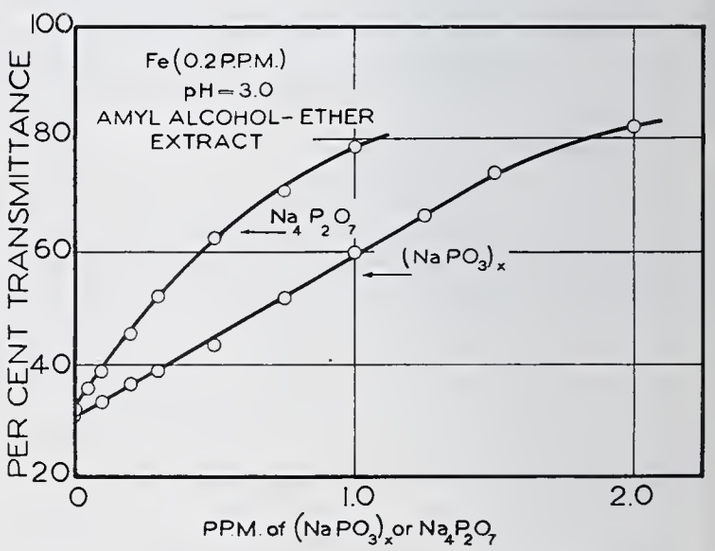

FIGURE 6. EFFECT OF METAPHOSPHATE AND PYROPHOSPHATE CONCENTRATION ON TRANSMITTANCE OF ISOAMYL ALCOHOL–ETHER EXTRACTS OF SOLUTIONS CONTAINING 0.20 P. P. M. OF IRON

pH = 3.0

For larger amounts of metaphosphate the iron concentration is adjusted to 2 p. p. m. and 5 ml. of 6 *N* nitric acid are added to standards and unknown. Extraction is not necessary.

Acknowledgments

Certain preliminary experiments were performed by Manuel Rosen working on an N. Y. A. project. Calgon, Inc., supplied the glassy sodium metaphosphate.

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Evaluation of Metal-Cleaning Compounds

A Quantitative Method

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A new quantitative method has been developed for the evaluation of metal-cleaning compounds. Since mineral oil fluoresces brightly under ultraviolet light and this fluorescence is capable of being photographed, a convenient means is provided for detecting and recording oil residues on metal surfaces both before and after cleaning.

THE property of removing oil from metals is relatively easy to recognize and evaluate qualitatively, but somewhat more difficult to evaluate quantitatively. Practically any alkali cleans most of the oil from the metal and unless a quantitative evaluation of the residual oil can be made, the true value of the metal cleaner is left as a matter for conjecture.

A survey of the literature for methods of evaluating the performance of metal-cleaning compounds did not disclose an accurate method for the detection of traces of oil. A real need existed for a quantitative test which would be both rapid and capable of permanent record for the comparison of cleaning products. The measurement of one or several physical or chemical characteristics of an alkaline cleaning solution is not sufficient to establish quantitatively its merit as a cleaning agent. Hence, a performance test involving the cleaning of uniformly soiled metal samples and the observation of the residual traces of oil appeared to be the best method of studying metal cleaners.

Principle of Testing Method

Mineral oil fluoresces brightly under ultraviolet light. Animal and vegetable oils which do not fluoresce in their own right can be made to fluoresce by the addition of an oil-soluble fluorescent dyestuff such as Fluorescent Oil Green H. W., obtainable from Wilmot & Cassidy, 108 Provost St., Brooklyn, N. Y. Since this white fluorescence of the various oils is proportional to the amount of oil adhering to the metal surface and since clean metal appears black under ultraviolet light, a natural scale of measurement is established for evaluating the efficiency of a metal-cleaning compound. Extremities of this scale are shown in Figure 1. The amount of oil adhering to the panel on the right, as determined from an average of ten such panels, is 0.000113 gram per sq. cm. The average oil deviation from the mean in this set of ten panels was 7.3 per cent by weight. The fluorescence is capable of being photographed, thereby providing a permanent record of the cleaning ability. By the use of this method fine distinctions can be made between cleaning compounds.

To establish the minimum visible amount of residual oil, gravimetric oil determinations were made on metal test panels representative of the lower limits of observation. It was found that 0.000004 gram of oil per sq. cm. is visually detectable. A test of this kind is probably most accurately defined as "visually quantitative".

Method of Oiling the Metal

The metal test strips, 5 × 10 cm. (2 × 4 inches) in size, are scrubbed by hand with a 0.50 per cent solution of Nacconol NR at 110° F., thoroughly rinsed in warm water, rinsed in alcohol, and allowed to dry.

Strips of wool flannel 4.7 × 9.7 cm. (1.875 × 3.875 inches) are saturated with a mineral oil composition such as is used in the rolling of brass. These oil-saturated wool strips are alternated with the metal strips to form a stack with protecting metal plates and oil-saturated wool strips above and below the stack of experimental strips.

The stack of plates is placed between the jaws of a hydraulic press, the edges of the stack of plates being carefully squared up. A pressure of 35 kg. per sq. cm. (500 pounds per sq. inch) is applied, and the pressure maintained until no more oil oozes out from the edge of the stack, the edges being constantly wiped with a clean cotton cloth. At the end of this process the pressure is released, and the strips are removed and retained in a perfectly horizontal position until taken out of the stack, one at a time, for the cleaning experiment.

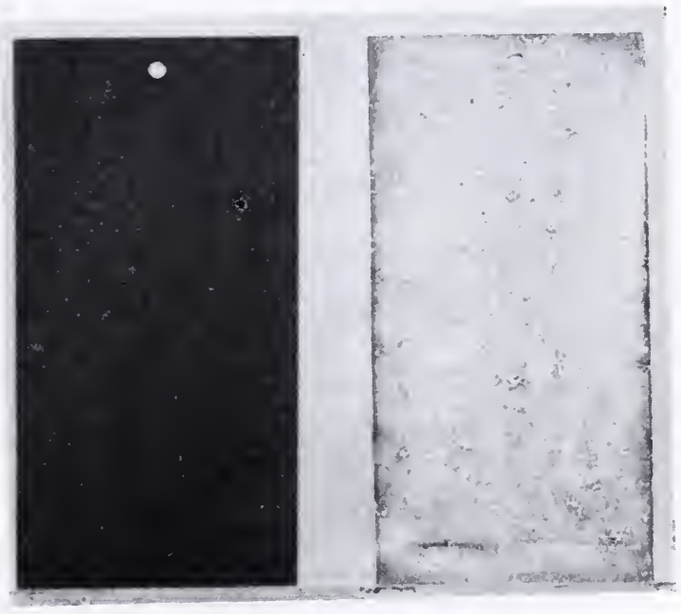

Figure 1. Luminogram of Steel with Mineral Oil

Left. Clean steel, no oil
Right. Oiled steel, 113 × 10^{-6} gram per sq. cm.

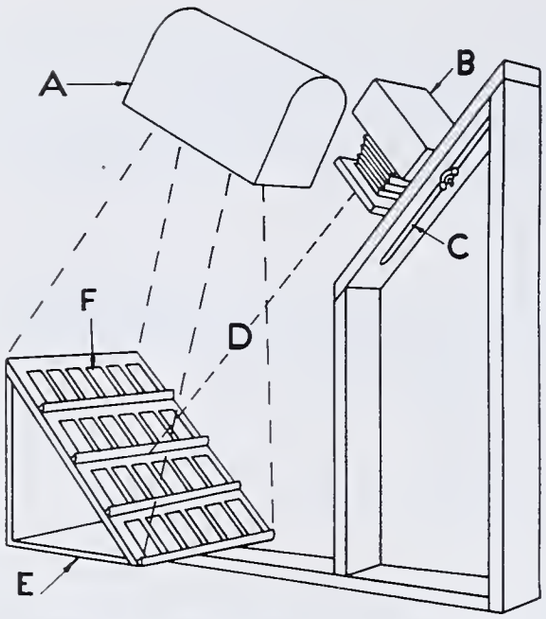

FIGURE 2. PHOTOGRAPHIC ARRANGEMENT FOR TAKING LUMINOGRAMS

A. Ultraviolet lamp
B. 4 × 5 view camera with K-2 filter and Tri-X film
C. Slot for camera adjustment
D. Distance from camera lens to easel, approximately 36 inches
E. Easel
F. Metal strips, 2 × 4 inches

Method of Cleaning

The cleaning results referred to herein deal entirely with "soaking" operations. The "soaking method" of metal cleaning was selected for this work, since it is widely used, simple, and most clearly shows the "chemical" cleaning ability of any metal-cleaning compound. There is no mechanical boosting action in the process to confuse the intrinsic cleaning ability of any product.

Eight hundred cubic centimeters of the cleaning solution are placed in a 1000-cc. beaker. The beaker is immersed in a constant-temperature bath at the desired temperature and the metal test plate is hung in the beaker from a hook for the required period of time. The metal is withdrawn and rinsed in running water at 100° F. for one minute and then hung in the air to dry. The metal strips are finally placed in a rack of such a nature that the strips do not touch each other until it is possible to photograph a complete series from any given set of experiments.

Method of Recording Results

After cleaning, the metal strips are either examined visually or photographed under ultraviolet light. A diagram of the arrangement of the apparatus for taking these pictures is presented in Figure 2. The ultraviolet light source used in the present work is a Hanovia analytic model quartz lamp, which provides a powerful source of ultraviolet light. Any strong source of ultraviolet light that is properly filtered to remove essentially all of the visible spectrum is satisfactory.

The camera is equipped with a Wratten K-2 filter, and Tri-X-film, and the pictures are taken at f. 4.5 with a 10-minute exposure. The camera is placed approximately 90 cm. (30 inches) from the subject, facing it directly. The ultraviolet radiation from the Hanovia lamp is incident to the surface of the subject at an angle of 45°, the lamp also being about 90 cm. (30 inches) from the midpoint of the subject. The film developer used in this work is DK-60A, which is designed to give maximum contrast. The paper is Eastman's Azo F. 3, on which an elon-hydroquinone developer is used. Temperature and developing time are carefully controlled in the development of both films and prints, but otherwise no special precautions are taken.

The pictures taken under ultraviolet light are referred to as Luminograms. A series of these luminograms is soon to be published (*1*).

Illustration of Method

A series of metal strips illustrating the gradation of scale covered by this test method is shown in Figure 3. This particular series deals with the cleaning ability of a single alkali, sodium metasilicate, and is reproduced here because it is typical of the pictorially quantitative record available. The strip on the left is a reference piece of clean steel. The series represents five tests at 140° F., the concentration of sodium metasilicate ranging from 2 to 10 per cent. The soaking time is 5 minutes. As the concentration increases there is a progressive reduction in the amount of oil residual on the metal surfaces.

Gravimetric oil determinations were run on this selected set of test panels prior to photographing, not as part of the method, but to demonstrate the sensitivity of the test. Original panels carried 0.000113 gram per sq. cm. of mineral oil.

Panel No.	Gram/sq. cm.
1	0.000038
2	0.000021
3	0.000014
4	0.000004
5	0.000004

FIGURE 3. LUMINOGRAM OF STEEL WITH MINERAL OIL, CLEANED WITH SODIUM METASILICATE

Left, reference piece of clean steel

Strip No.	1	2	3	4	5
Sodium metasilicate concentration, %	2	4	6	8	10
Residual oil, gram per sq. cm. × 10^{-6}	38	21	14	4	4

The present test not only indicates the amount of oil resident on the metal surface but also illustrates the distribution of the oil. If a metal surface carries only a small amount of oil by weight, but the oil is concentrated in a relatively small area, a good deal of damage can be done to any superficial coating that may be applied. It is very difficult to define the distribution of the oil by methods other than the one described in this paper.

Summary

The new testing technique, developed for the evaluation of the performance of metal-cleaning compounds, not only aids in selecting the proper types of cleaning compounds for specific cleaning jobs, but also establishes new criteria for a clean metal surface, thereby raising the standard for the technology of metal cleaning.

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Determination of Cobalt and Manganese by Photometric Methods

LOUIS WALDBAUER AND NELL M. WARD, State University of Iowa, Iowa City, Iowa

COBALT can be determined in the presence of manganese when the latter is present up to as high as 99.40 per cent, by means of the chloroform extract of the cobaltinitroso-beta-naphthol compound in the photelometer, using a green filter. Manganese can be determined by separation as the manganese ammonium phosphate with subsequent conversion to the manganese formaldoxime compound and then determined in the photelometer without a filter. The method is satisfactory even when the cobalt is present in amounts as high as 99.50 per cent in the original sample.

In the course of other work, it became imperative to determine small amounts of cobalt in the presence of large amounts of manganese, and small amounts of manganese in the presence of large amounts of cobalt. None of the methods available in the literature seemed satisfactory and hence the following methods were worked out. The procedures and techniques are simple and precise.

TABLE I. DETERMINATION OF COBALT (0.30 MG.) IN THE PRESENCE OF MANGANESE

Cobalt	Manganese	Readings	Cobalt
%	%	*Microamperes*	*Mg.*
100.00	00.00	45.00	0.30
37.50	62.50	45.00	0.30
23.08	76.92	45.00	0.30
13.04	86.96	45.00	0.30
5.66	94.34	45.00	0.30
1.19	98.81	45.00	0.30
0.60	99.40	45.00	0.30

Cobalt

The method described for the determination of cobalt is based upon the extraction of cobaltinitroso-beta-naphthol with chloroform. It is particularly designed for samples having low cobalt and high manganese content.

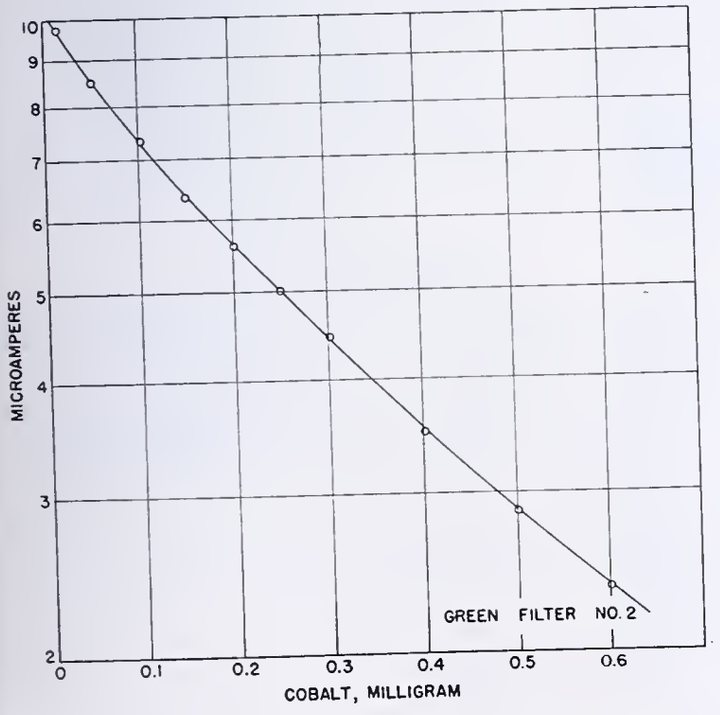

FIGURE 1. STANDARDIZATION CURVE FOR COBALT

A standard curve was established for cobalt, using a photoelectric photometer with samples varying from 0.01 to 0.60 mg. of cobalt by the following procedure: Enough 5 *N* hydrochloric acid was added to 10 ml. of ammonium citrate reagent in a 250-ml. flask to make it just acid, then the solution of the sample was introduced. After heating just to boiling, 25 ml. of alpha-nitroso-beta-naphthol reagent were added, and the solution was allowed to stand for 2 hours (*7*) and then filtered. The filter paper and the precipitate were washed with distilled water to free them as much as possible from the color of the reagent and dried at 100° C. The precipitate was extracted from the filter paper, using chloroform in 10- to 15-ml. portions until they were absolutely colorless. The volume of the extract was made up to exactly 100 ml. with chloroform. The alpha-nitroso-beta-naphthol reagent was prepared by boiling 1.0 gram of the compound with 200 ml. of water and 10 ml. of 5 *N* sodium hydroxide, filtering, and diluting to 2 liters. The ammonium citrate reagent contained 1000 grams of citric acid, 250 ml. of water, and 1000 ml. of ammonia solution (sp. gr. 0.90). A green filter was used in the photometer (Cenco-Sheard-Sanford photelometer).

The sample containing the cobalt and manganese was treated as directed in the preceding paragraph and the standard curve was used to determine the amount of cobalt present.

The results obtained are given in Table I. These data showed that the method was precise within the range indicated and that determinations were reproducible.

According to the literature, divalent cobalt is oxidized to trivalent by alpha-nitroso-beta-naphthol and precipitated as cobaltinitroso-beta-naphthol (*2*), and the cobalt is separated from the manganese without the reagent affecting the manganese (*2, 3*). It is a matter of small importance whether the precipitate carries down absorbed material, since it does not produce a colored compound with the reagents used.

Manganese

The following method for the determination of manganese, which is particularly designed for samples having low manganese and high cobalt content, is based upon the formation of a purple colored compound produced when the manganese and the formaldoxime reagent react in an alkaline solution.

A standard curve for manganese was established with samples varying from 0.01 to 0.75 mg. of manganese. The sample was placed in a 100-ml. volumetric flask, diluted to 50 to 60 ml., and made just neutral with 5 *N* ammonia solution. A 4 to 5-ml. excess of the ammonia solution was then added, and 1 to 2 ml. of formaldoxime reagent (*1*), and sufficient water to make the final volume exactly 100 ml. The formaldoxime was prepared by dissolving 4 grams of hydroxylamine hydrochloride in 100 ml. of water and adding 4 to 5 ml. of 40 per cent formaldehyde. No filter was used in the photelometer.

A series of curves was established in which the faint yellow color produced by the cobalt was compensated for by adding to this standard known amounts of cobalt. This was necessary, since cobalt will precipitate with manganese in the procedure used in actual analysis.

TABLE II. AMOUNTS OF COBALT AND MANGANESE FOR ESTABLISHING CURVES

Manganese	Cobalt			
	Curve I[a]	Curve II	Curve III	Curve IV
Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*
0.00	0.00	0.10	0.20	0.30
0.10	0.00	0.10	0.20	0.30
0.20	0.00	0.10	0.20	0.30
0.25	0.00	0.10	0.20	0.30
0.30	0.00	0.10	0.20	0.30

[a] Standard curve for manganese.

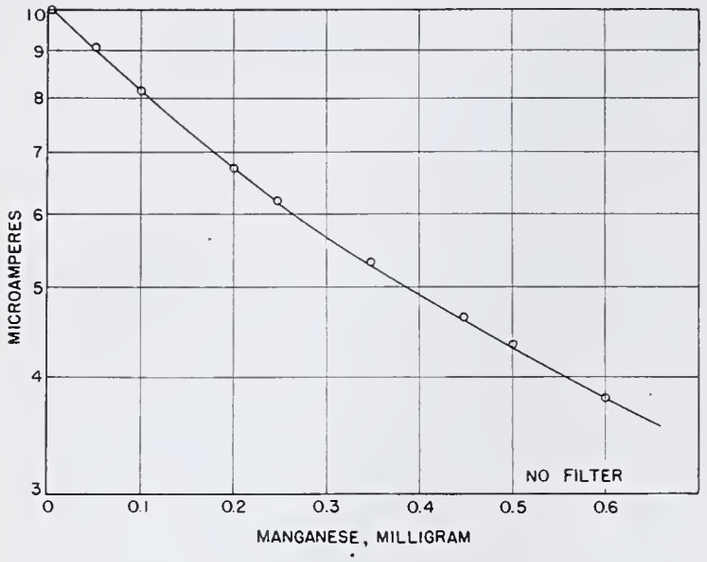

FIGURE 2. STANDARDIZATION CURVE FOR MANGANESE

TABLE III. ESTABLISHMENT OF STANDARD MANGANESE CURVES COMPENSATED WITH COBALT

(No filter)

Manganese	Microampere Readings			
	0.00 mg. Co[a]	0.10 mg. Co	0.20 mg. Co	0.30 mg. Co
Mg.				
0.00	99.50	99.00	98.50	98.00
0.10	80.67	80.50	80.25	79.50
0.20	67.67	67.25	67.00	67.00
0.25	62.00	62.00	61.75	61.50
0.30	58.00	57.25	...	57.00

[a] Standardization curve for manganese.

Table II suggests the combinations for samples for the series of curves.

These known samples were treated according to the method given above, and the reference curves were obtained from the photelometer readings (Table III).

When a cobalt blue filter was used in the photelometer, a wider spread of curves was obtained (Figure 3).

To a sample containing the manganese and cobalt in a volume not greater than 60 to 65 ml., 4 to 5 grams of solid ammonium chloride were added and sufficient water so that when about 25 ml. of ammonia solution (sp. gr. 0.90) were added, the final solution was highly ammoniacal and its volume approximately 90 ml. To the almost boiling solution, 10 ml. of saturated ammonium phosphate solution were added dropwise with constant whirling (*5*). The solution was then cooled and 10 ml. of 95 per cent ethyl alcohol were added. As soon as the precipitated manganese ammonium phosphate (*6*) was well crystallized, it was filtered and washed with portions of a wash solution made of 250 ml. of concentrated ammonia, 100 ml. of saturated solution of sodium phosphate, 100 ml. of ethyl alcohol, and 550 ml. of water. If the precipitate was definitely colored with the cobalt it was dissolved in 5 *N* hydrochloric acid and reprecipitated. The final precipitate was dissolved in 5 *N* hydrochloric acid, the paper washed with water, and the solution diluted to exactly 250 ml. A 25-ml. portion was transferred to a 100-ml. flask and treated with ammonia solution and formaldoxime reagent as directed in the preceding paragraph, and the photelometer readings were taken.

The amount of cobalt present in a 25-ml. aliquot portion of the solution was determined by the chloroform extraction method previously described. The amount of manganese was determined by references to the proper curve.

The results obtained, given in Table IV, showed that the method was precise within the range indicated and that the determination was reproducible.

Cobalt precipitated with the manganese in all concentrations used. If the precipitate had a cobalt content greater than the manganese content, it was simply dissolved and reprecipitated (*4*).

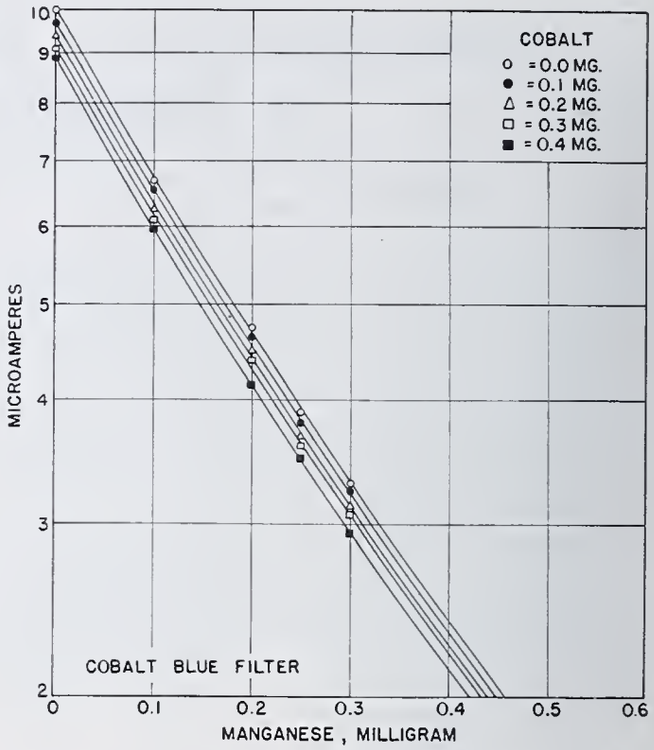

FIGURE 3. STANDARDIZATION CURVES FOR MANGANESE WITH COBALT PRESENT

Conclusions

Cobalt can be determined in the presence of 99.40 per cent of manganese by a simple and precise method. Manganese can be determined in the presence of 99.50 per cent of cobalt. Indications are that successful results could be obtained at still higher concentrations.

TABLE IV. DETERMINATION OF MANGANESE (0.25 MG.) IN THE PRESENCE OF COBALT

(No filter)

Mn	Co	Readings
%	%	*Microamperes*
33.33	66.67	62.00
20.00	80.00	62.00
11.11	88.89	62.00
4.76	95.24	62.00
0.99	99.01[a]	61.75
0.50	99.50[a]	62.00

[a] Samples were reprecipitated.

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A CONDENSATION of the dissertation submitted by Nell M. Ward to the graduate faculty in partial fulfillment of the requirements for the Ph.D. degree.

Equipment for Determining Moisture in Coal

New Type of Equipment

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MANY methods and types of equipment have been suggested for the determination of moisture in coal. Although the methods of the American Society for Testing Materials are the standards on which moisture values for buying and selling of coal are based, there is a need for more rapid methods of determination, particularly in the control of commercial preparation processes. The semiautomatic moisture tester manufactured by The Brabender Corporation has been used to advantage for determining moisture in grains, food products, powdered materials, etc., and it was thought that it might be applied successfully to the determination of moisture in coal. Through the courtesy of The Brabender Corporation one of these moisture testers was made available for the authors' use. This paper is, therefore, a report of results obtained by using this equipment for determining moisture in four face samples of Illinois coal, ranging in moisture content from about 5 to 19 per cent, and one commercial sample of Franklin County coal taken at the dewatering screen of a preparation plant.

Apparatus

The semiautomatic moisture tester is a combination warming oven and balance (Figure 1), so arranged that weighings may be made without removing the sample from the oven. It is so calibrated that, when using 10-gram samples, readings may be taken directly in per cent moisture. The warming oven is electrically heated, with thermostatic control which is adjustable for temperatures from 85° to 175° C. A small motor blower provides air circulation through the warming chamber during drying. The model used did not have a variable speed control for the blower motor. As run, the atmosphere of the drying chamber was changed about two times per minute. A rotating table, with provision for the simultaneous drying of ten samples, enables the operator to bring each sample into position for weighing. The fact that readings may be taken without removing samples from the drying oven enables the operator to determine drying curves. In addition, the apparatus has been found convenient in studying the effect of temperature and particle size of coal on rate of drying and on oxidation of samples.

Samples

Four face samples and one commercial sample of coal from Gallatin, Franklin, Macoupin, and Henry counties, representing coal beds 5, 6, 6, and 1, respectively, were used in this study. The face samples were secured from freshly exposed faces by cutting about 5-cm. (2-inch) channel samples that weighed 23 to 32 kg. (50 to 70 pounds). These were crushed in the mine to pass a 4-mesh sieve, and 2.8- to 3.8-liter (3- or 4-quart) samples were riffled out and sealed in friction-top cans. Two of these samples were used for the studies described in this paper. One of these portions was air-dried in the laboratory to determine air-dry loss, after which it was reduced in a Braun Type 6 C P pulverizer (coffee mill type) to −20-mesh and then to −60-mesh in a ball mill. Proximate analyses were made on the 60-mesh coal. From the other portion, samples of 8-, 20-, and 60-mesh coal were prepared without air-drying for total moisture determinations in the semiautomatic moisture tester and for total moisture determinations by the A. S. T. M. procedure (*2*) on the −20-mesh coal. The coal was further reduced to −8- and −20-mesh in the Braun pulverizer and to −60-mesh in the ball mill.

The sample used for studying the determination of moisture on air-dried coal was secured at the dewatering screen of a preparation plant that processes Franklin County coal. This coal was sealed in a gallon tin at the plant and brought to the laboratory where it was air-dried without preliminary crushing. It was then reduced to −8- and −20-mesh in the Braun pulverizer and further to −60-mesh in a ball mill.

Procedure

Proximate items, total sulfur, and calorific values were determined on the four face samples by usual A. S. T. M. procedures (*1*), including "as received" moisture by air-dry loss plus A. S. T. M. moisture (air-dry basis). Total moisture values, without preliminary air-drying, were determined on 20-mesh portions of these coals by the A. S. T. M. total moisture procedure (*2*). Total moisture values were determined also in the special moisture tester on three sizes, 8-, 20-, and 60-mesh, at three temperatures, 105°, 125°, and 150° C. Readings were taken at 5-minute intervals up to 30 or 40 minutes, after which readings were taken at 10-minute intervals up to 100 minutes. These values were plotted against time, and the peak of the curves so obtained was taken as the moisture value. Air-dry loss and A. S. T. M. moisture values (air-dry basis) were determined on the sample of commercial coal. The moisture value was obtained for comparison with values obtained with the special moisture tester on 8-,

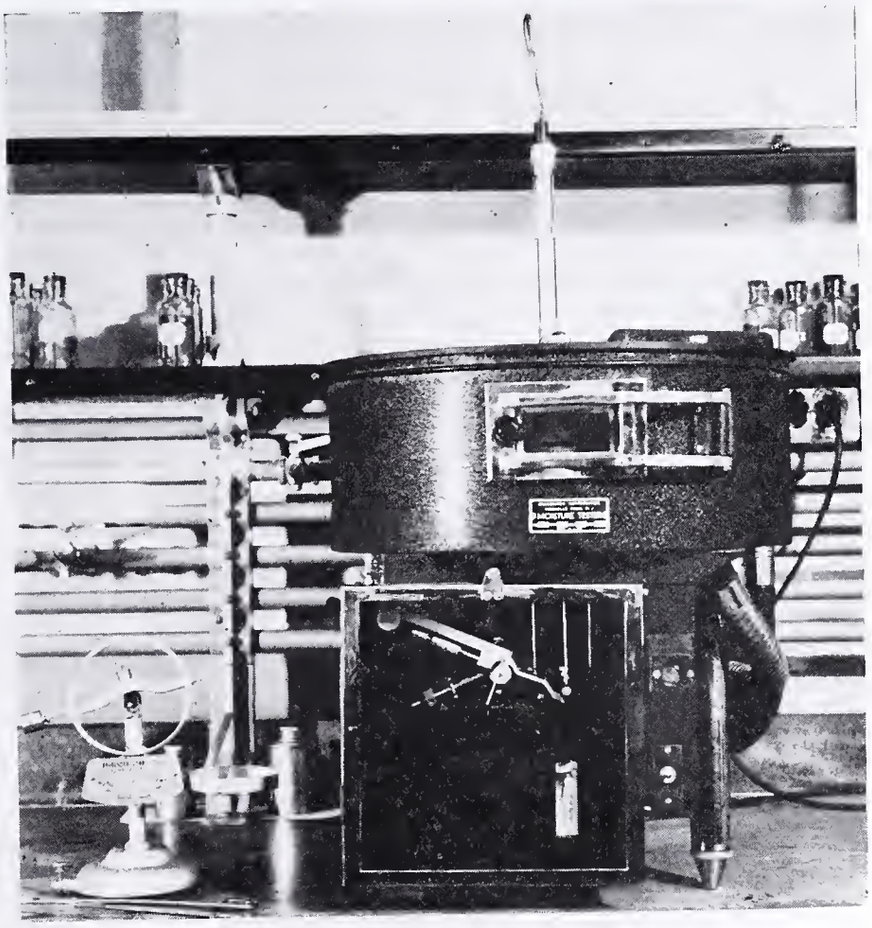
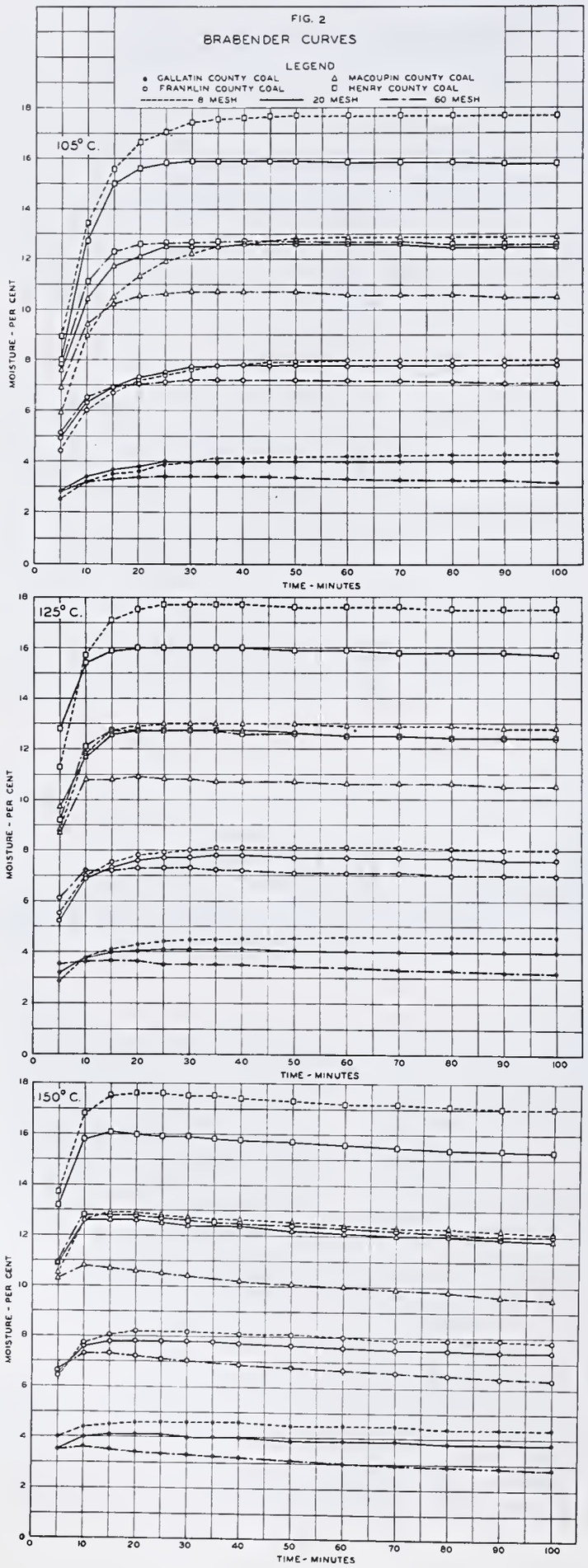


FIGURE 1. SEMIAUTOMATIC MOISTURE TESTER

20-, and 60-mesh portions of air-dried coal at 105°, 125°, and 150° C. All determinations were made in duplicate in the same run.



Results

Table I presents chemical analyses, location by county, coal bed numbers, and rank of the four face samples studied. In Table II are summarized moisture values for the face samples obtained by A. S. T. M. procedures, and by use of the special equipment. Figure 2 presents drying curves obtained for the face samples, and Figure 3 presents drying curves obtained for the air-dried sample of commercial coal.

Discussion

The four face samples used in this study were selected to represent different moisture levels of coal encountered in Illinois. The work consisted of three parts: (1) a comparison of moisture values obtained in the special moisture tester with those obtained by A. S. T. M. procedures and the times necessary to obtain these values; (2) a study of the effect of laboratory preparation of samples on moisture values; and (3) a study of the effect of temperature on moisture determination in coal.

Reference to Table II shows that moisture values obtained by use of the special moisture tester check A. S. T. M. total moisture values within A. S. T. M. tolerances (*3*) with only one exception (C-2425 at 150° C.) when using identical samples (compare A. S. T. M. total moisture with special tester values on −20-mesh samples). The times necessary for obtaining values in the special equipment were shorter, ranging from 10 to 40 minutes, as compared to 90 minutes' drying time specified for the standard A. S. T. M. procedure for 20-mesh coal. Smooth drying curves were obtained (Figure 2), rising rapidly in the earlier stages during loss of moisture, flattening when all moisture was expelled, and then dropping off slowly, probably owing to oxidation of samples. Readings on duplicate samples taken in the early stages of drying were not always within A. S. T. M. tolerances but they were well within these tolerances (*3*) at the level portions of the curves.

Data in Table II and drying curves in Figure 2 show the effect on moisture of laboratory preparation of samples. In all cases moisture values obtained in the special moisture tester were highest for 8-mesh samples, decreasing with increased preparation necessary to reduce the coal to 20- and 60-mesh. However, moisture values for 8-mesh samples were lower in most cases than "as received" values, indicating that moisture was lost in crushing the 4-mesh coal to 8-mesh. It is interesting to note, however, that the values for these 8-mesh samples check the "as received" values more closely than do the A. S. T. M. total moisture values obtained on 20-mesh coal. Necessary drying times increase as the particle size of samples increases, but the oxidation trend appears to decrease.

The effect of temperature on the determination of moisture in these face samples by the special apparatus is shown by data in Table II and Figure 2. Increasing the temperature decreases the time necessary for complete expulsion of moisture. Oxidation of samples is definitely greater at higher temperatures, as is evidenced by the dropping curves following peak levels. As the temperature is increased this oxidation becomes evident more quickly and takes place to a greater extent. For the most part moisture values obtained at the three temperatures used for the same mesh-size samples checked within A. S. T. M. tolerances (*3*).

A comparison of moisture values was made for an air-dried sample of prepared Franklin County coal using both the special equipment and the regular A. S. T. M. procedure. Drying curves for this sample at 105°, 125°, and 150° C. on three sizes, 8-, 20-, and 60-mesh, are shown in Figure 3. The

TABLE I. CHEMICAL ANALYSES

Laboratory No.	C-2416		C-2445		C-2452		C-2425	
County	Gallatin		Franklin		Macoupin		Henry	
Coal Bed No.	5		6		6		1	
Rank	A		B		C		C	
Analyses	As received	Dry	As received	Dry	As received	Dry	As received	Dry
Moisture, %	5.1		8.0		13.5		19.3	
Ash, %	9.4	10.0	9.5	10.3	8.1	9.4	8.9	11.0
Volatile matter, %	36.8	38.8	33.5	36.4	35.2	40.7	31.6	39.2
Fixed carbon, %	48.7	51.2	49.0	53.3	43.2	49.9	40.2	49.8
Total sulfur, %	3.03	3.19	1.55	1.69	3.75	4.34	3.85	4.77
Calorific value, B. t. u.	12,584	13,267	11,968	13,014	11,055	12,777	10,332	12,802

TABLE II. SUMMARY OF MOISTURE VALUES FOR FACE SAMPLES

Laboratory No.	C-2416		C-2445		C-2452		C-2425	
County	Gallatin		Franklin		Macoupin		Henry	
Moisture, as received[a], %	5.1		8.0		13.5		19.3	
Moisture, A. S. T. M. total[b], %	4.1		7.6		12.6		15.7	
	%	*Min.*	%	*Min.*	%	*Min.*	%	*Min.*
Moisture, Brabender total								
105° C. 8-mesh	4.3	70	8.0	60	12.9	60	17.7	50
20-mesh	4.0	25	7.8	40	12.6	40	15.9	30
60-mesh	3.4	25	7.2	30	10.7	30	12.7	30
125° C. 8-mesh	4.6	60	8.1	35	13.0	25	17.7	25
20-mesh	4.1	25	7.8	35	12.7	20	16.0	20
60-mesh	3.6	10	7.3	20	10.9	20	12.7	15
150° C. 8-mesh	4.6	20	8.2	20	12.9	15	17.6	20
20-mesh	4.1	15	7.8	15	12.6	10	16.1	15
60-mesh	3.6	10	7.3	10	10.8	10	12.8	10

[a] Air-dry loss + air-dry moisture.
[b] Total moisture on −20-mesh coal without preliminary air-drying.

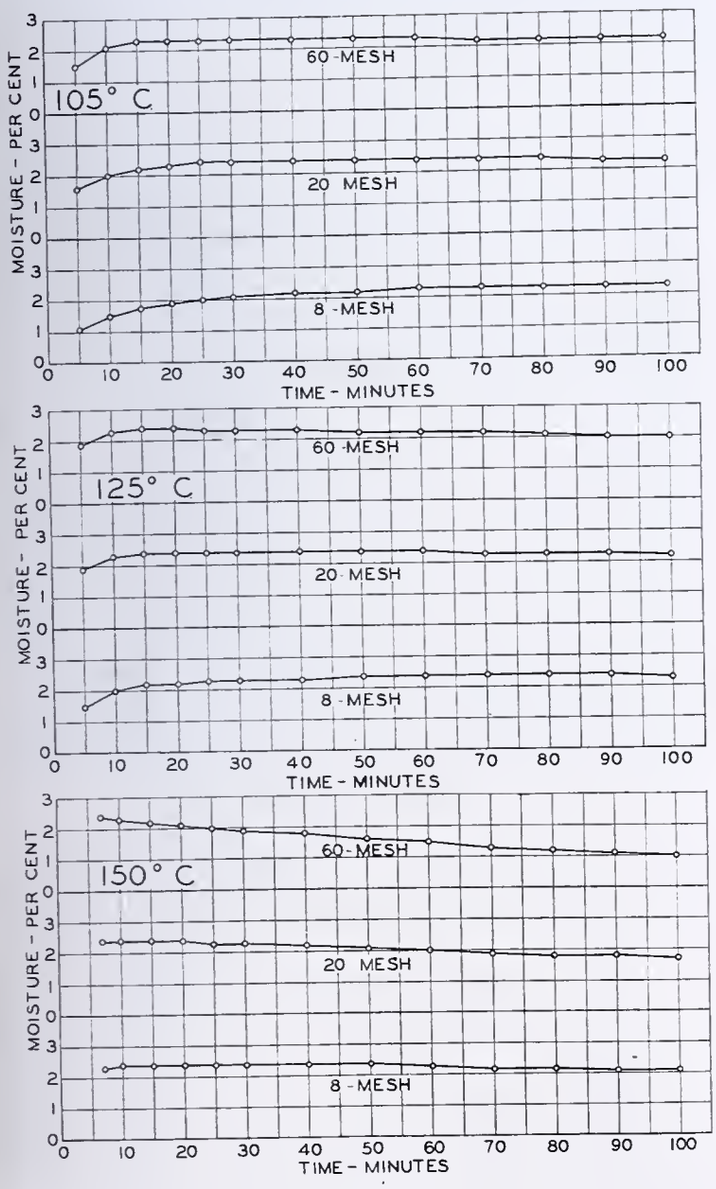


FIGURE 3. BRABENDER CURVES ON AIR-DRIED COAL
A. S. T. M. moisture 2.3 per cent

moisture value obtained by the A. S. T. M. method was 2.3 per cent for this sample. Special values checked the A. S. T. M. value within specified tolerances (*3*) in all cases. Finer sizes dried more rapidly, and higher temperatures accelerated loss of moisture. It is also shown that air-drying eliminates troublesome loss of moisture during grinding to finer sizes.

While many of the data presented herein are in accord with common information, yet they do present a rather vivid picture of drying rates, oxidation trends, and effect of laboratory grinding and temperature as related to the determination of moisture in coal. There is a definite need for a direct rapid method of determining total moisture for control purposes. Judging from data presented, it appears that the semiautomatic moisture tester will give reliable moisture values for the samples as tested. However, the problem of suitable sampling for total moisture determination remains. The authors feel that the fact that reliable values may be obtained on 8-mesh coal may simplify somewhat the sampling problem, particularly in so far as laboratory crushing for analysis is concerned. Drying of 8-mesh coal may be somewhat slow at 105° C. but it appears that higher temperatures may be used safely in the special tester for accelerating expulsion of moisture, since it is possible to note when oxidation becomes serious.

Conclusions

The semiautomatic moisture tester gave moisture values, at temperatures and for sizes studied, which check within A. S. T. M. tolerances. Times for these determinations may be considerably shorter than those specified in the standard A. S. T. M. procedures.

Laboratory crushing resulted in loss of moisture from samples, this loss increasing with increased crushing. Air-drying preliminary to crushing appeared to reduce this loss effectively.

Higher temperatures accelerated expulsion of moisture from samples and increased oxidation.

Acknowledgments

The cooperation of the mine management where samples were secured is gratefully acknowledged. Appreciation is expressed to The Brabender Corporation for making the equipment available for this study, to F. H. Reed for helpful suggestions during the course of this work, and to C. C. Boley, B. C. Parks, J. M. Schopf, and W. F. Wagner of the survey staff as well as Thomas Garwood of the C. W. and F. Coal Company for help in securing samples.

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PRESENTED before the Division of Gas and Fuel Chemistry at the 103rd Meeting of the AMERICAN CHEMICAL SOCIETY, Memphis, Tenn. Published with the permission of the chief, Illinois Geological Survey.

Determination of Water in Insulating Oil

Manometric Procedure

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IT WAS shown in studies on the electric hygrometer (*1*) that because of an indeterminate hysteresis effect the hygrometer unit could not be used to determine the water content of insulating oil. However, during the course of these studies, a constant-volume or manometric procedure was devised which shows great promise as a rapid and accurate procedure for the determination of small quantities of water in oil. The method consists of two pressure readings—namely, the initial pressure of the gases removed by evacuation from the oil sample and a final pressure reading after exposure of the evolved gases to a film of lithium chloride monohydrate. Adsorption of the organic oil vapors is reduced to a minimum because of the small quantity of lithium chloride monohydrate employed (approximately 1 mg. in an almost invisible film) and equilibrium between the hydrate and water vapor is attained very rapidly. The manner in which the unknown amount of water vapor is accommodated by a fixed weight of hydrate will be clear from the description of the apparatus.

The theoretical relation between the vapor pressure of water in contact with 0.5 mg. of anhydrous lithium chloride (*3*) is shown in Figure 1.

In region *OA*, water vapor can exist in the presence of anhydrous lithium chloride and the vapor will obey Boyle's law. At *A*, at a pressure of 0.38 mm. and a temperature of 30° C., lithium chloride monohydrate will be formed and further addition of water will result in no increase in pressure, providing the two solid phases, lithium chloride anhydrous and lithium chloride monohydrate, are present. At *B*, all the anhydrous lithium chloride has disappeared and the pressure will now increase to that of a saturated solution of lithium chloride hydrate, during which addition of water to the system none will be taken up by the lithium chloride hydrate. At *C* the saturated solution appears and a constant pressure results on still further addition of water until the solid phase disappears at *D*. In region *DE* the solution becomes more dilute with corresponding increase in vapor pressure.

Experimentally, the pressure along *CD* could be demonstrated, but the length of *CD* was found to be shorter than theoretically demanded by the solubility data. The explanation of this effect could not be found unless the solubility of the hydrate is greater in the film than in bulk. For this reason, region *CD* must be obtained experimentally for any selected quantity of lithium chloride. The equilibrium value of the pressure listed in (*3*) was readily found by approaching the final state from either direction. However, in region *OABC*, the same pressure reading could not be obtained in approaching the equilibrium value from either direction. When at the start all the water was condensed on the lithium chloride, a low pressure was found and the reverse was true if the water diffused from the vapor space to the lithium chloride. For these reasons, the vapor pressure of the saturated hydrate (region *CD*) was adopted as the reference pressure.

In preparation for an analysis the entire apparatus, of which a diagrammatic sketch is shown in Figure 2, was evacuated for 5 minutes with all stopcocks open except *B* and *G*, while heating lithium chloride, *L*, to approximately 300° C. with heater *W*. Stopcocks *D* and *K* were closed, the heater was removed, and the dry ice bath, *X*, was raised about trap *J*. Stopcock *B* was opened to admit air dried by desiccant in *A* through capillary *Y*, and 20 ml. of insulating oil were introduced by hypodermic syringe through serum rubber stopper *H* and stopcock *G* into degassing chamber *F*. Pumping was continued for 5 minutes while the dry air entering the degassing chamber through the porous disk, *E*, carried the water into the cold trap, *J*. By actual experiment, the volume of air passing through the disk was found to be 3 ml. at one atmosphere. This volume, however, corresponds to approximately 3 liters at the reduced pressure. Stopcock *I* was

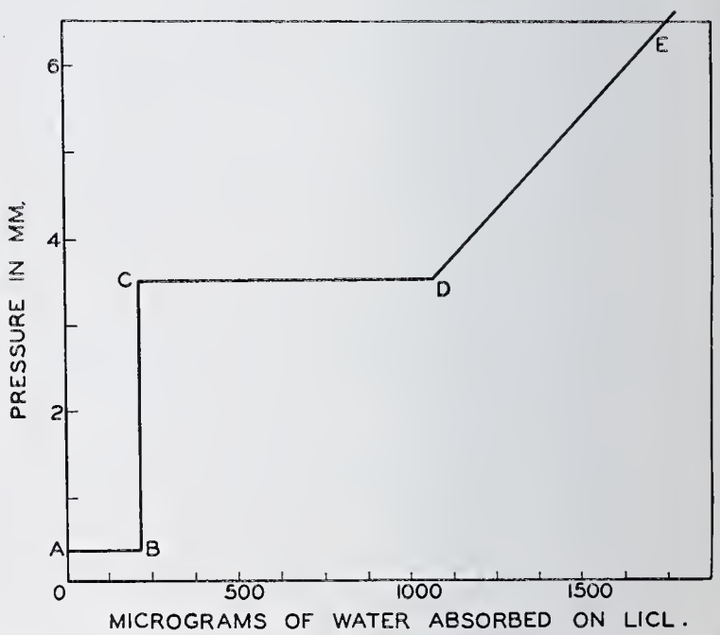

FIGURE 1. THEORETICAL RELATION BETWEEN WATER VAPOR PRESSURE AND WATER COMBINED WITH 500 MICROGRAMS OF LITHIUM CHLORIDE

[1] Present address, Westinghouse Electric & Manufacturing Co., East Pittsburgh, Penna.

TABLE I. COMPARISON OF RESULTS BETWEEN MANOMETRIC AND COMBUSTION PROCEDURE

Sample No.	Sample	Manometric Procedure		Combustion Procedure
	Grams	*Micrograms*	*P. p. m.*	*P. p. m.*
471	15	340	23	30
471	15	360	23	
475	18	640	35	33
475	15	490	33	
481	12	775	62	62
481	6	445	62	
480	9	515	57	56
478	6	615	102	100
476	15	590	39	43
476	15	595	40	
472	30	695	23	22
472	30	705	23	
Std.	6	800	133	146
Std.	6	785	132	

A. Dehydrite drying tube
B, D, G, I, K, N. Two-way stopcocks
C. Waste oil flask
E. Porous disk
F. Degassing chamber
H. Serum rubber stopper
J. Dry ice trap
L. Lithium chloride chamber
M. Manometer
O, Q, S, U. Three-way stopcocks
P, R, T, V. Auxiliary flasks
W. Heater
X. Dry ice bath
Y. Capillary
Z. Nichrome heater

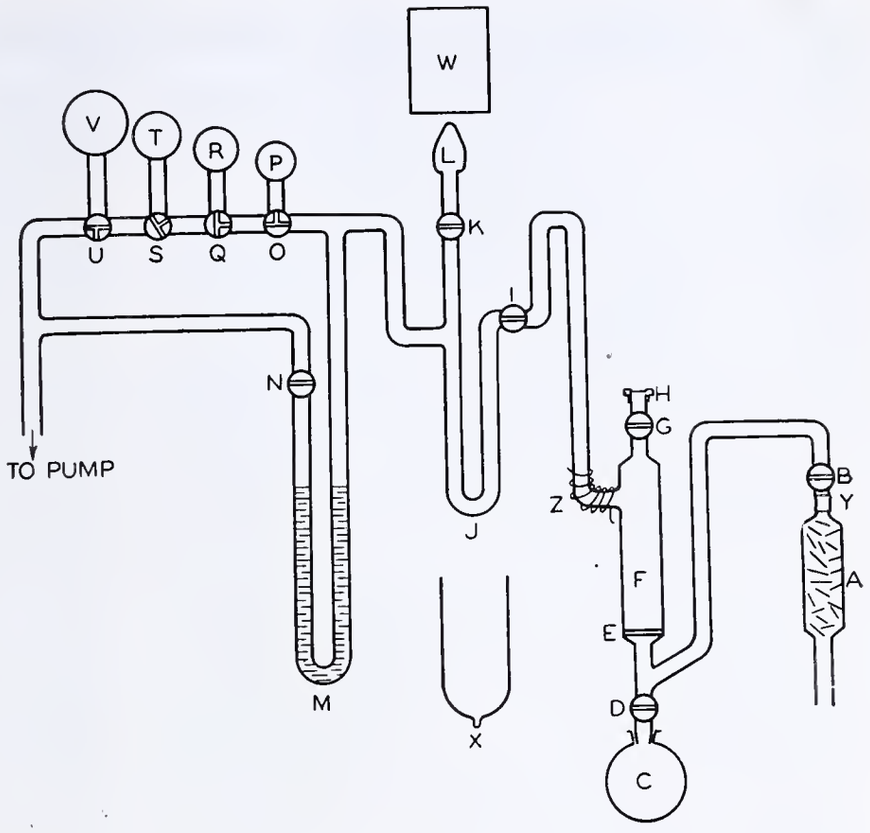

FIGURE 2. DIAGRAMMATIC SKETCH OF APPARATUS

then closed and pumping continued until the manometer reading was zero—about one minute. Stopcock N was closed, also the three-way cocks O, Q, S, and U by turning to the position shown at S, the cold bath, X, was lowered and trap J was warmed to 50° C. with a water bath for one minute. If the vapor pressure indicated on the manometer, M, was greater than 12 mm. of mercury it was reduced by allowing the water vapor to expand into the added volume of auxiliary flask P by turning stopcock O to the position shown at Q. If this volume was insufficient, flask R could be included in the system by turning stopcocks O and Q to the positions shown. When necessary, flasks T and V may be added to the system.

The water bath was removed and after allowing 5 minutes for the trap to reach room temperature the manometer reading and the gas volume were recorded. Stopcock O was closed by turning to the position shown at S and stopcock K was opened. After allowing 5 minutes for the water vapor to reach equilibrium with the lithium chloride in L, the manometer reading was again recorded together with the temperature. In preparation for a succeeding analysis the oil in F was forced into the evacuated flask, C, through stopcock D.

The water content of the oil was then simply calculated from the gas law, employing the water pressure P_{H_2O} obtained by combining the initial, final, and lithium chloride monohydrate saturated solution pressure readings. In equation form, P_{H_2O} may be derived as shown below, where P_{oil} signifies gases other than water vapor.

$$P_{initial} = P_{H_2O} + P_{oil} \quad (1)$$

$$P_{final} = P_{oil} + P_{LiCl \cdot H_2O} \quad (2)$$

$$P_{oil} = P_{final} - P_{LiCl \cdot H_2O} \quad (3)$$

Substituting 3 in 1

$$P_{H_2O} = P_{initial} - P_{final} + P_{LiCl \cdot H_2O} \quad (4)$$

All pressure readings were taken with a cathetometer capable of reading to 0.1 mm.

The choice of the magnitude of the fixed volumes V, T, R, and P as well as the quantity of lithium chloride will depend on the nature of the liquid to be analyzed. Insulating oil ranges in water concentration from 0 to 150 p. p. m. except in rare cases where emulsions are encountered. The volumes selected in Figure 2 were V = 250 ml.; T = 125 ml.; R = 70 ml.; P = 30 ml.; manifold = 10 ml. The weight of lithium chloride was approximately 1 mg. and it would accommodate between 250 and 950 micrograms of water—i. e., the length of CD in Figure 1. It was introduced as 2 drops of a 1 per cent solution of the salt in 95 per cent ethanol from a pipet which delivered 20 drops per ml.

In Table I, experimental results are given on samples of synthetic transformer oils of the chlorinated aromatic type, which had been withdrawn from service. The corresponding water content as obtained by the combustion procedure (*2*) is also included. Duplication of results was readily obtained with the manometric procedure with the same or different weight of sample. It is gratifying to note the good agreement of the results from the two independent methods. The manometric procedure appears to be capable of the greater precision.

The apparatus has been so designed that water may be determined in concentrations ranging from 8 to 170 parts per million, using a 30-gram sample. Few samples of oil will be found which exceed the maximum concentration and by increasing the weight, samples in the lower range may be readily accommodated. In the field, by replacing the mercury by a high-grade vacuum pump oil as the manometer fluid, a factor of approximately 16 is obtained in the displacement of the fluid. Thus, the pressure readings may be made with a metric rule with sufficient accuracy for most purposes. A complete analysis may easily be carried out in 30 minutes.

Acknowledgment

The authors wish to express their appreciation to W. F. Davidson, director of research, for his interest and advice in this work.

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Flask Design and High-Speed Stirring

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THE relative efficiency of flasks was judged in a previous paper (*3*) on the basis of comparative heats of reaction, and a creased flask with an inverted bottom was found best. The present work consists of a survey of the direction and force of the currents in a cross section of the flask, a comparison of the results from oxidation of *p*-nitrotoluene obtained in the special flask with those in an ordinary flask and with those observed by Huber and Reid (*1*), and finally a description of a considerably improved high-speed stirring apparatus for laboratory use.

The direction and force of the currents generated by a propeller which forces the liquid downward at the center of the special flask already mentioned have been tested. The results in a cross section through the center show a thin section, not more than 5 mm. wide, adjacent to the wall in which a current of very high velocity moves upward; a region adjacent to this section in which the upward force is only about a tenth that in the high-velocity belt; a wider section in which no pressure is evident; and an area nearer the propeller where a moderate suction is evident.

Existence of this fast-moving current next to the wall explains a number of facts which hitherto have not been clear—for example, the reports (*3*) that sintered glass fused on and Vigreux indentations pressed in the wall, and that a neck sharply recessed in the flask reduced the efficiency, are now understandable. These obstructions were directly in the current of high velocity where their effect was greatly magnified. It is therefore more than ever apparent that the wall serves chiefly, if not solely, as a surface for the rapid transit of a current of very high velocity to a position above the propeller; and this surface must be as smooth as possible and free from any obstructions, even when these are as distant from the propeller as the base of the neck.

The effectiveness of a flask of proper design as compared with an ordinary flask has been measured by the percentage of *p*-nitrobenzoic acid obtained by oxidation of *p*-nitrotoluene with permanganate. This reaction is good for a comparative study, for it progresses without stirring and is therefore not dependent on a special type of agitation. More particularly is it of value because it was studied also by Huber and Reid (*1*) in their interesting work on high-speed stirring. When this reaction was carried out in two different special creased flasks, a yield of 51 per cent was obtained after one hour's stirring at 9000 r. p. m. The yield in an ordinary three-necked flask under the same conditions was 30 per cent.

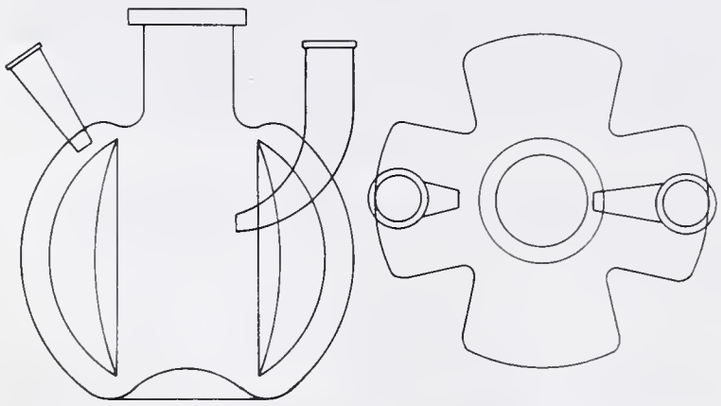

FIGURE 1. SPECIAL FLASK USED IN EXPERIMENTS ON OXIDATION OF *p*-NITROTOLUENE

A comparison of this result with that of Huber and Reid (*1*) is complicated by the fact that the stirrer which they employed was different (a centrifugal instead of a propeller type), the diameter of their stirrer was somewhat larger, the volume of their reaction mixture was smaller, and the container was an ordinary beaker with baffles rather than a flask. The first represents merely one of the differences, besides the container, which is a factor in stirring. The second difference can be adjusted approximately by comparing values at equal peripheral velocities, on the reasonable expectation that the liquid which leaves the agitator will then have approximately an equal velocity in each instance. The third difference, which involves capacity, might also be corrected by making the reasonable assumption that a larger volume of liquid will mean a proportionally fewer number of contacts of each particle with the agitator. It is convenient, however, to make the comparison at equal peripheral velocity and thereby show increased capacity due to the shape of the container as well as better yield. The data show (Table I) that the special flask handled 71 per cent more liquid and gave a slightly better (2 per cent) yield than did the apparatus of Huber and Reid. Since the yield in each case was less than 100 per cent and the maximum conversion within one hour had not yet been reached, it can safely be considered that each apparatus was accomplishing all that it could at that peripheral velocity, and that the values show the superiority of the present apparatus.

TABLE I. COMPARISON OF AN ORDINARY FLASK, A SPECIAL CREASED FLASK, AND THE APPARATUS OF HUBER AND REID

	Ordinary 3-Necked Flask	Special Creased Flask	Huber and Reid's Apparatus
Type of stirrer	Propeller	Propeller	Centrifugal
Diameter of stirrer, cm.	3.18	3.18	3.7
Total volume of reaction mixture, ml.	785	785	459
Yield at 9000 r. p. m., %	30	51	54
Yield at equal peripheral velocity 1498 cm. per second, %	30	51	49

Such differences can be of practical importance. With larger quantities they represent a power factor which might become so large as to offset the advantages which would accrue from high-speed stirring. In reactions where high-speed stirring is absolutely necessary, such a difference would greatly influence the cost of production. The higher the speed the more important does the design of the container and stirrer become.

The unit for laboratory operation has been considerably improved. This apparatus now runs smoothly and quietly, will operate over long periods of time without requiring adjustment, and is proving a laboratory asset. It has been used in work already reported (*2*).

Experiments

HIGH VELOCITY CURRENTS (J. D. and B. D.). A capillary tube of 2-mm. bore was inserted through the side neck of a three-necked flask which had four creases, the flask was filled with water, and the stirrer was rotated at 9000 r. p. m. Observations were made across the section of maximum flask diameter. When the capillary was touching the wall the water rose to a height of 25 cm. above the flask. When moved about 5 mm. from the wall, it fell to less than one tenth of that value. This method of surveying was originally intended as another means of judging the efficiency of stirrers of the propeller type.

Oxidation of *p*-Nitrotoluene (B. D.). The flask (Figure 1) was made from an ordinary 1-liter round-bottomed flask by putting in four creases, pushing the bottom inward, slightly recessing the neck, making an inlet tube which extended to a point above the stirrer where suction occurred, and inserting a thermometer tube which extended beyond the width of the current of high velocity. The last two changes eliminated some pockets present in the ordinary three-necked flask and took advantage of the previous survey of currents. The special inlet tube was not, of course, needed for the experiments described in this paper but is of value in other reactions. The slight recession of the neck is a compromise between the flat base which would be ideal from the efficiency standpoint and the sharply recessed position which eliminates filling the neck with liquid. Even with this modification, a rubber seal had to be placed on the bearing in order to eliminate loss of liquid.

The propeller was constructed as described in the previous paper. The diameter was 3.18 cm. (1.25 inches). The six segments were set at an angle of 45°. It was located 12.5 mm. (0.5 inch) above the center of the inverted bottom of the flask. This propeller could not attain a speed of 10,000 r. p. m., but the agitation was greater than that attained by a four-bladed propeller which was 2.86 cm. (1.25 inches) in diameter which rotated easily at that velocity. If the angle at which the blades were set was less than 45°, the agitation was visibly lessened.

The motor (0.25 h. p.) and assembly were the same as described in the previous article (*3*).

The reaction mixture consisted of 43.5 grams of potassium permanganate, 16.7 grams of *p*-nitrotoluene, 7.5 grams of sodium hydroxide, and 750 grams of water. Two grams of manganese dioxide from a former experiment were added in order to ensure constant conditions with respect to all surfaces. The mixture except for the permanganate was heated to 70°, the oxidizing agent added, and the stirring increased to 9000 r. p. m. The reaction was exothermic and the flask had to be cooled constantly by an external bath. Stirring was continued for an hour at 9000 r. p. m. with the temperature maintained at about 75° C., after which the mixture was cooled to 10° C. and filtered through asbestos in order to remove *p*-nitrotoluene and manganese dioxide. The filtrate was then acidified with sulfurous acid, and the precipitate was filtered, redissolved in hot dilute sodium hydroxide, and filtered again. The acid was recovered by acidification and filtration, and was dried in an oven at 70° C. to constant weight. The manganese dioxide residue was extracted twice with 50 ml. of hot alcohol, from which the unused *p*-nitrotoluene was recovered by precipitation with water.

In two experiments the yield of acid obtained was 10.1 grams each time, of which 0.4 gram was material that had been lost in the washings. The recovered *p*-nitrotoluene was 4.4 and 4.3 grams, respectively. The loss in each case amounted to about 24 per cent. These results were substantially duplicated in a 3-necked flask which had four vertical creases, an inverted bottom, and a flat (not recessed) surface at the junction of neck and flask. A comparison run made in an ordinary three-necked flask without creases yielded 6.1 grams of acid and 10.1 grams of unchanged *p*-nitrotoluene. The loss was 10 per cent.

The percentage yields for the same peripheral velocity calculated from the data of Huber and Reid are listed in Table I, along with the average from the experiments in the creased and the result with the ordinary flask. The proportions of reagents employed in this work are the same as those used by Huber and Reid, but the comparison is not exact because the temperature at which they operated was that of the bath rather than of the reaction mixture. The temperature rise must have been considerable from both the heat of reaction and the mechanical work. The showing of the special flask is therefore probably better than the figures indicate.

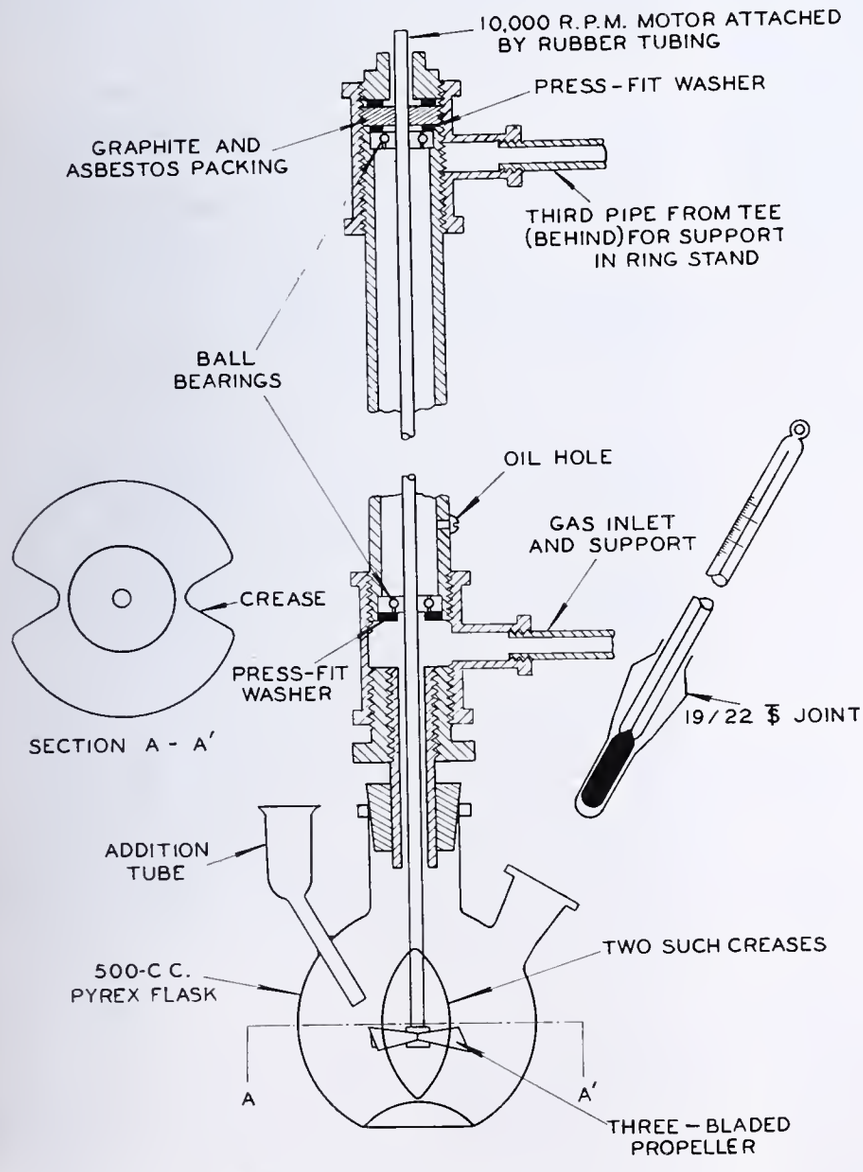

Figure 2. Improved Apparatus for High-Speed Stirring

Improved Apparatus (J. D.)

The design of an apparatus now being used regularly in this laboratory is shown in Figure 2.

This apparatus consists of two ball-bearing assemblies supported in a 15 × 2.2 cm. (6 × 0.875 inch) brass pipe and coupling arrangement. The upper T is tapped in order to make a continuous screw. The upper ball-bearing rests on a shoulder made by boring the inside of the pipe, so that a press-fit results. A washer, likewise turned to a press-fit, holds the ball-bearing in place. The lower ball-bearing is similarly fitted and held in place. The washer at the bottom must be carefully made since there is no other support for the bearing. The shaft (6.25 mm., 0.25 inch) is not attached to but fits closely in the bearings. The shaft with stirrer can therefore be removed without having to dismantle the support. This apparatus is mounted on a laboratory ring stand, which in turn sits on four rubber stoppers in order to dampen transmission of vibration to the desk top. This last precaution is not always necessary for quiet operation.

Dry nitrogen can be admitted through the lower pipe when desired. For reactions with organosodium compounds the nitrogen was not bubbled through the mixture but maintained at a very slight pressure by means of a mercury or oil trap.

The flask now in use with this apparatus has two instead of four creases. The change is not believed to detract seriously from the value of the flask and makes construction somewhat easier for the smaller sizes.

For some purposes and particularly at moderate speeds a simple yet adequate bearing can be made from ordinary glass tubing in which a glass or other stirring rod rotates. The seal is effected by a short piece of rubber tubing which projects beyond the lower end of the glass bearing just far enough to touch

and press gently on the rod. The upper end of the bearing is flared a trifle in order to eliminate undue friction. A drop of glycerol lubricates and improves the seal.

This device is practically the reverse of the well-known bearing with the rubber seal at the top, used often for stirring at reduced pressures. The seal at the top is not tight enough to permit use of the apparatus in preparation of organosodium compounds. That at the bottom has given entire satisfaction. The device is particularly good in place of a mercury seal because it offers less drag in stirring and spills no tiny particles of mercury. The seal should be a little distance above the flask, a change easily made with an adaptor, in order to reduce the softening action of organic solvents on rubber. Neoprene has proved better in this respect than ordinary rubber. A single rubber piece has been used in as many as ten or twelve runs in the preparation of organosodium compounds before it was necessary to replace it.

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Contribution from the Research Laboratory of Organic Chemistry, Massachusetts Institute of Technology, No. 270.

Apparatus for Crystallization and Filtration at Low Temperatures

F. W. QUACKENBUSH and H. STEENBOCK, University of Wisconsin, Madison, Wis.

BECAUSE of the technical difficulties involved, fractional crystallization has seldom been employed for the purification of low-melting substances. Brown and his co-workers (*1*, *2*) surmounted these difficulties by carrying out the necessary operations in a special room at a constant temperature of −20°, and were able to demonstrate the effectiveness of low-temperature fractionation as a means of separating unsaturated fatty acids. Wheeler and Riemenschneider (*4*) employed the method successfully in the preparation of pure oleic acid.

In this laboratory, the method has been adapted to a relatively small unit which permits both crystallization and filtration at temperatures as low as −75° C. The unit consists of a suitable assembly within a well-insulated chamber. The chamber is provided with means for external control, thus eliminating discomfort to the operator, and the apparatus is sufficiently simple to be practicable in any laboratory. Two units were constructed which differed chiefly with respect to size and means of effecting filtration. In the larger unit the solvent with suspended crystalline material was forced onto the funnel by means of air pressure. In the smaller unit transference of the funnel was effected by manually rotating the crystallization flask and its supporting shaft.

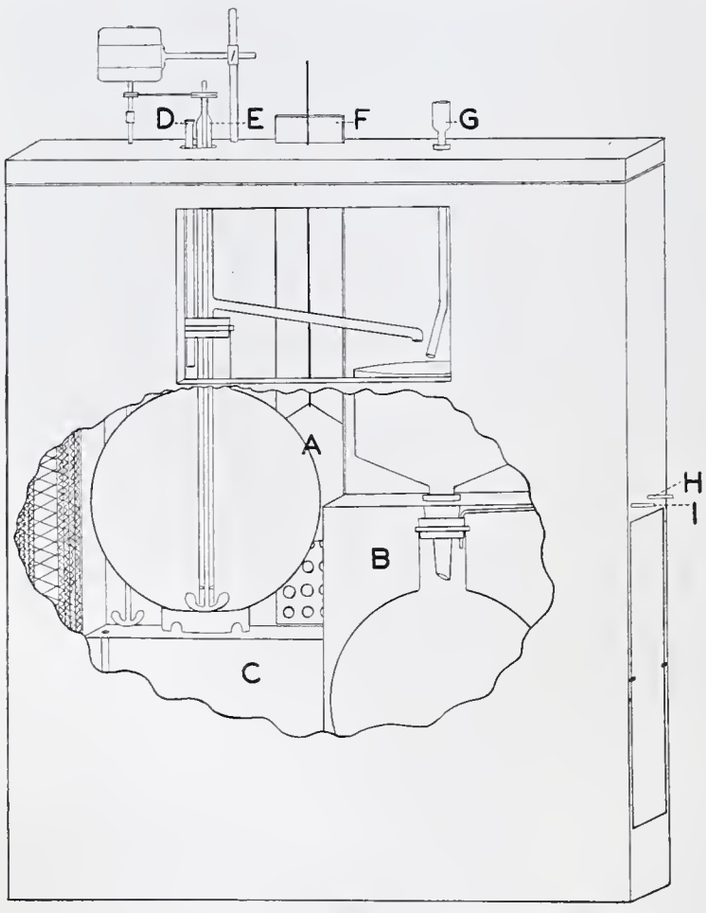

Figure 1. Large Crystallization Chamber

Construction of the Larger Apparatus

The apparatus consisted of an insulated chamber (Figure 1) the interior of which was divided into three compartments: *A*, for crystallization and filtration; *B*, for collection and removal of the filtrate; and *C*, a reservoir. The chamber (outside dimensions 62.5 × 40 × 75 cm., 25 × 16 × 30 inches, high) was constructed of galvanized sheet iron and wood and insulated with a 7.5-cm. (3-inch) thickness of packing-box paper and rock wool. An observation window (17.5 × 22.5 cm., 7 × 9 inches, triply glazed) permitted visibility of operations.

Compartment *A* contained the cooling liquid in which the crystallization flask and Büchner funnel were partially submerged. It was provided with a mechanical stirrer and a chute, *F* (2.5 × 7.5 cm., 1 × 3 inches), through which dry ice was introduced to produce the cooling. Circular openings, 1.25 cm. (0.5 inch) in diameter, at the lower end of the chute were covered with a sliding door to aid in controlling the rate of cooling. The crystallization flask (5-liter, round-bottomed) was set on the floor in a rigid steel collar (12.5 cm., 5 inches, in diameter × 2.5 cm., 1 inch, high), and was held in position by a removable wooden collar which was fitted over the neck of the flask and was anchored to the walls of the compartment. Two glass tubes extended into the flask from the exterior of the chamber. The smaller tube, *D* (14 mm.), which terminated in the neck of the flask, served for the introduction of the solution to be fractionated, for the insertion of the thermometer, and as an inlet for air during filtration. The T-tube, *E* (16 mm.), which extended to within 1.25 cm. (0.5 inch) of the bottom of the flask, served both as a bearing for a mechanically-driven glass stirrer and as a conduit for transferring the contents of the flask to the approximate center of a 20-cm. (8-inch) Büchner funnel. The stem of the funnel was held by a rubber stopper within a conical collar which extended from the floor of *A* into *B*.

Compartment *B*, which was accessible through a door at the end of the chamber, contained a 5-liter flask into which the stem of the Büchner funnel extended. A small glass tube, *I*, connected the flask to an aspirator.

Compartment *C* (25 × 25 × 20 cm., 10 × 10 × 8 inches, high) was gas-tight and served as a reservoir for the cooling medium from *A* during the removal of a solid fraction from the funnel. A tube (1.25-cm., 0.5-inch steel pipe) originating at the deep end of *A* extended to within 1.25 cm. (0.5 inch) of the floor of compartment *C*. A glass tube, *H*, connected the top of compartment *C* with a compressed-air line. It served for the admission or removal of air to transfer the cooling medium from one compartment to the other. A drain pipe extended from the bottom of *C* to the exterior.

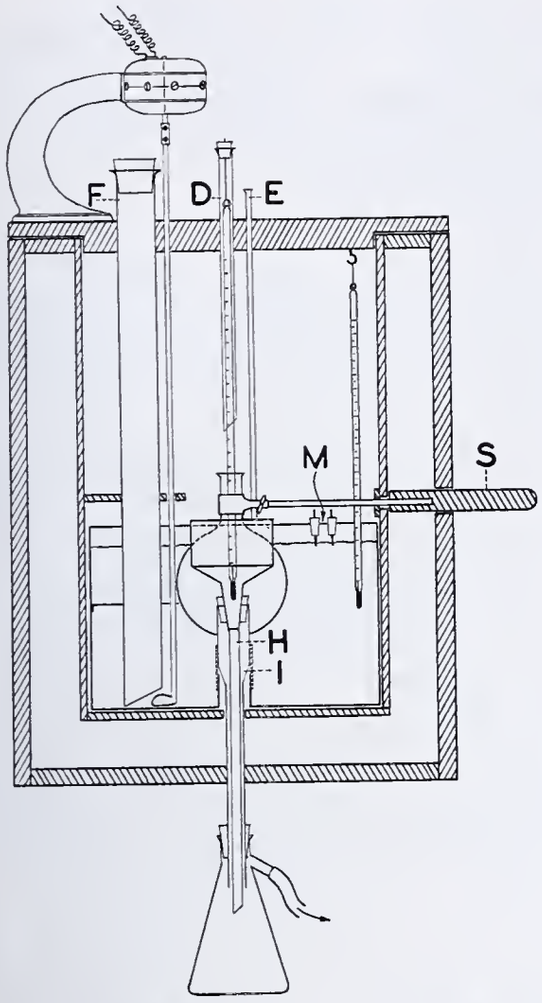

FIGURE 2. SMALL CRYSTALLIZATION CHAMBER

A small funnel, *G*, the neck of which extended through the top of the chamber to the Büchner funnel, served for the introduction of a warm solvent to dissolve solid fractions.

Operation

To perform a fractionation, tube *H* was clamped shut and alcohol sufficient to reach the neck of the crystallization flask (16 liters) was introduced into compartment *A*. A filter paper was fitted into the Büchner funnel, and the cover placed on the chamber. The solution to be fractionated was run into the crystallization flask through tube *D* and a thermometer then inserted through the tube. The stirrers were set in motion and dry ice was introduced through the chute, *F*, until the desired temperature was obtained. To lower the temperature to −75°, 5.4 to 6.7 kg. (12 to 15 pounds) of dry ice were required. To effect a filtration, the thermometer was removed, an atomizer bulb attached to *D*, and the contents of the flask were forced through the side arm of tube *E* into the funnel. Meanwhile, tube *I* was attached to an aspirator. The crystallization flask was rinsed with a small portion of solvent and when properly cooled the latter was forced into the Büchner funnel to wash the precipitate. The clamp was removed from *H* and the liquid from the bath allowed to flow into reservoir *C*. The flask containing the filtrate in *B* was replaced by a clean flask and the filtrate was returned to the crystallization flask. Warm solvent was introduced through tube *G* to wash the crystalline fraction from the funnel. The cooling medium in *C* was then forced back into *A* and more dry ice was added to produce the temperature desired for the next fraction.

Smaller Apparatus

The smaller chamber (Figure 2) (outside dimensions 40 × 40 × 50 cm., 16 × 16 × 20 inches, high) was constructed of wood and insulated with a 5-cm. (2-inch) thickness of packing-box paper. An observation window (15 × 15 cm., 6 × 6 inches) was triply glazed. The chamber was provided with a crystallization flask which was mounted on a rotatable support, *S*, a Büchner filtering apparatus, a steel tank (25 × 25 × 17.5 cm., 10 × 10 × 7 inches) for the cooling medium, and a mechanical stirrer. Three glass tubes entered the chamber through the cover: tube *D* (14 mm.) led to the crystallization flask, tube *E* (10 mm.) to the Büchner funnel, and tube *F* (40 mm.) to the bottom of the tank. From the Büchner funnel a glass tube, *H*, extended through a larger tube, *I*, to the suction flask underneath the chamber. The joints were made leakproof with tight-fitting rubber connections. A 500-cc. crystallization flask was used with a 10-cm. (4-inch) Büchner funnel. For smaller amounts of solution these were replaced with correspondingly smaller equipment. Capillary tube holders, *M*, provided convenient means for determining melting points during operation of the chamber.

Fractionations were performed much as with the larger unit. The solution was introduced into the crystallization flask through tube *D*, 5 liters of alcohol were poured into the tank, and sticks of dry ice were added through tube *F*. To cool the bath to −70°, 1.8 kg. (4 pounds) of dry ice were required. At the lower temperatures, cooling was hastened by raising *F* from the bottom of the tank. To effect filtration, support *S* was rotated manually, thereby pouring the contents of the crystallization flask into the funnel. The flask was rinsed with fresh solvent and, after proper cooling, the latter was poured over the precipitate. As with the larger apparatus, the filtrate was returned to the crystallization flask and warm solvent was used to remove the solid mass from the funnel. Since the funnel remained in contact with the cooling medium during all operations, quantitative removal of the solid material required relatively large amounts of hot solvent.

Applications

An application of the apparatus is shown in a typical experiment on the concentration of linoleic acid from a mixture of the fatty acids from corn oil (Table I).

The fatty acids from 450 grams of corn oil (Mazola) were dissolved in 4.5 liters of freshly distilled acetone and the solution allowed to cool in the chamber. After discarding a fraction obtained at −20°, crystalline fractions were collected at 10° intervals between −40° and −70°. Prior to each filtration the temperature was held constant for a minimal period of 30 minutes.

Assuming oleic acid to be the sole contaminant in the crystalline fractions, the iodine numbers indicated that the concentration of linoleic acid in the separating crystals reached a maximum of 88 per cent at −60°. These results compare favorably with those obtained by Brown and Stoner (*1*) in a room held at −20°.

The apparatus has also been applied effectively in the separation of isomers of linoleic acid (*3*) and in the preparation of oleic, linolenic, and arachidonic acids. It should be found useful in the purification of other types of compounds crystallizable only at low temperatures.

TABLE I. CRYSTALLIZATION OF CORN OIL FATTY ACIDS FROM ACETONE

Temperature ° C.	Weight of Fraction *Grams*	% of Total	Iodine Value (Wijs, 1 Hour)	Linoleic %
−40	71.0	17.0	116.0	28
−50	97.0	23.2	143.3	58
−60	88.5	21.2	169.9	88
−70	30.1	7.2	169.2	87
−70 (filtrate)	35.0	8.4	162.3	..

Acknowledgment

The authors are indebted to Lever Brothers Company for a grant in support of this work.

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PUBLISHED with the approval of the director of the Wisconsin Agricultural Experiment Station.

Enclosed Laboratory Apparatus for Effecting Repeated Crystallizations from Solvents

JOHN D. PIPER, N. A. KERSTEIN, AND A. G. FLEIGER
The Detroit Edison Company, Detroit, Mich.

IN 1937 the authors (*5*) described a type of enclosed glass apparatus in which repeated crystallizations of compounds from their own mother liquors were effected while these compounds were completely protected from atmospheric contamination. Many hygroscopic and oxygen-susceptible compounds were successfully purified in various modifications of this type of apparatus for use in research on dielectrics. Crystallization from the mother liquor was found, however, to be an ineffective method for removing colored impurities from some compounds, notably lauryl sulfonic acid (*4*). The apparatus described in the present paper was designed to permit the use of a solvent as well as to enable repeated crystallizations to be effected conveniently in a closed system.

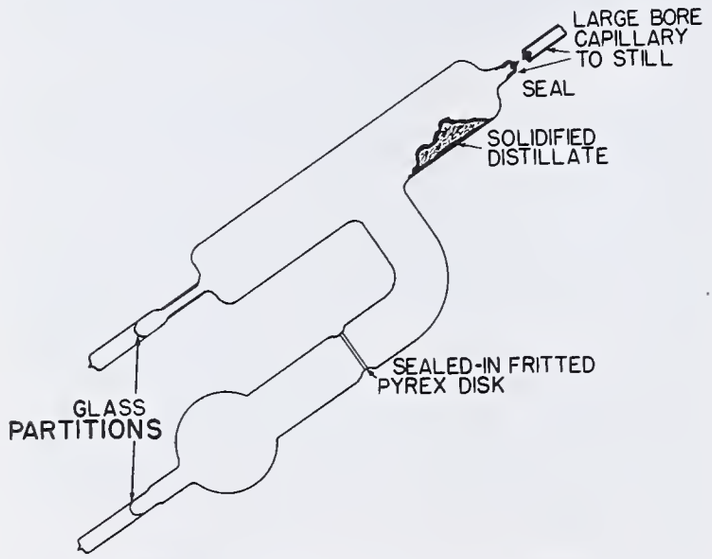

FIGURE 1. APPARATUS IN FILLING POSITION

Figure 1 shows the apparatus in the filling position just after it has been separated from a vacuum still for which the apparatus served as the receiver. The fritted Pyrex disk was made of coarse Pyrex powder, that was ground, sintered, and sealed in the tubing according to the techniques described by Kirk, Craig, and Rosenfels (*3*) and by Cool and Graham (*2*). Inasmuch as fritted disks in Pyrex tubes are now commercially available (*1*), the somewhat time-consuming task of constructing and sealing-in the disk is no longer necessary.

Figure 2 shows the apparatus with the solvent bulb attached and with the space between the glass partitions evacuated preparatory to breaking the partitions with the hammer enclosed within the bulb. The solvent must be more volatile than the impurities to be removed from the compound. The pressure within the bulb is usually the vapor pressure of the solvent, which is distilled into the bulb under vacuum.

After the partitions are broken the solvent is transferred to the compartment containing the compound and the bulb is detached by sealing off the large bore capillary and pulling the softened glass apart. Solution of the compound is next effected; if necessary, the entire apparatus is warmed. The solution is then passed through the fritted disk and is allowed to crystallize with the apparatus in the position shown in Figure 3. This prevents insoluble impurities from entering the compartment from which the purified compound is finally removed. If necessary, a cooling bath is used as is shown.

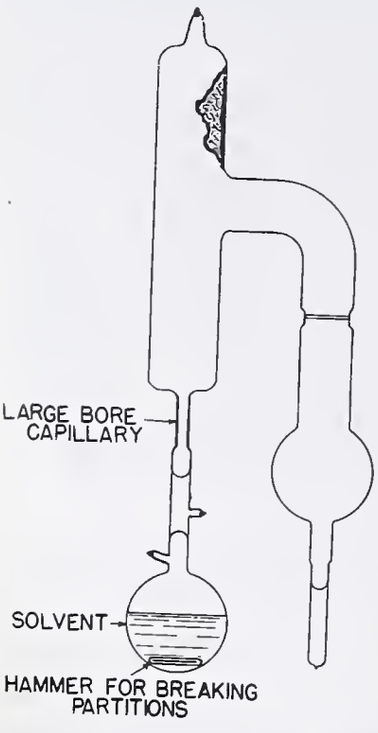

FIGURE 2. APPARATUS WITH SOLVENT BULB ATTACHED

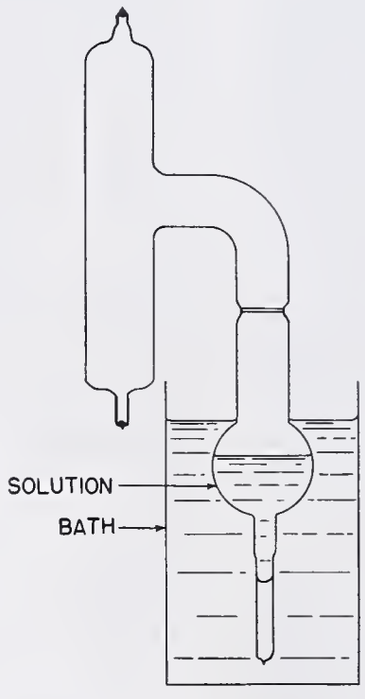

FIGURE 3. CRYSTALLIZATION POSITION

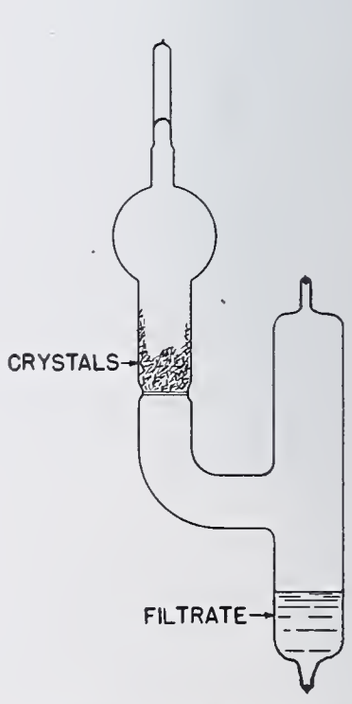

FIGURE 4. DRAINING POSITION

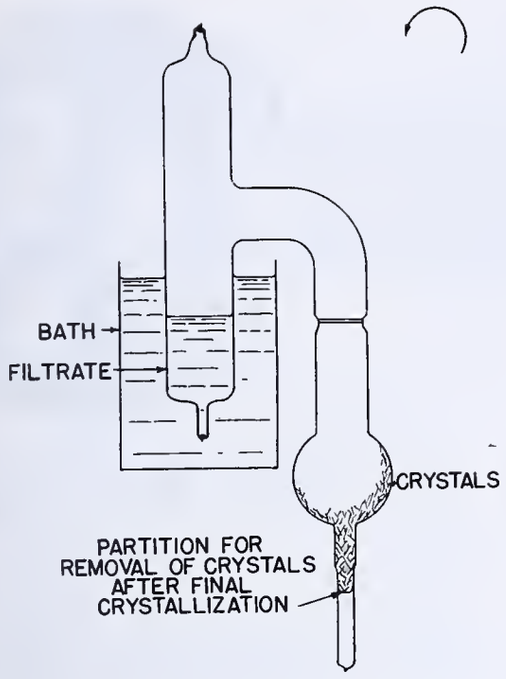

FIGURE 5. DISTILLATION POSITION

The first crop of crystals is obtained by inverting the apparatus and allowing the solution to drain through the fritted disk as is shown in Figure 4. Next the apparatus is rotated 180° in the direction shown by the arrow.

Figure 5 shows the apparatus in the position in which a part of the solvent is returned to the crystals by distillation. To effect the distillation either a heating bath is applied to the compartment containing the solution, as is shown, or a cooling bath is applied to the compartment containing the crystals. Re-solution and recrystallization are then effected in that order, and the apparatus is again inverted, this time in the direction of the arrow shown in Figure 5. The cycle is repeated as many times as is thought desirable. In the purification of lauryl sulfonic acid, which prior to crystallization was subjected to molecular distillation in a small all-glass still, three crystallizations from purified hexane were sufficient to remove all noticeable traces of colored impurity that persisted in the distillate.

Obviously, by applying the technique previously described (4), the apparatus is also suitable for crystallizing compounds from their own mother liquors.

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An All-Glass Buret System

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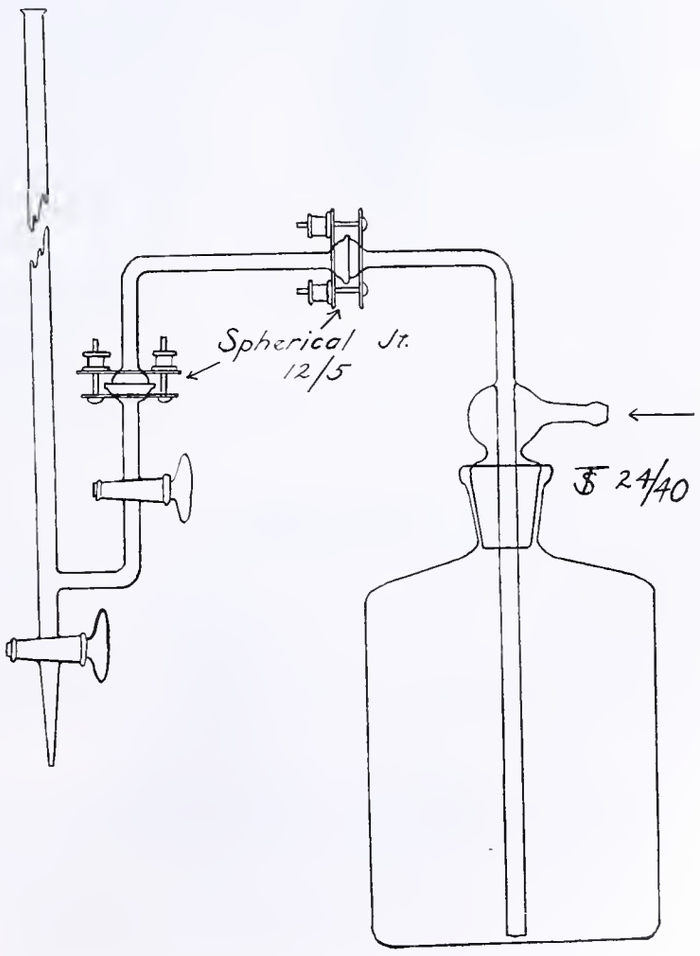
The Lankenau Hospital Research Institute, Philadelphia, Penna.

THE accompanying sketch illustrates an all-glass buret supply system which is convenient for solutions that find only occasional use. The necessary flexibility of the system is furnished by the use of ball and socket joints. The assembly may be easily dismantled and cleaned and used for different solutions and thus possesses an advantage over existing types of all-glass buret assemblies which are so complex that each buret is usually left permanently with the reagent bottle. The device has been used successfully with glacial acetic acid solutions, as well as with aqueous solutions of iodine, etc.



The system is easily constructed from standard items by one able to make simple seals. (These items, constructed from Pyrex may be obtained from the Ace Glass Co., Vineland, N. J., which also will fabricate the complete setup.) There are required one 24/40 gas inlet adapter and two complete 12/5 spherical (ball and socket) joints. The reagents are stored in 1-liter glass-stoppered (24/40) bottles. The buret may be filled by suction or by a hand-pressure bulb, or by suitably altering the design, gravity flow may be employed. It is convenient to have a series of burets of various sizes which may be used interchangeably as needed.

Technical Microscopy in the Rubber Industry

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FOR supplying information not obtainable by the usual methods the microscope is useful and often essential; not only will it disclose details which would otherwise be unseen, but by its unique methods of examination measurements of certain properties of materials can be readily secured which are not otherwise obtainable.

When one considers the value of the microscope to the rubber industry specifically, the study of the size of particles is usually the first and frequently the only application which comes to mind, although other uses have been suggested and described (*7*, *8*, *22*, *25*, *27*, *33*, *38*). The present paper not only describes the importance of the microscope in determining particle size and shape, but also covers its applications in the solution of other problems which ordinarily are not considered to lie in the field of microscopy. [Chamot (*9*) describes many interesting applications in different industries which indicate the unusual capabilities of microscopic methods.]

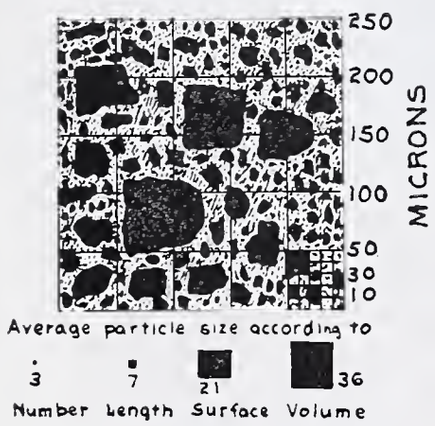

FIGURE 1. POWDERED COAL
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In a well-organized industrial laboratory, the microscope is used to supplement and aid other methods for examination of raw materials, control of factory processing, correlation of physical properties with service performance, and solution of factory difficulties. The specific examples cited in this paper represent uses which have originated in connection with the manufacture of rubber goods but they serve also to illustrate methods applicable to problems of the same type originating in other industries.

Examination of Raw Materials

For the accurate evaluation of pigments used in rubber the size and shape must be known accurately. Foreign material must be identified when it is present. Most of the microscopic methods used for measurement of particle size are described in an excellent summary by Chamot and Mason (*10*). Those methods invariably depend primarily on direct observation of the individual particles, but indirect methods are also useful. For example, Pohle (*33*) employed a "mass color effect" for determining the presence or absence of small particles; thin sections of rubber compounds loaded with pigments containing a high proportion of "colloidal" particles, such as channel blacks, exhibit a reddish-yellow transparency which may be distinguished easily from the neutral gray observed where the particles are largely noncolloidal. In the Goodrich laboratory also, this Tyndall phenomenon has been found useful. This effect was utilized quantitatively by Stutz and Pfund (*37*) and later by Stutz (*36*) alone in measuring the average particle size of zinc oxides.

The methods of determining particle size may logically be divided into two classes, those applicable to particles above the limit of resolution of the microscope and those applicable below that limit, although few commercial pigments contain particles which in size lie entirely in one class or the other.

GENERAL ASPECTS OF THE MEASUREMENT OF PARTICLE SIZE. In 1923 Perrott and Kinney (*32*) discussed the factors of fundamental importance in the use of particle size measurements. From one set of measurements obtained by microscopic observation, these authors showed that an "average particle size" may be calculated with respect to number, length, surface, or volume, and that the significance of an average particle size depends on which average is chosen for use. For example, in Figure 1 is shown a diagrammatic representation of the powdered coal with which Perrott and Kinney worked and the several averages obtained, these ranging from the average of 3 microns based on number to the 36 microns based on volume. In Figure 2 is shown diagrammatically the approximate distribution of particles in the sample of powdered

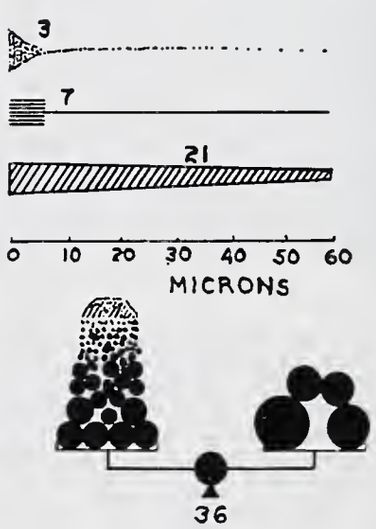

FIGURE 2. DISTRIBUTION OF PARTICLES IN POWDERED COAL

[1] Present address, National Rubber Machinery Company, Akron, Ohio.

coal by number, by diameter, by surface, and by weight. By number the small particles clearly predominate but by weight it is observed that relatively few of the larger particles are sufficient to balance a very large number of the smaller particles. Although this powdered coal is a material much larger than rubber or paint pigments, the difference in the "average sizes" is relatively as great for smaller particle sized pigments, since the nonuniformity is fundamentally responsible for the differences in the averages based on number, diameter, surface, or volume.

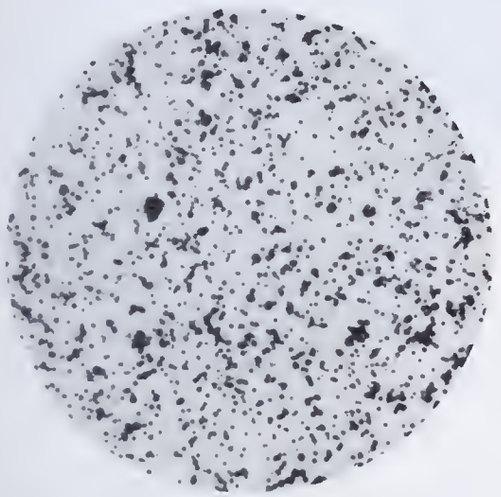

FIGURE 3. CARBON BLACK

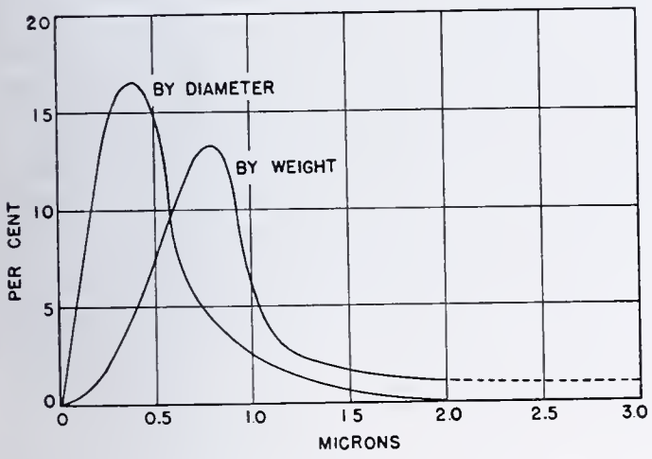

FIGURE 4. SIZE-DISTRIBUTION CURVES OF CARBON BLACK

The differences between these averages obviously indicate the necessity for obtaining that average particle size which is most significant in a specified use. Haslam (*23*) attempted to determine the rational choice for rubber technology. Using zinc oxides of different particle sizes, he found the $\bar{d}_1$ "diameter" to give essentially no correlation, $\bar{D}$ to give some correlation, and $\bar{d}_3$ to give fair correlation with the physical properties which the oxides imparted to the rubber compositions. [$\bar{D}$ expresses "the diameter of a hypothetical sphere such that the volume of n such spheres is equal to the actual volume of n particles" (*6*).] No calculations of the $\bar{d}_4$ diameter or results based on that value were presented, but the author's experience has indicated the $\bar{d}_4$ diameter to be of primary importance in rubber technology. By its use one can determine best the ratio of the active to the inert fractions of a given pigment. This is illustrated by the following example:

Figure 3 is a photomicrograph of a carbon black of rather large particle size, while Figure 4 shows size-distribution curves of this

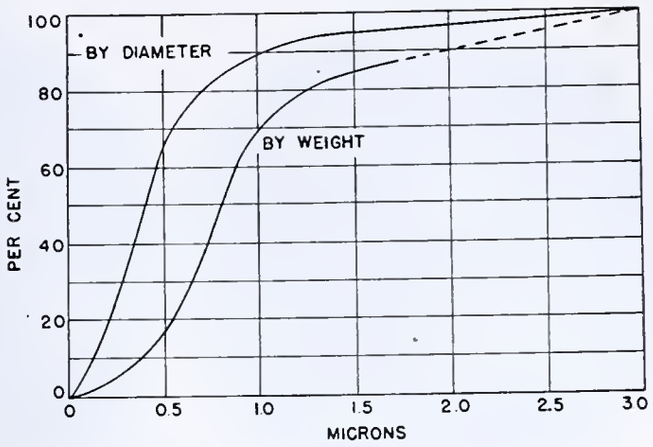

FIGURE 5. CUMULATIVE PER CENT CURVES OF CARBON BLACK

pigment. One curve is the size-distribution based on diameter, while the other is based on weight. The difference between the two curves is obviously large, this difference being evaluated quickly from the cumulative per cent curves (Figure 5). On the $\bar{d}_2$ curve the "average particle diameter" is 0.4 micron, a value on either side of which lies half of the sum total of the diameters of the particles; but the "weight" curve indicates that only about 10 per cent by weight of the particles lie below 0.4 micron while 90 per cent by weight comprise the fraction above this point. Which curve is most significant in evaluating the behavior of this pigment?

On the basis of physical tests of rubber compositions, 0.6 micron may be reasonably picked as the approximate dividing line between a nonreinforcing and a reinforcing pigment. At that point, the curve based on diameter indicates that nearly 75 per cent of the particles of this material possess reinforcing characteristics, a conclusion which is inconsistent with the properties imparted to rubber by this material. In contrast, the curve based on weight indicates that only about 25 per cent of the particles comprise a reinforcing fraction, as shown in Figure 6, and this is more consistent with the performance of the pigment in rubber. This may be visualized in another way: 20 volumes of this carbon black added to 100 volumes of rubber incorporate 5 volumes of reinforcing particles and 15 volumes of particles with no reinforcing value.

To procure the data necessary to calculate any of these "average sizes" is a laborious task. For example, Green (*19*) mentions 7 hours as the required time for five samples of zinc oxide. Obviously, these time-consuming methods should be avoided, yet values must be supplied which will accurately indicate the behavior to be expected of the sample in rubber. Since the microscopist in a rubber laboratory is called upon to report on the particle size of many pigments

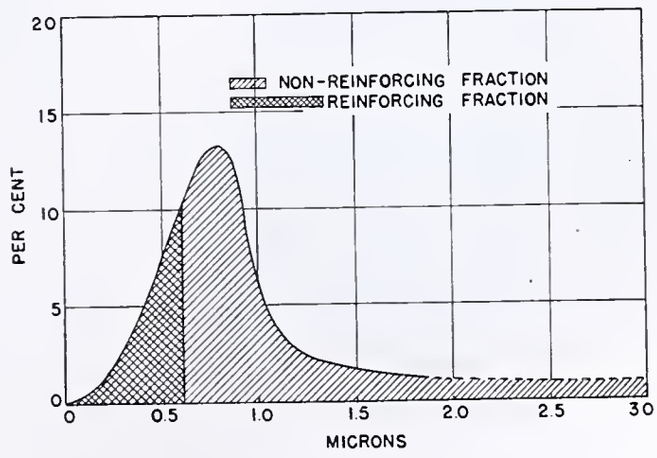

FIGURE 6. REINFORCING CHARACTERISTICS OF CARBON BLACK

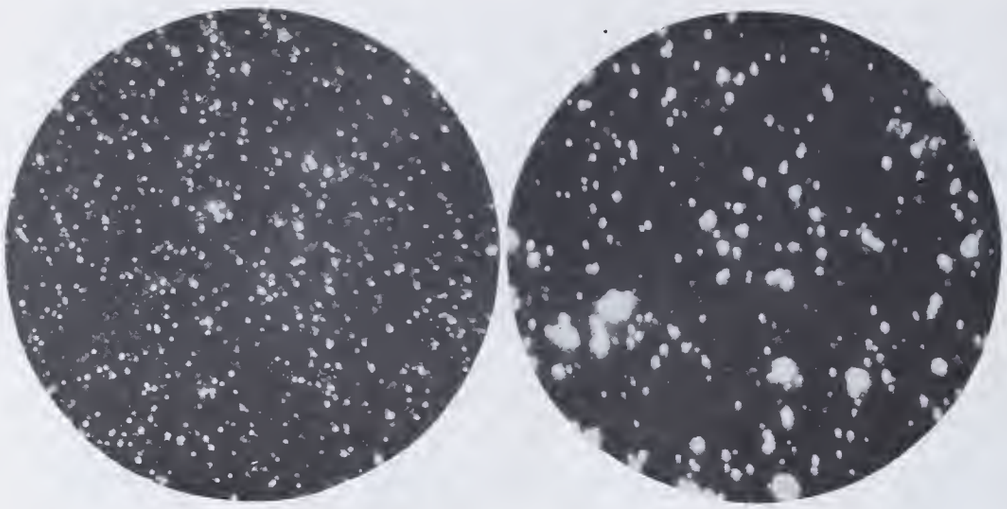

FIGURE 7. IRON OXIDES (×1000)

each day, the question of technique and method to be used thus becomes of paramount importance.

The first step in the measurement of particle size is the preparation of a suitable mount. The method employed in the Goodrich laboratory has been described recently by the author (*2*). For microscopic examination of the mount, nothing short of perfect optical conditions can be tolerated if an accurate final result is to be obtained. For pigments of 5 microns and under, dark-field is used, not only that every particle may be more readily observed but also because of the possibility of obtaining some idea of the size of particles below the limit of resolution.

For illumination a 108-watt, 6-volt ribbon-filament lamp fitted with an aspheric condenser is employed, placed to give a parallel, correctly centered beam. A cardioid dark-field condenser is fitted to the substage of the microscope. The most commonly used optics is a 4-mm. dry apochromat (N. A. 0.95) with correction collar, and an ×20 compensating eyepiece fitted with an ocular micrometer with graduations equivalent to 1.25 microns. For a few pigments a dark-field condenser—e. g., the Zeiss of "Leuchtbild"—which permits the use of an objective of N. A. 1.30 is useful. Critical illumination must be employed and the cover glass correction must be adjusted to the minimum of spherical aberration. The optical requirements throughout are simple but rigid.

On the sample under examination, the observer proceeds to construct mentally a size-distribution curve. If necessary he prepares a mount of a standard sample from which a size-distribution curve has been prepared accurately and uses this as a means of refreshing his memory. Using the specific optics just mentioned particles down to about 0.4 micron (theoretically 0.33 micron) may be resolved. The observer notes the minimum and maximum size and makes an estimate of the per cent by weight above and below 0.4 micron. The size of the particles below 0.4 micron, which are not resolved under the described conditions, may be judged on the basis of the decrease in intensity of diffracted light with decreasing particle size. The fraction above 0.4 is, in most pigments, of greater consequence in fixing the average particle size than the fraction below 0.4 micron, but neither fraction may be neglected. Having performed mentally these separate steps, the observer makes an estimate of the average particle size based on weight. Figures 7 and 8 summarize some of the steps in this procedure. Figure 7 is a photomicrograph of a standard iron oxide (left), and of an iron oxide (right) which was sent in for evaluation, and Figure 8 is a copy of the report which was sent to the Raw Materials Division on this particular sample. It is extremely rare that such a particle size evaluation fails to indicate precisely the properties which an unknown pigment will produce in rubber.

The success of this method depends primarily on the proper use of a dark field, which permits a rapid determination of the distribution of particle sizes. In the author's opinion dark-field methods should be more commonly employed. This lack of use may have resulted, partially at least, from various discouraging notes in the literature—for example (*16*), "While centering the condenser and objective is important with a bright-field condenser, it is far more important ..... with a dark-field condenser", a statement which implies that slipshod manipulation will result in some sort of an image with bright-field while with dark-field perfect technique is imperative; it is obvious that perfect technique should be employed with all methods. Even technicians otherwise faultless have contributed to the impression that dark-field is of little value (*21*). "Nothing is gained and in fact a great deal lost, by attempting to employ dark ground illumination, or the ultramicroscope. In this latter case it is impossible to distinguish small aggregates from individual particles." Since by dark-field it is readily possible to distinguish agglomerates or aggregates from individual particles it is regrettable that this most useful capability of dark-field should be reported erroneously.

This general procedure is satisfactory for the majority of rubber pigments, but for one important class—namely, those rubber reinforcing pigments in which all the particles lie below the limit of resolution of oil-immersion objectives (about 0.20 micron)—a different procedure is needed.

METHOD FOR PARTICLE SIZE MEASUREMENT BELOW THE RESOLVING POWER. Bright-field microscopy using ultraviolet light offers some advantage in increased resolution (*24*) of small particles but has not proved of extreme value in the study of channel gas blacks. It has been appreciated that size-distribution curves of these materials would be of great value, as they are with larger particle sized pigments, but it is only recently that these curves have been obtained. Parkinson (*31*), in an admirable investigation by centrifugal fractionation, reports the size-distribution curve for channel black. Equally fascinating are the results obtained with the electron microscope, the work of Wiegand and Prebus (*11*). It seems probable that electron microscopy may be utilized eventually even for control purposes, but at present the results are most interesting in their theoretical application in the study of channel black.

November 21, 1938.

Mr. John Doe,
Raw Materials Dept.

Code XYZ

This sample of iron oxide is considerably coarser than our type. It shows a larger maximum size, a higher average size and a different size-distribution. Undoubtedly it possesses less tinting strength than our standard iron oxide.

The particles in Code XYZ have the same shape as those in the type, i.e., essentially spherical.

	Min.	Max.	Aver.	% by Weight Below 1 μ
Standard iron oxide	0.1	2.0	0.7	80
Code XYZ	0.1	5.0	1.7	20

Microscopic Laboratory.

Per

FIGURE 8. REPORT ON IRON OXIDE

In the Goodrich laboratory, one aim has been the development of a method for particle size evaluation in this region below the resolving power which would be accurate and suitable for control purposes. For these pigments, the first method (*3*) used was an adaptation of the Siedentopf-Szigmondy (*35*) procedure with gold sols. A small, known amount of carbon black was dispersed in the cleanest available

rubber and the number of particles of black in a small volume were counted in a dark-field microscope. From the concentration of black, the density of the black, and the number of particles counted in the known volume, the average diameter could be calculated. This average is the $\bar{D}$ diameter.

FIGURE 9. CHANNEL BLACKS (×15)

The method was not very satisfactory because of the inevitable presence of small particles in the rubber itself, which made necessary a large and somewhat uncertain correction factor. Gehman and Morris (*17*) made a notable advance in this art by dispersing carbon black in rubber, then making a cement with a clear, colorless mineral oil such as Nujol. Thus by the use of an optically empty diluting medium the concentration of carbon black particles is reduced to the point where they may be separately observable in a dark-field microscope, and the correction for the foreign particles in the mixed dispersion medium is minimized. This count method has been investigated by a committee for the A. S. T. M. with the aim of discovering the sources of error which at first prevented concordant results among different laboratories (*5*).

This "proposed" method is now used extensively for particle size measurements in the region below about 0.20 micron. It is used frequently also for pigments in which the particles are predominantly above this limit of resolution and for which other methods should be used because of the distinct nonuniformity of sizes. In such instances, this D diameter is smaller than an average size based on weight (D_4). The erroneous results in rubber technology have been pointed out earlier in this paper. Nevertheless, this method is invaluable for the measurement of particle sizes below the limit of resolution and for determining differences, for example, in various lots of the same type of pigment. Its principal disadvantage lies in the amount of time required and in the tediousness of the counting. In order to avoid these practical difficulties a relatively fast and accurate method has been developed in the Goodrich laboratory.

This method for channel blacks was developed in 1930 and may be characterized as a combination of the Siedentopf-Szigmondy principle and the method of the author published in that year (*1*). It depends on the observation of two microsections of rubber, containing the same concentration of the pigment under examination, placed in juxtaposition on the slide. In carrying out this method, 90 parts by weight of the cleanest available rubber and 10 parts of the specimen carbon black are mixed on a small rubber mill with scrupulous care to avoid loss and contamination. Perfect dispersion must be secured, this being judged by a prescribed method (*1*). Next, a small amount of the master batch is diluted by mixing with clean rubber on a 12-inch roll mill, to a finished concentration of 0.1 per cent of black. The presence of the inevitable foreign particles in rubber is of minor consequence with concentrations of carbon black as high as 0.1 per cent. The stock thus prepared is mounted under the same cover glass, side by side with a similar stock containing a standard carbon black, the particle size of which has been measured, for example, by the count method. In the completed preparation, which should be about 1 micron thick, the two small pieces should just touch but not merge. This is shown in Figure 9 at low magnification.

In a sense this procedure is an application of the principle of the comparison microscope, for by it two separate objects, of the same pigment concentration and thickness of section and illuminated with the same condenser, may be observed with the same objective. The preparation of Figure 9 is shown at higher magnifications in Figure 10. These two specimens represent two channel blacks, one of which has an average of 0.065 and the other of 0.092 micron as measured by the count method. Observation in the microscope shows interesting differences. First, at low magnifications (×100 to ×400) the larger sized black gives in general a more brilliant image than the smaller. Closer examination reveals that this occurs largely because of the higher content in the 0.092 sample of the large sized particles in these pigments. Second, at higher magnifications (×800 to ×1500) it may be observed that the total number of particles is greater in the 0.065 sample, which may be expected because the average size is smaller. This high magnification observation is revealed only imperfectly in Figure 10 (right).

An unknown carbon black is thus compared with one or two standards, close observation being given to the relative number of the larger particles and the total number of particles in equal volumes. It is possible by this method to estimate rather accurately the average particle size of the unknown, the method having a precision of the order of 0.01 micron in the range from 0.05 to 0.10. The figure obtained is equivalent to the $\bar{D}$ diameter and because of the uniformity in particle size of channel blacks, it is sufficiently good to

FIGURE 10. CHANNEL BLACKS
Left, ×100
Right, ×100

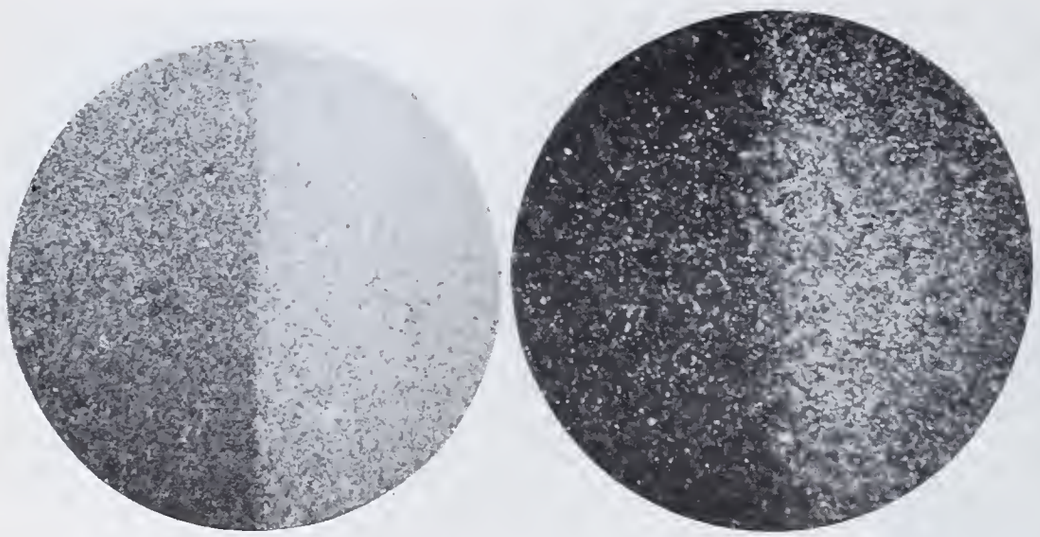

FIGURE 11. THERMAL DECOMPOSITION BLACKS (LEFT) AND ZINC OXIDES (RIGHT) (×300)

provide an evaluation of the material in rubber, even for such a significant property as tread wear.

As a matter of general interest two other pairs of small particle sized pigments are shown in comparison by this method (Figure 11), two thermal decomposition blacks and two zinc oxides. The method extends considerably the use of the microscope as an effective and rapidly operable tool for the comparative study of pigments which are below its resolving power.

One of the blacks is P-33 carbon black and the other an experimental P-33 (smaller). For these samples and permission to publish, grateful acknowledgment is made to R. B. Moore, formerly with the Thermatomic Carbon Company, and to A. W. Campbell of the Commercial Solvents Corporation.

The zinc oxides are Kadox and an experimental Kadox (smaller). For these samples and permission to publish, grateful acknowledgment is made to the New Jersey Zinc Company.

OBSERVATION OF PARTICLE SHAPE. For many years it was suspected (*15*, *39*, *40*) and gradually became known, that any pronounced departure from a spherical shape of the individual pigment particles produces a rubber compound with characteristic properties. Most clays, some magnesium carbonates, some zinc oxides, some whitings, and a few other pigments in rubber impart properties which may be related directly to the needlelike or the platelike shape of the particles. Among the properties which are profoundly affected by a nonspherical shape is the hysteresis of a rubber compound. In Figure 12 are shown the first hysteresis loops of two rubber compounds, one of which contains 20 volumes of a spherical zinc oxide while the other, that showing the greater hysteresis loss, contains an equal quantity of a zinc oxide of predominantly acicular shape. The respective oxides are shown in Figure 13.

Such observations indicate that, to be useful for the rubber technologist, a report on an unknown pigment sample must include the fullest possible information on the particle shape as well as the average particle size. To obtain this information on pigments with particles above the limit of resolution is relatively simple. The percentage of particles which are acicular may be readily estimated when the usual preparation (*2*) is examined in a dark-field microscope. When a pigment sample is composed principally of thin plates, as in some samples of clay, for example, it is often desirable to rub out on a slide a small amount of the pigment in an oil having a high refractive index. (A thick mixture of clarified cassia oil and tolu balsam, n = about 1.61, is satisfactory.) When this preparation is then examined in dark-field, the slight flow of the particles aids in securing an accurate idea of the predominant particle shape. With particles below the re-

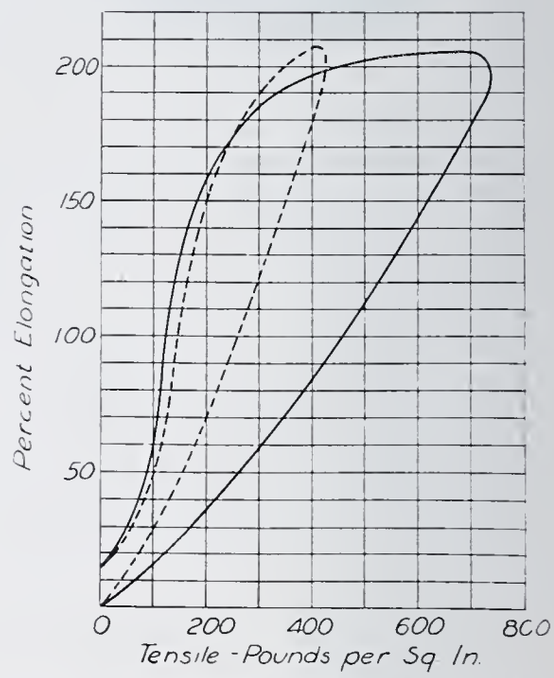

FIGURE 12. HYSTERESIS LOOPS OF TWO RUBBER COMPOUNDS

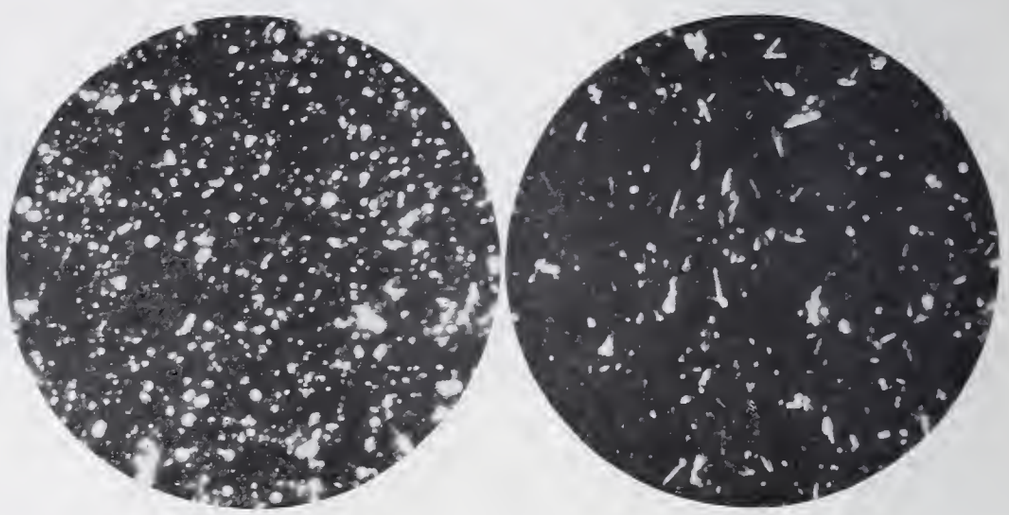

FIGURE 13. ZINC OXIDES (×1000)

solving power, since the direct observation of shape is of course not possible, the Szegvari azimuth diaphragm will be found useful.

This discussion has emphasized that the microscopic laboratory has the responsibility of providing precise, rapid, and useful information on particle size and shape for all divisions of the factory. The methods which have been described make it possible to maintain that efficient operative function. Rapid operation by these methods yields more satisfactory results than can be obtained by hours of measuring particle sizes by the laborious methods described in the literature. Many of the unsatisfactory conclusions in the attempted utilization of particle size measurements have resulted from the complications and tediousness of the method used. There are only three essential conditions to be fulfilled: good preparations, perfect optical conditions, and a trained eye and brain. Accurate conclusions of the effect of pigment particles in rubber may then be drawn from microscopic observation.

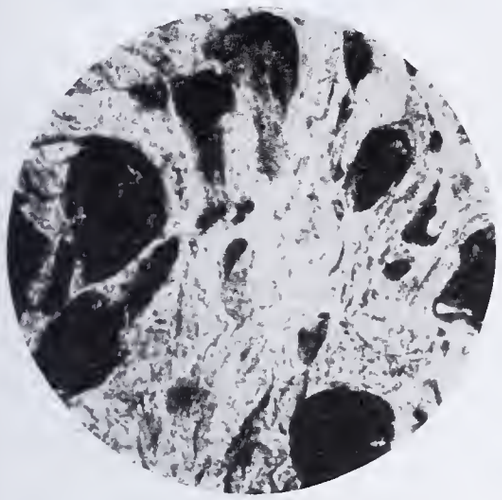

FIGURE 14. UNDISPERSED AGGLOMERATES (×200)

Control of Factory Processing

To the colloid chemist the term "dispersion" denotes the degree of subdivision of the particles in a suspension or a sol. To the rubber technologist dispersion or degree of dispersion denotes the extent to which the individual particles of a pigment are wet by and covered with rubber. The microscope provides the best direct means of determining the degree of dispersion of a pigment in a rubber stock. Various procedures have been described for preparing samples for the microscopic study of dispersion (*1*). The method developed and described by Roninger (*34*) is unique in its interesting application of metallographic methods to the study of rubber articles. The advantages of this method in the study of dispersion of pigments in rubber have been further investigated in the New Jersey Zinc Company Research Laboratory (*29*).

The usefulness of any method depends on the correlation between the microscopic appearance of a small sample and the gross properties of a large factory batch or finally the finished rubber article. All evidence indicates that the small sample is representative of the whole.

The method described by the author (*1*) for preparing rubber specimens for microscopic examination of dispersion has one advantage which experience shows to be of extreme practical importance. This superiority resides in the slight distortion produced in the rubber stock during squeezing, which assists in determining the character of any pigment agglomerates. By employment of this method the actual cause of poor dispersion may be learned—i. e., whether the result of using a pigment having inherent poor dispersibility or of improper or insufficient processing. This method also possesses a distinct advantage in time, since it is possible to prepare a section in 5 minutes or less, where other methods require from 30 minutes to an hour or more.

Until recently what might be termed the grosser aspect of dispersion has been the chief study in this field—i. e., the study of undispersed agglomerates of carbon black (*4*), zinc oxide (*12*, *20*), etc., ranging in size from a few to several hundred microns, exemplified in Figure 14. In 1938, Park and McClellan (*30*) summarized the work of a number of investigators, all of whom had suggested more or less specifically that the stiffening which occurs upon heating of carbon black–rubber stocks is caused by flocculation of the particles of black. The authors presented microscopic evidence that that flocculation does occur. Figure 15 was obtained according to Park and McClellan's procedure. On the left is a rubber stock containing 8 parts by volume of well-dispersed channel gas black, while on the right is the same rubber stock after heating for one hour at 135°. This sort of orientation of carbon black particles in rubber may be considered as an aspect of dispersion different from the gross dispersion which has been discussed.

Correlation of Physical Properties and Service Performance

The study of pigment dispersion, which vitally affects tread wear, is only one example of the usefulness of microscopic methods in determining and controlling the factors responsible for the service performance of a rubber article.

Wider experience in all phases of rubber goods production and use has also promoted the application of microscopy to the broader field involving the service performance of all types of rubber articles.

The automobile tire presents an almost limitless field for this kind of study. In the correlation of tread wear, for example, with the particle size of reinforcing pigments, the microscope has made many contributions. In the study

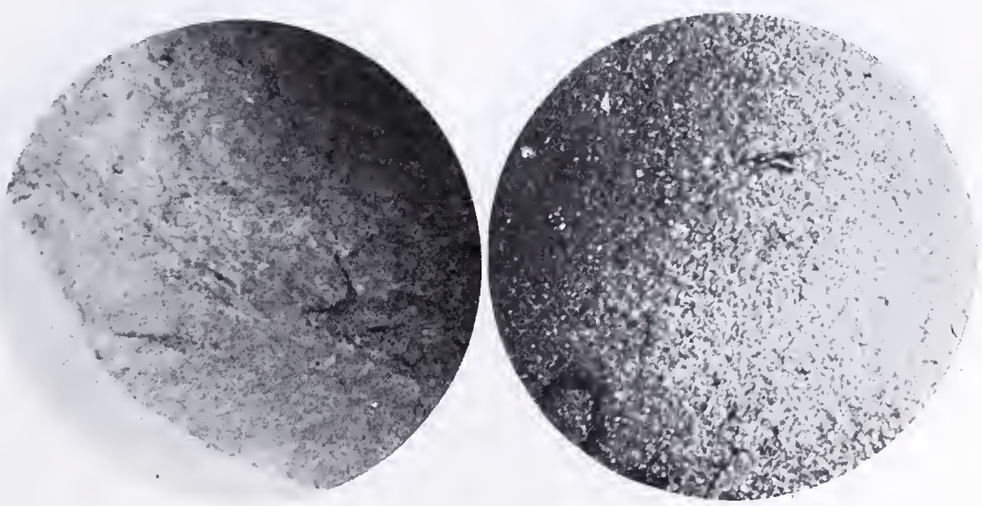

FIGURE 15. RUBBER STOCKS (×200)

of the tire carcass, microscopic methods have afforded the best means of determining the degree of impregnation of fabric by rubber (*13*, *34*) and have been extremely useful in studying the adhesion of rubber to the fabric of the carcass.

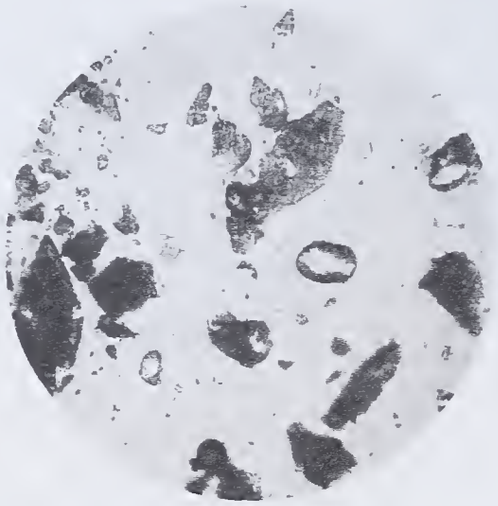

FIGURE 16. PORCELAIN IMPURITY (×100)

Almost all rubber articles are subjected constantly to experimental and development changes in order to produce goods which give superior service. In studying these experimental articles microscopic observations provide invaluable information on the cause of premature failure or of extraordinary service.

Solution of Factory Difficulties

In this important function of microscopy success depends to a large extent on a wide knowledge of chemical and physical principles, on an abundant experience in factory processes, on a broad acquaintance with many materials, and most of all on ingenuity in applying all this knowledge and experience to the problem in hand. The microscope is not a magic instrument which prints the answer to a question when an object is put on its stage. But if properly used with regard to its optical principles, it often gives the necessary clue which, combined with knowledge and reason, will solve many an otherwise unsolvable problem.

In a certain rubber stock appeared a hard, white, brittle foreign substance, small in amount but very objectionable. A single glance at the crushed substance in polarized light in the microscope (Figure 16) revealed definitely that it was porcelain or a porcelainlike substance. With the character of the impurity established, its source was traced to a raw material and finally to the porcelain-lined equipment used in the supplier's plant several hundred miles away.

A piece of white stock on which appeared some black streaks (Figure 17, left) was brought into the laboratory. One man claimed that the black streak was grease, while another, responsible for the greasing of the machine where this stock was processed, claimed that the calender operator had confused and mixed two stocks. The microscope made it possible to decide between these two alternatives. A small sample of the black contamination was removed and examined in a polarizing microscope; the appearance (Figure 17, right) proved unquestionably that the black streak was grease. Previous experience had shown that soapstone inevitably is present in high concentration in grease on machinery in a rubber factory and this provided the clue which solved the problem.

In many instances of this kind the microscope is invaluable; for it makes possible a quick, positive solution which in turn enables the operator immediately to correct an unsatisfactory situation.

In the examinations which are frequently required for identifying small amounts of foreign material, practically every facility offered by the microscope or microscopic methods is eventually used. When the observer is familiar with the foreign material, one glance may be sufficient to allow him to identify the substance. If not, he may make qualitative microchemical analyses according to the methods of Chamot and Mason, make spot tests according to Feigl, determine optical anisotropy or refractive index on small pieces under the microscope, or utilize some special property such as the fluorescence of the substance.

In many instances information obtained by ordinary means is almost valueless unless supplemented by information to be obtained with the microscope.

For example, a technical compounder wishes to know what pigments are present in a competitor's article. The stock is analyzed chemically and he receives a report as follows:

Probable Formula	
Rubber	54.50
Softeners	6.50
Total S	3.00
ZnO	4.00
Carbon black	14.00
$CaCO_3$	10.00
$BaSO_4$	8.00
	100.00

With this information alone, it would still be an almost endless task to reproduce the stock because there are several zinc oxides, carbon blacks, whitings, and barium sulfates available for use in rubber. The stock is examined under the microscope and the specific kind of the several materials is determined. The final report then reads as follows:

Probable Formula	
Rubber	54.50
Softeners	6.50
Total S	3.00
American process zinc oxide	4.00
Gastex	14.00
By-product whiting	10.00
Blanc fixe	8.00

Obviously the compounder now possesses definite information which, because more accurately defined, makes his task simpler. [Geohegan (*18*) describes the importance of similar information in the paper mill.]

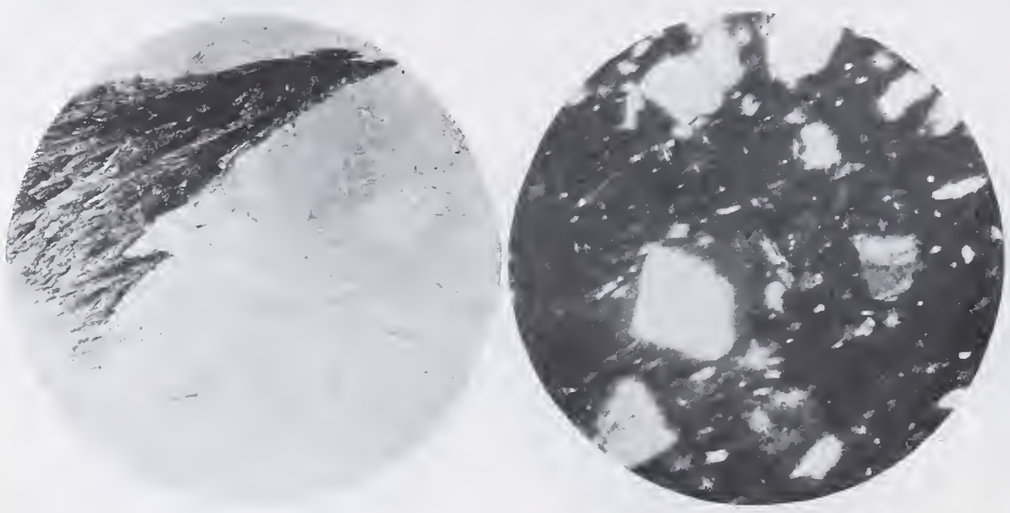

FIGURE 17. GREASE IMPURITY

Left, ×4
Right, ×100

FIGURE 18. SILICEOUS IMPURITY (×4)

Sometimes microscopical observation alone, with its possibility of securing a roughly quantitative idea of the pigment present, provides sufficient information for the technical compounder to begin the problem of reproducing a stock with the desired properties.

Microscopical studies must often be supplemented with knowledge outside the domain of optics, in order to furnish the clue which will solve a difficulty.

Sheet packing occasionally showed annoying white streaks (Figure 18). The foreign material was examined microscopically and found to be a siliceous material similar to clay. This fact alone did not solve the problem, but by observation of the manufacturing operation the essential clue was discovered. In producing this article a thin layer of an aqueous graphite paste was spread on its surface. As this paste dried, the siliceous particles in the graphite, being preferentially wet by the water, were carried to the surface. This siliceous matter, when dry, appeared as a white streak on the graphite-coated surface. The difficulty was eliminated by substituting a dispersion medium more compatible with graphite than with silica.

Technical men are interested in the subject of bloom. The steps which occur in the development of bloom have been described (*14*). Bloom can and does occur in both uncured and cured rubber. The accompanying photomicrographs of sulfur in a cured "pure gum" compound (Figures 19 to 21) were made to illustrate the steps in the development of bloom.

After a rubber stock is cured, the sulfur in excess of that actually soluble in the rubber precipitates in small particles throughout the mass. Solution of the small particles next occurs, followed by diffusion through the rubber and growth of the larger particles, which are dendritic in form, as shown in Figure 19 (upper left). This Ostwald ripening continues, and in the next stage (upper right) the form is still dendritic but the separate crystals making up the branches on the central staghorned tree are larger than those in the more delicate formations. At this stage diffusion to the surface commences—note that the relatively massive growths of Figure 19 (upper right) are connected to the surface of the sheet—and crystals of sulfur begin to appear on the surface. Eventually this sheet of rubber becomes perfectly clear inside and all its excess sulfur crystallizes on the surface in the form of perfect rhombs (lower left). Any bloom on rubber, whether sulfur, age resister, or other material, eventually develops as perfect crystals characteristic of the chemical composition. Figure 19 (lower right) is an example of an age resister bloom, showing perfect crystal form.

Bloom is seldom desired and its appearance usually indicates that a material has been incorrectly used or a process has not been properly conducted. Because a bloom usually consists of one pure chemical substance, its identification may be accomplished by determining one or more physical or chemical properties which are characteristic of that substance. Melting point, refractive index, qualitative chemical tests, solubilities, and crystal form may be used for identification. Since the actual quantity of bloom is generally small, the identifying procedure is executed either with the microscope—e. g., refractive index measurements—or by a spot test method.

A bloom sometimes causes some defect in the rubber article on which it appears—for example, the cause of peculiar bad aging in small isolated areas on a colored thin sheet article, evident to the naked eye (Figure 20, upper left), was investigated. Immediately after cure, some small dark spots could be observed on the surface and it had been concluded the dark spots were evidence of local high concentration of color. Examination under the microscope disclosed new and important facts. By

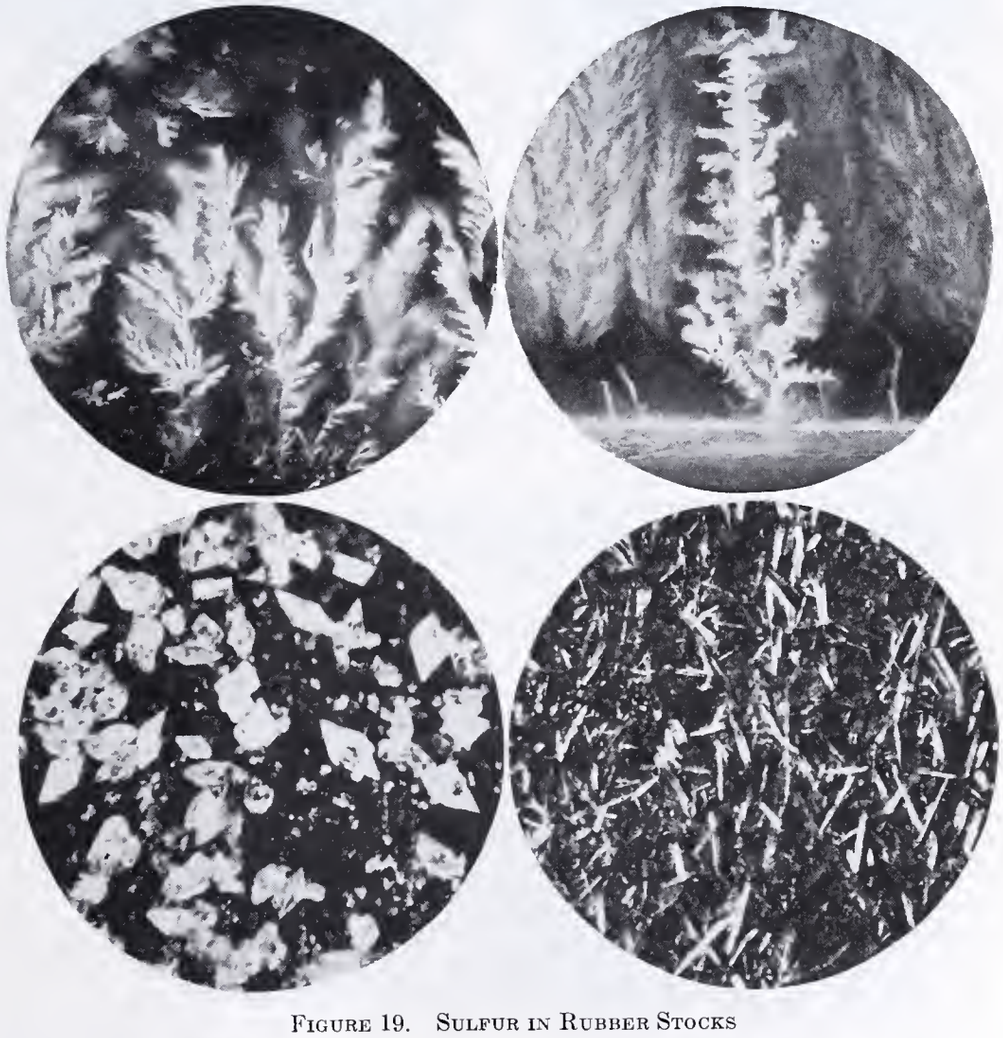

FIGURE 19. SULFUR IN RUBBER STOCKS
Upper, ×20
Lower, ×50

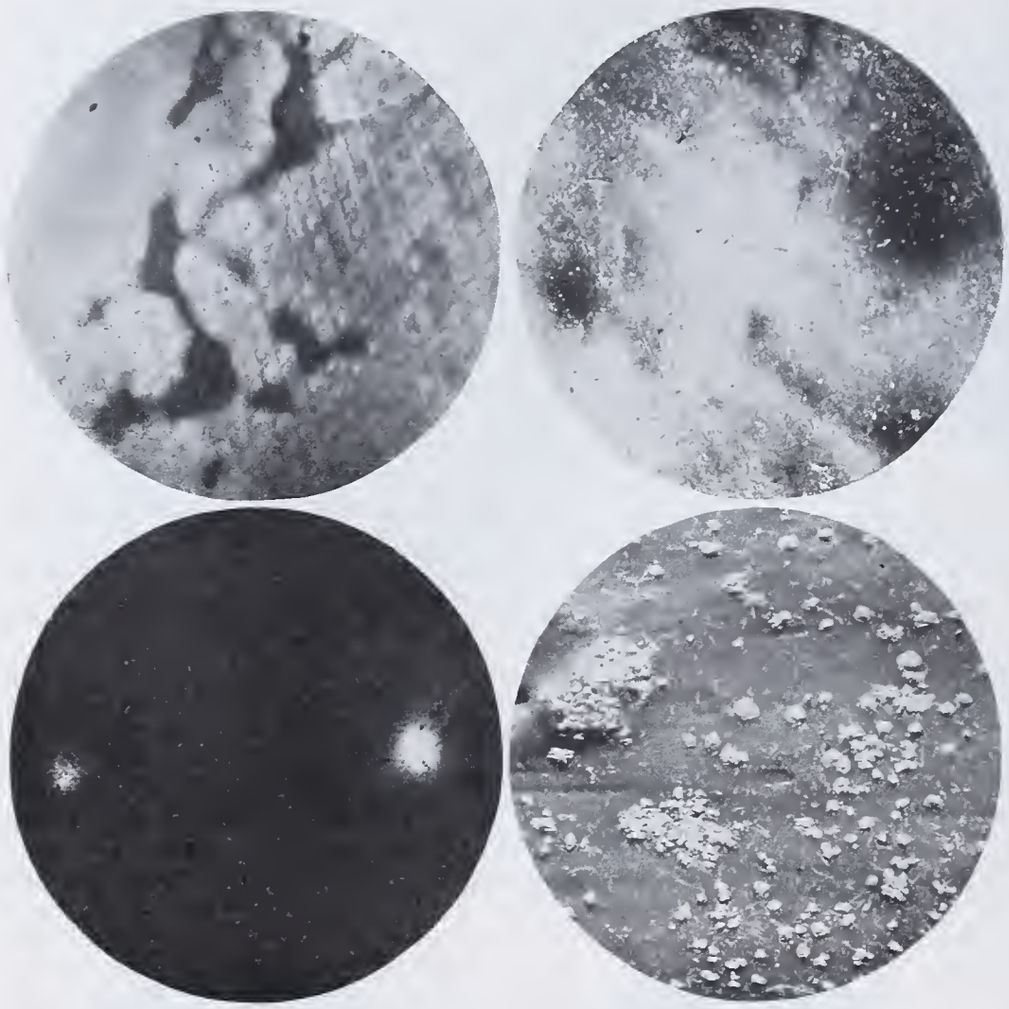

FIGURE 20. BLOOM IN RUBBER STOCKS

Upper left, ×4
Upper right and lower, ×50

reflected light it was found (upper right) that the spots were dark, but by transmitted light (lower left) the spots appear lighter than the surrounding area. This indicated not a high concentration of the coloring pigment but rather an excessive amount of some colorless substance which had pushed the coloring pigment aside. This substance was probably either sulfur or accelerator. By a microscopical examination of the uncured stock at high magnification (lower right) it was observed that the sulfur had bloomed profusely in small areas. This fact, which had not been disclosed by ordinary methods of observation, explained the poor local aging. At the beginning of the cure—i. e., when the rubber stock came in contact with a hot mold—the stock cured in these bloomed areas before the sulfur could become uniformly distributed by migration. Local overcuring consequently resulted. Knowing this explanation, it became possible to take proper measures to eliminate the cause.

Another case illustrates how one simple, accurate observation in the microscope may be sufficient to provide the clue which solves a difficulty. The article shown in Figure 21 (left) was apparently bloomed and a technical compounder requested an identification of the bloom. Observation under a Greenough binocular microscope showed that the condition was not a bloom at all. It was disclosed that the article had been turned inside out, and had been in bright light. Under the influence of the stretching and of the light, the surface had cracked rapidly, leaving small fibers of the rubber stock across the cracks (Figure 21, right). These small threads reflected a relatively large amount of light, giving a whitish appearance much like bloom; the error in analysis by naked eye observation alone was unavoidable.

Occasionally a customer returns a product with some complaint about its quality. Such a case occurred with a certain article which had presumably failed at a lamination. Careful microscopic observation of the failure (Figure 22) showed conclusively that the article had not failed but had been cut, probably accidentally; under the microscope the knife marks were plainly visible and the crack showed an appearance entirely different from that of a failure at a lamination.

Another case resulted in the deducing of an unusual chain of chemical reactions from one primary fact discovered microscopically. A certain lot of sheet packing had apparently developed mildew to an annoying extent. The white, fibrous efflorescence on the surface (Figure 23) unquestionably appeared like mildew to the eye, and still looked like mildew when it was examined in a Greenough microscope at 20 or even 100 diameters. When, however, the material was removed, mounted, and examined at higher powers, there were found no spores or oil sacks which are characteristic of mildew. Examination with polarized light disclosed an abundance of double refracting prisms which immediately suggested calcium sulfate ($CaSO_4.2H_2O$) which, however, could not have been present in the original composition. The identity of this postulated material was confirmed both by the extinction angle of the crystals and from qualitative microanalysis. Knowing the composition of the efflorescence still did not solve the mystery of its presence, but a combination of facts, some already known and some acquired at the time, made a logical, if unusual, chain of reactions fit into the final result. On the surface of the sheet some sulfur bloom had undoubtedly developed. The sheeting for a time had been stored in a roll, slightly damp, and in a warm place. Under these conditions the bloomed sulfur had oxidized, eventually forming dilute sulfuric acid with the moisture present, which in turn gradually reacted with the whiting in the sheet, forming calcium sulfate which

FIGURE 21. IDENTIFICATION OF BLOOM

Right, ×50

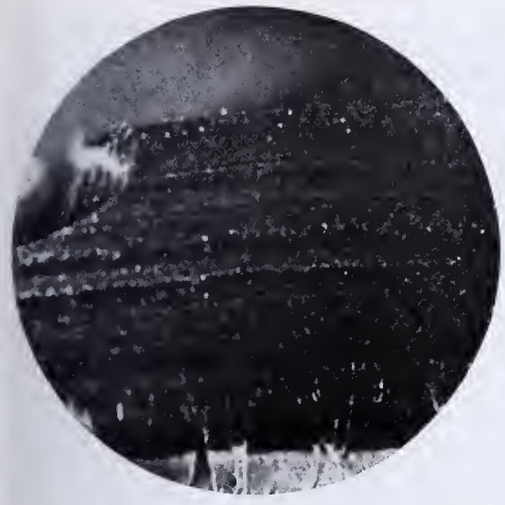

FIGURE 22. FAILURE DUE TO CUT (×20)

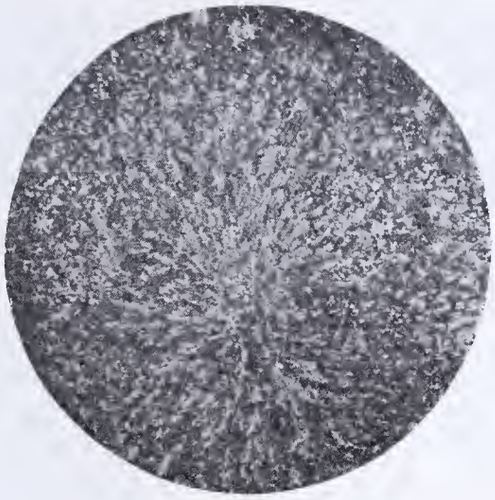

FIGURE 23. SHEET PACKING (×50)

returned sample was more than 2 years old. The fact was presented to the customer that the article had been lying on his shelf for that length of time and he agreed that the aging was not excessive.

This list of case histories in which problems, apparently not solvable by other means, have been solved by means of the microscope, could be largely extended. Space does not permit illustrating, by examples, every facility which the microscope affords. Frequent questions arise concerning the compatibility of rubber with rubberlike materials such as neoprene or Thiokol; the examination of a suitable preparation in a dark-field microscope is probably the most

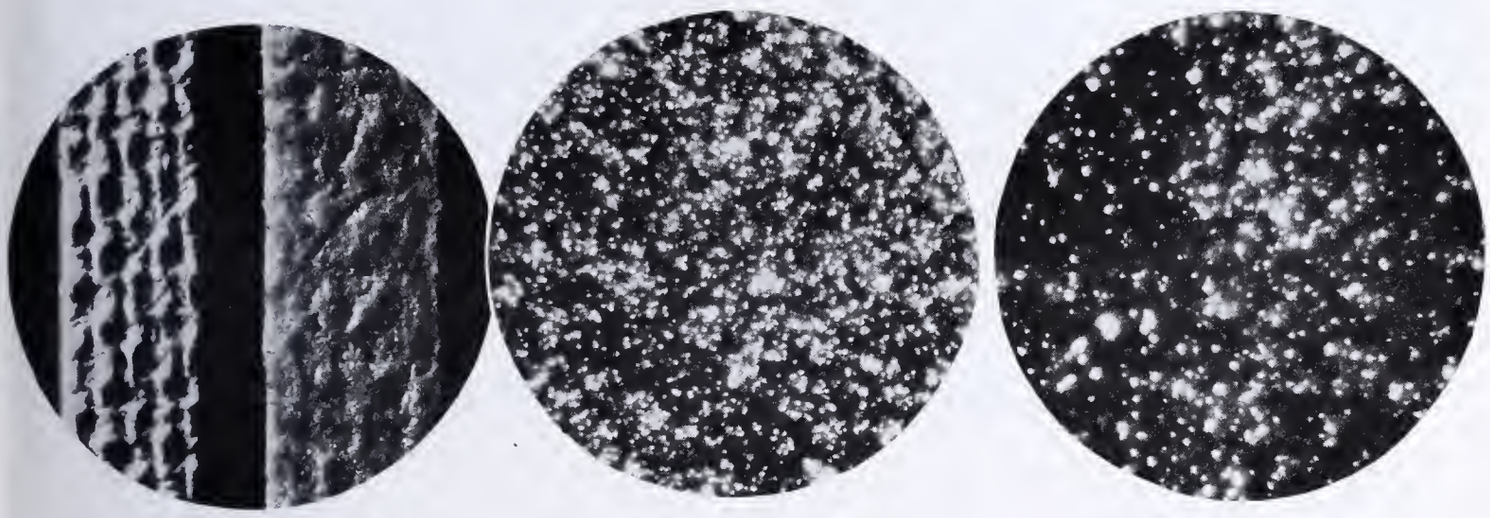

FIGURE 24. WHITE THREAD
Center and right, ×1000

eventually crystallized out in the form of more or less perfect prisms.

Articles returned by the customer for adjustment sometimes prove to be of a competitor's manufacture, and microscopical methods are often invaluable in disclosing this fact. For example, two samples of white thread were brought into the laboratory to determine whether they were of the same or different manufacture. The samples were first examined under a low-power microscope and Figure 24 (left) shows clearly that their surface appearances were different. Microsections were made of two samples (Figure 24, center and right) and again a difference could be observed; the pigmentation was not the same in particle size and possibly not in concentration. Finally a microqualitative analysis was made on the ash and the compositions were found to be entirely different. One was a standard Goodrich product, while the other was proved definitely to be that of a competitor.

Another case of a similar nature shows that even the science of deduction may be enlarged to unusual limits with the aid of the microscope. Some small cups were returned with the complaint that they showed premature aging. This seemed to the technical compounder who was responsible for their quality to be inconsistent with this knowledge of their aging behavior; however, the article, shown on the left in Figure 25, unquestionably was badly aged when compared with the new cup shown on the right. In the effort to find out whether the aging was premature, the fact was uncovered that the source of zinc oxide used for the rubber stock for this cup had been changed more than 2 years previously. Microsections were made and microscopical examination of the old and the new proved positively, from the character of the particles of the zinc oxides, that the

FIGURE 25. AGING OF SMALL CUP

direct and positive means of determining the existence of distinct phases and certainly one of the most rapid. Microcataphoresis methods have been used in the study of certain pigments and latex. Some problems require the use of metallographic methods; in many cases where vertical illumination is used polarized light is serviceable. Fluorescent microscopy is an elegant method, revealing information which is otherwise unobtainable (*26*, *28*).

Although many problems possess traits in common, a large proportion of cases display new features which require strictly individual and original treatment. This paper presents evidence that the microscope and microscopic methods disclose invaluable information, far less readily obtainable by other means and indeed frequently unobtainable by procedures other than those peculiar to the microscope.

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Determination of Alkoxyl Groups in Cellulose Ethers

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The Vieböck modification of the Zeisel method for the determination of alkoxyl groups has been studied in detail with special reference to the application of the method to the lower alkyl ethers of cellulose. An improved apparatus is shown and improvements which have been made in the technique are described. Particular attention is given to the use of solvents, such as phenol and organic acid anhydrides. It is not recommended that these be used. Attention is called to the necessity of using hydriodic acid of 56.5 to 57 per cent concentration when water-insoluble ethylcelluloses are to be determined.

THE rapidly increasing commercial importance of ethylcellulose and methylcellulose makes desirable a more universally acceptable method for the accurate determination of alkoxyl groups of cellulose ethers. It is particularly desirable that the method be suitable for a wide range of ethoxyl and methoxyl values.

During the past six years, the semimicromethod described herein has been used by the authors. The conclusions arrived at are based upon routine daily determinations performed on methyl- and ethylcelluloses of widely varying degrees of substitution.

The original Zeisel method (*10*) as modified by Pregl (*6*) has been generally adopted for determinations on a micro scale. It consists in heating the ether sample with a strong solution of hydriodic acid to form an alkyl iodide, which, by means of a stream of carbon dioxide, is swept through a condenser and finally absorbed in a solution of silver nitrate. The resulting silver iodide is separated and weighed. This procedure is cumbersome and is subject to the usual errors in a gravimetric technique.

The method was adapted to volumetric analysis by Vieböck and Schwappach (*8*) and Vieböck and Brecher (*7*) who substituted a mixture of bromine, acetic acid, and sodium acetate for the silver nitrate in the absorption tubes. Ethyl iodide absorbed in this mixture is oxidized to iodate, as shown in Equations 1 to 3. After reduction of the excess bromine with formic acid, the iodate is determined iodometrically, using potassium iodide and standard thiosulfate as shown in Equations 4 and 5.

$$ROC_2H_5 + HI = C_2H_5I + ROH \quad (1)$$

$$C_2H_5I + Br_2 = C_2H_5Br + IBr \quad (2)$$

$$IBr + 2Br_2 + 3H_2O = HIO_3 + 5HBr \quad (3)$$

$$HIO_3 + 5KI + 5H^+ = 3I_2 + 3H_2O + 5K^+ \quad (4)$$

$$I_2 + 2Na_2S_2O_3 = 2NaI + Na_2S_4O_6 \quad (5)$$

Apparatus

The details of the modified Zeisel apparatus are shown in Figure 1 and the complete assembly is shown diagrammatically in Figure 2. The apparatus consists of a boiling flask, *A*, fitted with side arm for introduction of carbon dioxide and connected to a column, *B*, which serves to separate aqueous hydriodic acid from the volatile alkyl iodide, which then passes through an aqueous suspension of red phosphorus in scrubber trap, *C*, and is ab-

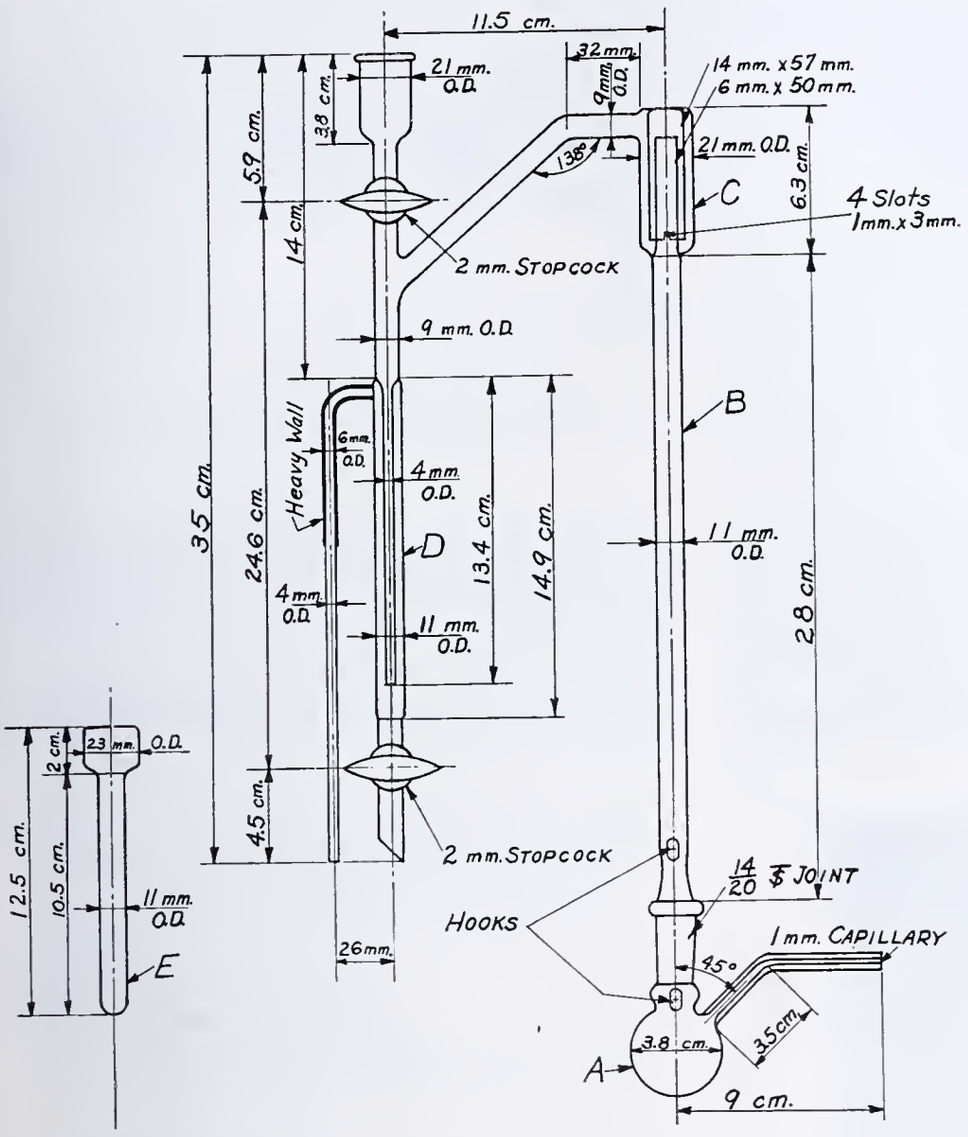


FIGURE 1. CONSTRUCTION DETAILS OF MODIFIED ZEISEL APPARATUS

sorbed in the bromine–acetic acid solution in absorbing tube *D*. A secondary adsorber, *E*, also containing bromine–acetic acid, ensures complete absorption of the alkyl iodide.

Many changes in the apparatus were made before the one shown in Figures 1 and 3 was finally adopted. Except for having a slightly smaller volume, the reaction flask, *A*, is the same as that suggested by Clark (*1*, *2*).

An apparatus having a long Vigreux column in conjunction with a trap containing glass wool instead of water was successfully used for several years. However, the glass wool was subject to attack from the hydriodic acid and, after a period of time, the effectiveness of the trap was somewhat lessened. More recently, it was found that a straight column and a water trap provide a simpler apparatus from the standpoint of construction and operation. The water trap, *C*, is similar to that of Vieböck (*7*, *8*), except it has no removable ground-glass joint. This trap is filled from the absorber end of the apparatus. The advantage of this type of trap is that there is no possibility of leaks through a ground-glass joint at the top.

The use of water to scrub out vapors of entrained iodine and hydriodic acid was first employed by Clark (*1*, *2*). Although this modification can be used successfully, an aqueous suspension of red phosphorus will eliminate any possibility of iodine being carried over into the absorbers. Recent experiments indicate that this method will give satisfactory results with materials containing considerable amounts of sulfur.

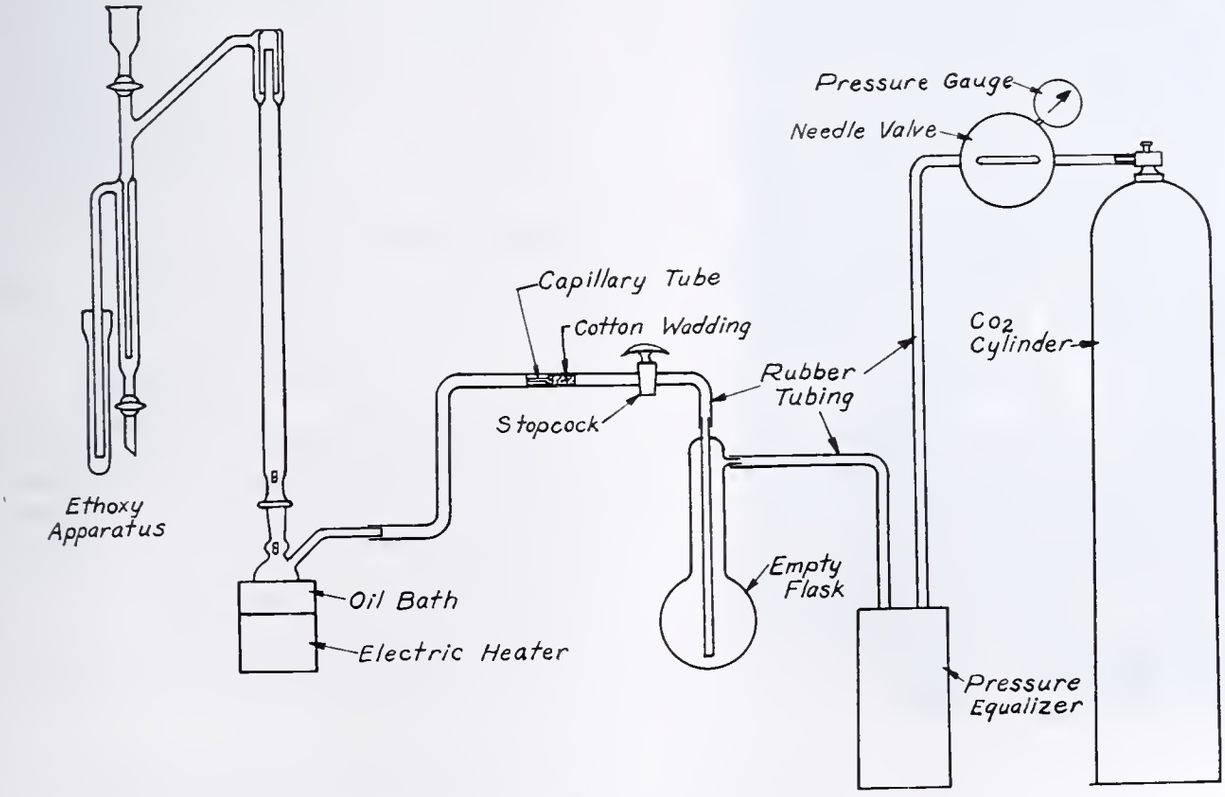


FIGURE 2. ASSEMBLY OF ETHOXYL APPARATUS

TABLE I. ETHOXYL DETERMINATIONS AT 40-MINUTE REACTION TIME

(Average of at least 2. Reaction mixture, 6 cc. of HI, sp. gr. 1.69)

	Gelatin Capsules[a]						Open Glass Capsules			
	p-Ethoxybenzoic acid[b]		p-Ethoxydiphenyl[c]		Ethylcellulose lot 4521	Ethylcellulose lot 996	p-Ethoxybenzoic acid[b]		Ethylcellulose lot 4521	Ethylcellulose lot 996
Added Reagents	Found	Calcd.	Found	Calcd.			Found	Calcd.		
None	27.0, 27.1	27.1	11.4	22.7	48.5, 48.8	48.0	27.2	27.1	47.1	47.1
2.5 cc. of phenol	26.8, 26.6	27.1	22.6	22.7	48.3	47.6	27.2	27.1	48.4	47.7
0.5 cc. of phenol	26.9	..	..	..	48.1	..	..	..	..	..
0.5 cc. of phenol + 0.5 cc. of propionic anhydride	26.9	..	..	..	47.6	..	..	..	..	..
0.4 cc. of propionic anhydride	..	..	..	..	48.5	..	..	..	..	..
1.0 cc. of propionic anhydride	..	..	..	..	46.6	..	..	..	..	..
Blank	0.3 cc. of 0.1 N $Na_2S_2O_3$						0.1 cc. of 0.1 N $Na_2S_2O_3$			

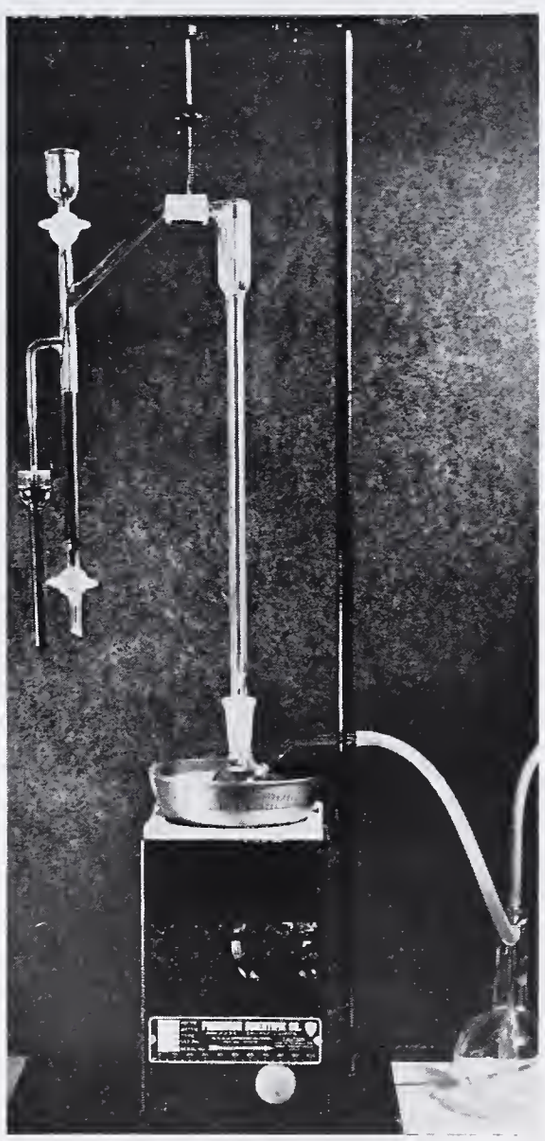
[a] Parke, Davis & Company Size 0.
[b] p-Ethoxybenzoic acid, Eastman c. p. grade, m. p. 196–198° C.
[c] p-Ethoxydiphenyl, Eastman c. p. grade, m. p. 71–72° C.

The use of ground-glass joints throughout is advisable; however, it is best to use as few joints of any kind as possible. In the apparatus proposed in this paper (Figures 1 and 3) only one joint is used between flask A and the final absorber, E.

E is attached to the side arm of the first absorber by means of a wire loop, making assemblage easy. A view of a single unit is shown in Figure 3 and a battery of any convenient number of such units may be run at the same time.

The source of carbon dioxide is a single cylinder, as indicated in Figure 2. A pressure of 2.27 kg. per sq. inch (5 pounds per square inch) is obtained by the use of a regular reducing valve. The gas is led through a surge chamber to minimize pressure fluctuations, then through an empty flask to a manifold containing the required number of outlets. From each outlet a rubber tube equipped with a stopcock and a capillary tube containing a cotton wadding filter, leads to A.

The heating bath consists of a long oil bath electrically heated and thermostatically controlled, with openings in the top large enough to allow immersion of the flasks in the oil approximately to the level of the contained hydriodic acid.



Courtesy, Dow Chemical Co.

FIGURE 3. INDIVIDUAL UNIT

TABLE II. METHOXYL DETERMINATIONS AT 40-MINUTE REACTION TIME

(Average of at least 2. Reaction mixture 6 cc. of HI, sp. gr. 1.69)

	Vanillin[a], Gelatin Capsules		Methylcellulose Lot M-1097	
Added Reagents	Found	Calcd.	Gelatin capsules	Open glass capsules
None	20.6	20.4	31.1	30.9
2.5 cc. of phenol	20.1	20.4	31.0	30.8

[a] Vanillin, Eastman c. p. grade m. p. 81–82° C.

TABLE III. EFFECT OF HYDRIODIC ACID CONCENTRATION UPON ALKOXYL CONTENT

Hydriodic Acid				p-Ethoxybenzoic Acid[c]	
Sp. gr. at 25° C.	Concn., % by wt.	Ethylcellulose[a]	Methylcellulose[b]	Found	Calcd.
1.694	56.7	48.6	31.0	27.0	27.1
		48.4	31.1	27.1	
1.672	55.8	47.1	31.0	26.9	27.1
		47.4	30.9	26.9	
1.665	55.5	47.7	31.1	26.9	27.1
		47.5	30.9	27.0	

[a] Ethocel, lot 4521.
[b] Methocel, lot M-1097.
[c] Eastman c. p., m. p. 196–198° C.

Experimental Work

This investigation has been devoted principally to a study of factors which affect the accuracy and simplicity of the method of analysis of methyl- and ethylcelluloses. The effect of hydriodic acid concentration was first studied. The effect of solvents, such as phenol, acetic anhydride, and propionic anhydride, alone and in various combinations, upon the determination was studied. Experiments were conducted to compare the suitability of gelatin capsules with glass containers for use in weighing samples. The analyses made upon samples of ethylcellulose, methylcellulose, and crystalline organic compounds to determine these effects are listed in Tables I and II.

The effect of concentration of hydriodic acid upon the value of the alkoxyl content is shown in Table III for water-insoluble

ethylcellulose, water-soluble methylcellulose, and a pure sample of *p*-ethoxy-benzoic acid. The data show that small variations in hydriodic acid content do not affect the reproducibility of values obtained with methylcellulose or with *p*-ethoxybenzoic acid, materials which are easily dissolved by the hydriodic acid, but do give low ethoxyl values for ethylcellulose when the specific gravity of the hydriodic acid used is less than 1.69 at 25° C.

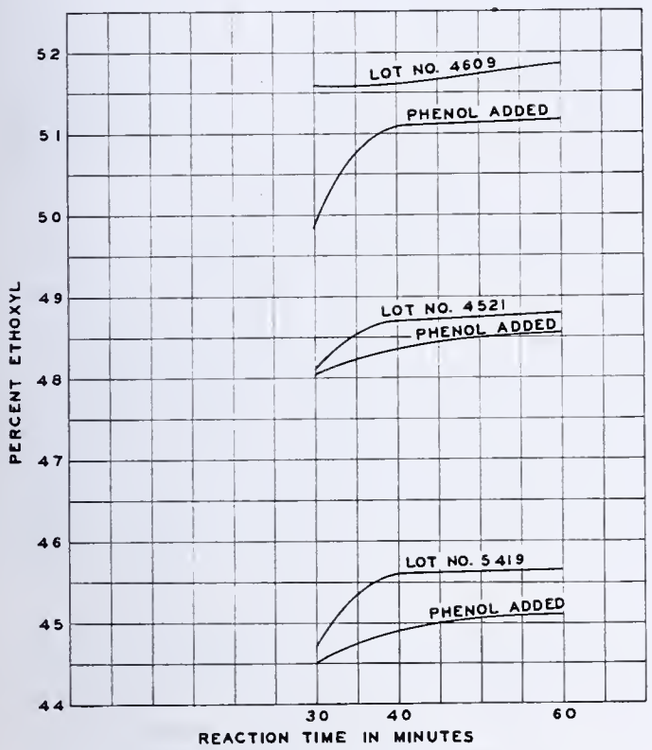


FIGURE 4. EFFECT OF PHENOL ON DETERMINATION OF ETHOXYL AT VARIOUS REACTION TIMES

The rest of the investigation was devoted to the determination of the effects which other variations in the method produced upon the accuracy of the method when applied to methyl- or ethylcellulose.

The effect of variations in the sample container used and of the addition of phenol and/or propionic anhydride to the reaction mixture is shown by Table I for determinations on ethylcellulose. The data also indicate the degree of reproducibility of results when 56.5 to 57 per cent hydriodic acid (sp. gr. 1.69 to 1.70 at 25° C.) is used. Similar data are given in Table III for methylcellulose.

The gelatin capsule gives a slight but uniform titration of only 0.2 to 0.3 cc. of 0.1 *N* sodium thiosulfate compared to about 0.1 cc. for glass. Uniformly higher results were obtained on both cellulose ethers when the gelatin capsules were used instead of open glass cups for adding the sample to the concentrated hydriodic acid. Of the many means suggested for introducing the sample into the flask, the use of the gelatin capsule has proved to be the most reliable. It functions to protect the sample from any action by the hydriodic acid until the boiling flask can be joined to the rest of the apparatus. As soon as the reaction mixture is heated, the gelatin dissolves completely, leaving the sample free to be decomposed by the acid. Capsules sampled over a period of 3 years' purchases (Parke, Davis & Company, Detroit, Mich.) gave a blank of not more than the 0.3 cc. noted in Table I.

Other methods for introducing the sample are open to the following objections: Cigaret paper (*1*) causes excess fuming of the hydriodic acid, tin foil (*5*) is awkward to use, while an open glass capsule (*3*) allows the sample to spill and float on the surface of the liquid with the danger of some of the material sticking to the sides of the flask and charring. The use of a closed glass capsule involves the hazardous problem of breaking it inside the flask.

Much has been written (*1*, *3*, *4*, *5*, *9*) about the advisability of using some sort of agent to aid in the solution of the sample. Experiments using phenol, acetic anhydride, and propionic anhydride were conducted to determine the effect of the addition of these materials to the reaction mixture upon the determination of methoxyl and ethoxyl at various reaction times. The results are shown in Tables IV and V and Figure 4.

The data of Table IV indicate that slightly lower ethoxyl values are obtained on ethyl cellulose when phenol is used. On *p*-ethoxybenzoic acid and *p*-ethoxyacetanilide, phenol exerts a retarding effect at 30 minutes' reaction time. However, at the recommended 40 minutes' reaction time, phenol has no effect when these two ethers are used as reference compounds. When *p*-ethoxydiphenyl is used as the reference compound, phenol is required because of the difficulty of dissolving this unsubstituted ether in the reaction mixture. The data of Table V show that phenol is without effect upon the determination of methoxyl content of methylcellulose or of vanillin as the reference compound. Both materials are readily attacked by the hot concentrated hydriodic acid.

Except for a few very insoluble compounds like *p*-ethoxydiphenyl, hydriodic acid alone is very satisfactory, especially when freshly distilled material having a concentration of 56.5 to 57 per cent is used with a gelatin capsule for weighing the sample and transferring it to the reaction flask. The experience of the authors is that phenol, acetic anhydride, or propionic anhydride give slightly low results, as the data of Tables I and II show.

TABLE IV. EFFECT OF PHENOL ON DETERMINATION OF ETHOXYL

Sample	Added Reagents	Time of Reaction 30 min.	40 min.	60 min.	Calcd.
Ethylcellulose Lot 5419	None	44.7	45.6	45.5	..
		44.7	45.6	45.5	..
	Phenol[a]	44.8	45.0	45.0	..
		44.3	44.8	45.2	..
Lot 4521	None	47.5	48.8	48.7	..
		48.7	48.5	48.8	..
	Phenol[a]	47.7	48.4	48.7	..
		48.4	48.3	48.4	..
Lot 4609	None	51.7	51.5	51.9	..
		51.5	51.6	51.8	..
	Phenol[a]	49.6	50.8	50.9	..
		50.1	51.4	51.2	..
p-Ethoxybenzoic acid[b]	None	26.9	27.1	..	27.1
		27.1	26.9	..	..
	Phenol[a]	25.4	27.0	..	..
		24.5	26.9	..	..
p-Ethoxyacetanilide[c]	None	24.9	24.9	..	25.1
		24.9	24.9	..	..
	Phenol[a]	24.4	25.0	..	..
		24.8	24.9	..	..

[a] 2.5 cc. of Dow U. S. P. phenol added.
[b] *p*-Ethoxybenzoic acid, Eastman C. P. grade, m. p. 196–198° C.
[c] Dow U. S. P. acetphenetidin, m. p. 135° C.

TABLE V. EFFECT OF PHENOL ON THE DETERMINATION OF METHOXYL

Sample	Added Reagents	Time of Reaction 30 min.	40 min.	Calcd.
Methylcellulose lot M-2264	None	30.1	30.2	..
		30.3	30.3	..
	Phenol[a]	29.8	30.0	..
		30.0	29.9	..
Vanillin[b]	None	20.4	20.3	20.4
		20.2	20.5	..
	Phenol[a]	20.1	20.5	..
		20.3	20.2	..

[a] 2.5 cc. of Dow U. S. P. phenol added.
[b] Vanillin, Eastman C. P. grade, m. p. 81–82° C.

Recommended Analytical Procedure

The method described in the following paragraphs was developed from the results of the preceding experiments.

REAGENTS. 1. Potassium acetate in acetic acid. Dissolve 100 grams of C. P. anhydrous potassium acetate in 1 liter of solution containing 900 cc. of glacial acetic acid and 100 cc. of acetic anhydride.

2. Dissolve 5 cc. of bromine in 145 cc. of the 10 per cent solution of potassium acetate in acetic acid. Prepare this solution fresh daily.

3. Potassium iodide, C. P. grade.

4. Sodium acetate. Dissolve 250 grams of C. P. anhydrous sodium acetate in 1 liter of distilled water.

5. Formic acid. Analytical reagent grade, 90 per cent, sp. gr. 1.20.

6. Hydriodic acid. C. P. constant-boiling mixture, b. p. 126° to 127° C. (57 per cent hydriodic acid), made very pure by the method presented herewith.

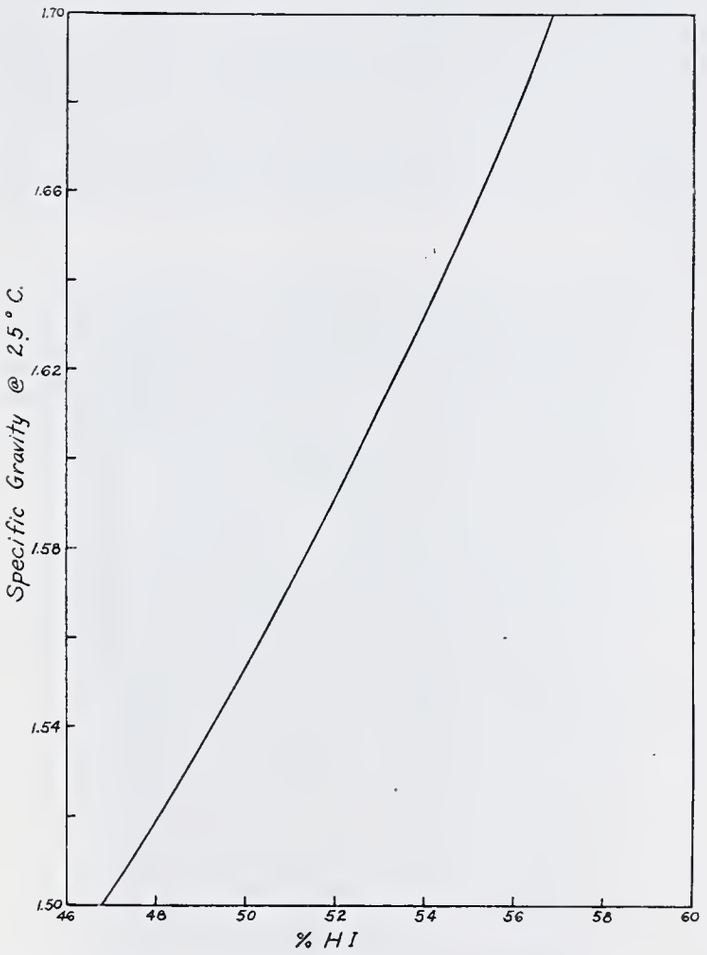


FIGURE 5. SPECIFIC GRAVITY OF CONCENTRATED HYDRIODIC ACID AT 25° C.

Add 70 grams of C. P. red phosphorus to a 2-liter three-necked round-bottomed flask which has been fitted with a reflux condenser, dropping funnel, and thermometer. Fill the flask half full with distilled water and heat with a low flame. Slowly add, through the dropping funnel, a solution of 800 grams of iodine in 800 cc. of hydriodic acid. Heat until the mixture boils (b. p. 127° C.) and continue boiling for one-half hour. Allow the excess red phosphorus to settle and decant the hydriodic acid–phosphorus mixture into a suitable distillation flask equipped with an efficient fractionation column. It is necessary to use an all-glass apparatus equipped with ground-glass joints. Pass a slow stream of carbon dioxide through the apparatus during the distillation. The inlet tube should dip beneath the surface of the liquid. Continue the distillation and collect for use only that portion which has a specific gravity between 1.690 and 1.700 (Figure 5). Store in small brown glass-stoppered bottles previously swept out with carbon dioxide, and seal stoppers with melted paraffin. Store in a dark place.

$$2P + 3I_2 = 2PI_3$$
$$PI_3 + 3H_2O = 3HI + H_3PO_3$$

7. Prepare 0.1 *N* sodium thiosulfate and standardize against 0.1 *N* iodine, which in turn is standardized against 0.1 *N* arsenious oxide or potassium iodate (Bureau of Standards).

8. Sulfuric acid, 10 per cent. Add 60 cc. of the concentrated acid to 940 cc. of distilled water.

9. Aqueous suspension of red phosphorus. Add about 30 mg. of C. P. red phosphorus to 50 cc. of distilled water.

10. Carbon dioxide. A commercial cylinder of the gas equipped with a reducing valve is a convenient source.

PROCEDURE. Unless they are already finely divided, grind ethylcellulose samples in a Wiley micro laboratory mill and then oven-dry at 105° C. for one-half hour. Fill the trap by pouring a small amount of an aqueous suspension of red phosphorus through the cup above *D*. Follow with a water rinse, using sufficient liquid to make the trap about half full. Add 6 to 7 cc. of the bromine–potassium acetate–acetic acid solution to the first receiver and 10 to 12 cc. to the second. The latter is attached to the apparatus by means of a wire loop. Weigh carefully a 50- to 60-mg. sample in a gelatin capsule (Parke, Davis size 0) and drop into the boiling flask. Add a few small glass beads and chips of clay plate and finally 6 cc. of constant-boiling 57 per cent hydriodic acid. Immediately attach the flask to the condenser, using a few drops of the acid to seal the joint. Bubble a slow stream of carbon dioxide through the side arm of the boiling flask (two bubbles per second are satisfactory), place the boiling flask in a 150° C. oil bath, and heat for 40 minutes. Wash the contents of the receiver into a 500-cc. flask containing 10 cc. of the sodium acetate solution. Dilute to 125 cc. with water and add formic acid, dropwise, with swirling, until the brown color of the bromine discharges, then add 3 additional drops. This usually requires a total of 12 to 15 drops. After standing 3 minutes, add 3 grams of potassium iodide and 15 cc. of 10 per cent sulfuric acid. Titrate the liberated iodine with 0.1 *N* sodium thiosulfate and apply the correction for a blank.

$$\frac{\text{Net cc. of 0.1 } N \text{ } Na_2S_2O_3 \times \text{conversion factor} \times 100}{\text{Weight of sample (corrected for ash and moisture)}} = \% \text{ alkoxyl}$$

Factor for OCH_3 = 0.000517, for OC_2H_5 = 0.00075

Discussion and Summary

The method as described has been used in this laboratory for several years, both as a routine control test for ethyl- and methylcellulose and as an accurate analytical procedure for determining ethoxyl and methoxyl values in crystalline organic compounds such as vanillin and *p*-ethoxybenzoic acid.

It is evident that with cellulose ethers it is neither necessary nor advisable to use a solvent in addition to the hydriodic acid, but by the time the analysis is completed, the compound being determined must be completely decomposed. With a very few organic compounds which are especially resistant to attack the use of a solvent like phenol or propionic anhydride is necessary. Nevertheless, the procedure as given is generally applicable to the lower alkyl ethers.

Further research is being done on this method to modify it for analyzing propyl and butyl ethers of cellulose. The method has not been applied successfully to benzylcellulose, because the boiling point of the benzyl iodide is higher than that of the hydriodic acid used.

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A Modified Micro-Fermentation Method for the Estimation of Thiamine

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THE following improved procedure for the estimation of thiamine by the micro-fermentation method, using Warburg-Summerson equipment (*3*) and special flasks (*1*) has proved to be highly accurate in the measurement of thiamine in plant and animal tissues and fluids. Many steps in this new procedure are identical with those in previous publications (*1*, *2*) and are repeated here so that the complete method may be available in one publication.

Reagents

The following aqueous solutions are necessary for this analysis:

SOLUTION A, 1 liter to contain 70 grams of C. P. citric acid ($C_6H_8O_7.H_2O$) and 90.8 grams of C. P. dipotassium phosphate.

SOLUTION B, 1 liter to contain 200 grams of C. P. anhydrous dextrose, 2.2 grams of C. P. monopotassium phosphate, 1.7 grams of C. P. calcium chloride ($CaCl_2.2H_2O$), 10 grams of C. P. magnesium sulfate ($MgSO_4.7H_2O$), 0.01 gram C. P. manganous sulfate ($MnSO_4.4H_2O$), 0.01 gram of C. P. ferric chloride ($FeCl_3.6H_2O$, phosphorus-low), and 0.067 gram of crystalline nicotinic acid.

AMMONIUM SULFATE SOLUTION, 15 PER CENT, prepared so that 1 liter of final solution contains 150 grams of ammonium sulfate.

GELATIN SOLUTION, 1 PER CENT, prepared so that 1 liter contains 10 grams of gelatin (Difco).

THIAMINE STOCK SOLUTION. Dissolve 50 mg. of thiamine hydrochloride (previously dried to a constant weight in a desiccator over phosphorus pentoxide) into a mixture of 26.4 ml. of undenatured 95 per cent ethyl alcohol, and 0.42 ml. of concentrated hydrochloric acid, and make up to 100 ml. with distilled water (*4*). This solution is stable for several months if kept at 4° C. in a glass-stoppered flask. Immediately prior to each run, substock and standard thiamine solutions are prepared from the stock thiamine solution as follows:

THIAMINE SUBSTOCK SOLUTION, 500 ml. to contain 5 ml. of 1 per cent gelatin and 1 ml. of thiamine stock solution. (This is best prepared by placing 1 per cent gelatin in a 500-ml. volumetric flask with 100 ml. of distilled water and shaking to wet the entire surface, then adding thiamine stock solution and distilled water up to volume.) Final concentration of thiamine is 1 microgram per ml.

THIAMINE STANDARD SOLUTION A, 100 ml. to contain 1 ml. of 1 per cent gelatin and 1 ml. of thiamine substock solution. (This solution is prepared by placing 1 per cent gelatin in a 100-ml. volumetric flask with 25 ml. of distilled water and shaking to wet the entire surface, then adding thiamine substock solution and water up to volume.) Final concentration of thiamine is 10 millimicrograms per ml. (1 millimicrogram = 10^{-9} gram).

THIAMINE STANDARD SOLUTION B, 100 ml. to contain 1 ml. of 1 per cent gelatin and 2 ml. of thiamine substock solution. (This solution is prepared in the same way as thiamine standard solution A.) Final concentration of thiamine is 20 millimicrograms per ml.

YEAST SUSPENSION, 2 PER CENT, prepared so that 100 ml. contain 2.0 grams of commercial baker's yeast.

SODIUM SULFITE SOLUTION, 4 PER CENT, prepared so that 100 ml. contain 4.0 grams of C. P. sodium sulfite ($Na_2SO_3.7H_2O$).

POTASSIUM IODIDE SOLUTION, 5 PER CENT, prepared so that 100 ml. contain 5.0 grams of U. S. P. potassium iodide.

SOLUBLE STARCH SOLUTION, 1 PER CENT, prepared so that 100 ml. contain 1 gram of C. P. soluble starch.

SULFURIC ACID SOLUTION, 32 PER CENT, prepared by diluting 33 ml. of 96 per cent C. P. sulfuric acid to 100 ml. with distilled water.

SULFURIC ACID SOLUTION, 8 PER CENT, prepared by diluting 25 ml. of 32 per cent sulfuric acid to 100 ml. with distilled water.

HYDROGEN PEROXIDE, 3 PER CENT.

SODIUM HYDROXIDE SOLUTION, 15 PER CENT, prepared so that 1 liter contains 150 grams of C. P. sodium hydroxide.

Solution A, solution B, the 15 per cent ammonium sulfate solution, and the 1 per cent gelatin solution are plugged with cotton, covered with foil, and sterilized in flowing steam at 100° C. for 30 minutes on three successive days. Once opened, the solutions should be stored at 4° C. The 2 per cent yeast suspension and the 4 per cent sodium sulfite solution are made up immediately before use.

Preparation of Samples

Clear liquid samples require no further preparation. Suspensions of solid samples may be made readily using a Waring Blendor. Preliminary tests must be made by diluting the sample with distilled water until a 1-ml. aliquot, made up to 100 ml., stimulates the production of carbon dioxide by yeast to an extent equivalent to 10 to 20 millimicrograms of crystalline thiamine (1 millimicrogram = 10^{-9} gram).

To one of two aliquot samples in 100-ml. glass-stoppered flasks, add 5 ml. of 4 per cent sodium sulfite and 20 ml. distilled water. To the second aliquot sample add 25 ml. of distilled water. Adjust the pH of both solutions with 15 per cent sodium hydroxide or 8 per cent sulfuric acid to 5.2 using buffered methyl red as indicator.

After placing cotton plugs in both flasks, heat in flowing steam at 100° C. for 30 minutes, cool, and destroy excess sulfite with 3 per cent hydrogen peroxide, using acidified starch iodide solution (5 per cent potassium iodide, 1 per cent soluble starch, and 32 per cent sulfuric acid) as an outside indicator (spot test, *2*), and making certain that all sulfite is destroyed by adding a 2-drop excess of 3 per cent hydrogen peroxide. This is a critical step in the procedure, because the presence of traces of undestroyed sulfite inhibits carbon dioxide production, thus preventing the correction for nonthiamine carbon dioxide-stimulating substances. This procedure is best carried out by preparing in a spot plate a number of solutions containing 1 drop each of the acidified starch-iodide indicator. To test for sulfite destruction after the addition of 3 per cent hydrogen peroxide drop by drop, transfer a drop of the test solution to the spot plate by means of a stirring rod. Destruction of sulfite is indicated by the appearance of a purple color in the spot plate. Adjust both the treated and untreated test solutions to pH 6.2 with 15 per cent sodium hydroxide using neutral litmus as indicator and make up to 100-ml. volume. The concentration of carbon dioxide-stimulating substances in the untreated solution should be equivalent to 10 to 20 millimicrograms of thiamine per ml.,

TABLE I. COMPARISON OF ASSAY RESULTS FOR THIAMINE BY REVISED MICRO-FERMENTATION AND THIOCHROME METHODS

Sample	Micro Fermentation	Thiochrome
	Microgram per gram or ml.	
Urine A	0.0663 0.0674	0.0652
Urine B	0.0620 0.0607	0.0582
Urine C	0.236 0.232	0.224
Urine D	0.0403 0.0395	0.0383
Urine E	0.0299 0.0309	0.0321
Urine F	0.0620 0.0610	0.0590
Urine G	0.0300 0.0300	0.0284
Urine H	0.0391 0.0385	0.0370
Urine I	0.103 0.105	0.100
Urine J	0.0573 0.0584	0.0609
Food A	0.399 0.407	0.386
Food B	0.486 0.477	0.497
Food C	0.469 0.479	0.464
Food D	0.524 0.512	0.502
Food E	0.803 0.808	0.808
Food F	0.519 0.507	0.507
Food G	0.905 0.913	0.866
Food H	0.744 0.769	0.739
Food I	0.566 0.579	0.553
Food J	0.510 0.500	0.471

whereas the concentration of these substances in the solution treated with sulfite may now be below this range. If the treated solution is below an equivalent of 10 millimicrograms, it is necessary to add 10 millimicrograms of thiamine (1 ml. of thiamine standard solution A) before making up to volume. Experience has shown this to be necessary generally with food samples but not with urine samples.

Fermentation Procedure

Prior to each run, grease all ground joints, open the capillary gas valves to the cups, and set the water bath to 30° C. In the main chamber of each reaction flask place 1 ml. of distilled water. In the inset place 1 ml. of the test solution or suspension which contains carbon dioxide–stimulating substances equivalent to 10 to 20 millimicrograms of thiamine, or 1 ml. of thiamine standard solution A or B. Then prepare the buffer-substrate-yeast suspension by mixing in a 100-ml. volumetric flask 15 ml. of solution B, 10 ml. of solution A, and 7 ml. of 15 per cent ammonium sulfate followed by 25 ml. of 2 per cent yeast suspension. Elapsed time is measured from the time the yeast suspension is added. Make the buffer-substrate-yeast suspension to volume and add 1 ml. to the inset of each flask. Fix the flasks to the manometers, place in the water bath, and flush with nitrogen through a manifold. Ten minutes have usually elapsed at this point if 12 Summerson units (24 Warburg units) are employed. Shake the flasks at 140 oscillations (70 round trips) per minute at an amplitude of 3 cm. Stop the passage of nitrogen after 50 minutes have elapsed, and close the capillary gas valves to the cups. Release excess pressure in each unit 5 minutes later. Make a zero reading at exactly 60 minutes, close the system, and make a final reading 60 minutes later.

Calculations are based on the comparison of gas evolved in the test solution after correcting for nonthiamine stimulating substances with the gas evolution due to standard thiamine solutions A and B, assuming a linear relationship between thiamine concentration and carbon dioxide evolution over the 10 to 20 millimicrogram range. The gas evolution due to standard thiamine solutions A and B must be determined at each run.

With this modified procedure repeated estimations of the thiamine content of various plant and animal tissues and fluids have shown an agreement within 2 per cent (Table I). Furthermore, these results agree within 5 per cent with those obtained by fluorometric assays of the same samples (Table I). This fermentation procedure is preferred to fluorometric methods in the analysis of samples containing very small quantities of thiamine or when information is desired on the fate of thiamine during the handling and preparation of foods and in tissue metabolism. Over 1000 successful thiamine determinations have been performed by the authors using the method described.

Summary

A revised procedure for the microestimation of thiamine in tissues and tissue fluids using the Warburg technique is presented. Interference by nonthiamine substances is corrected by sulfite treatment. Residual sulfite is eliminated by a peroxide treatment. This procedure is especially applicable to the assay of tissues and fluids low in thiamine (10^{-8} gram of thiamine per ml.) and in the measurement of thiamine degradation products in tissue metabolism and in the treatment of foods.

Acknowledgment

Technical assistance from Elizabeth Weeks and Helen C. Scrufutis is acknowledged.

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Colorimetric Microdetermination of Iron

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THE observation of van Klooster (*1*) in 1921 that nitroso R salt forms a green color with ferrous iron was later confirmed by the writer (*2*).

Further studies on the reaction between ferrous iron and nitroso R salt, started in connection with various potassium analyses where iron occurred as a contaminant, suggested the possibility of using this reagent for the determination of ferrous iron. The method, as described below, has been found satisfactory for determining iron in plant tissues.

Reagents

Standard Iron Solution. Dissolve over a hot plate 1 gram of metallic iron ("rust-free" wire) in a 1000-ml. flask containing 10 ml. of concentrated sulfuric acid and 100 ml. of water, cool, and make to volume with water. Standards containing 0.2 to 20.0 micrograms of iron per ml. are prepared from this solution.

Nitroso R Salt. Place 0.5 gram of nitroso R salt in a 100-ml. volumetric flask containing 70 ml. of water, dissolve by gentle agitation, and then complete the volume with redistilled (iron-free) acetone. The reagent keeps well for many months.

Sodium Acetate. Place 544.3 grams of sodium acetate (iron-free) in a 1000-ml. volumetric flask, then pour in 300 ml. of water, and shake gently at intervals while heating on a hot plate until complete solution. Make to volume with water and filter.

Hydroxylamine Sulfate. Place 10 grams of hydroxylamine sulfate in a 100-ml. volumetric flask containing 80 ml. of water, shake until complete solution, and complete the volume with water.

Procedure

Ash about 5 grams of dry plant tissue in a platinum crucible. Dissolve the ash with 10 ml. of 5 *N* hydrochloric acid by heating slowly over a hot plate. Add to the platinum crucible approximately 20 ml. of water. Heat the mixture to about 90° C., transfer to a 100-ml. flask, cool nearly to room temperature, dilute to the mark, and filter.

Place a 10-ml. aliquot of the solution in a test tube and neutralize with 5 *N* sodium hydroxide in the presence of a very small piece of litmus paper. Remove the latter with a glass rod and add 1 ml. of hydroxylamine sulfate, 1 ml. of nitroso R salt, and 2 ml. of sodium acetate. (Either 2 ml. of sodium acetate or 1 ml. of 1.5 *N* ammonium hydroxide can be used for adjusting the solution to pH 8 to 10.) Make to 15 ml. in a graduated test tube and allow to stand for 20 minutes or longer. Transfer to a cell, preferably 2.5 mm. in thickness, and determine the color intensity with a photoelectric colorimeter, using a No. 47 light filter with transmission limits 445 to 505 millimicrons. A 2.5-mm. cell can be obtained by using a plunger 7.5 mm. thick in a 10-mm. cell as supplied with the Summerson-Klett photoelectric colorimeters.

In case of formation of a precipitate by calcium with sulfate or

Ferrous iron forms with nitroso R salt (1-nitroso-2-hydroxy-3,6-naphthalene disodium sulfonate) at favorable range of pH values (8 to 10) a green pigment which is in direct proportion to the amounts of ferrous iron present in the solution. The sensitivity of the reagent is very great, reaching the low limits of 0.2 microgram of ferrous iron per ml. and ranging between 0.2 and 50 micrograms or more. Hydroxylamine sulfate was found highly satisfactory for the reduction of ferric to ferrous iron and sodium acetate or ammonium hydroxide for obtaining a satisfactory pH range for the development of the green pigment. Iron may be determined by means of a photoelectric colorimeter with the use of appropriate light filters.

phosphate ions after the addition of sodium acetate, centrifuge, decant the supernatant liquid, and discard the precipitate. Turbid solutions interfere considerably with the accuracy of the method.

Results

Table I and Figure 1 present values obtained with a Summerson-Klett photoelectric colorimeter for different concentrations of iron with three different amounts of nitroso R salt. The data indicate that 0.5 ml. of nitroso R salt, as reported in Table I, was sufficient only for quantities of iron ranging from 0.25 to 10.0 micrograms; and that 1.0 ml. of nitroso R salt covered twice as great a range of ferrous iron concentrations—that is, from 0.25 to 20 micrograms. With a volume of 2.0 ml. of nitroso R salt a range of iron concentrations from 0.25 to 25.0 micrograms was covered satisfactorily. A greater extension of the range from 25.0 to 30.0 micrograms of iron with 2.0 ml. of nitroso R salt was possible but with an error ranging from 2 to 10 per cent, resulting probably from the decreased sensitivity of the photoelectric colorimeter at the higher regions of its logarithmic scale. The range of iron concentrations from 0.25 to 20 micrograms is the most satisfactory for photometric or colorimetric determinations.

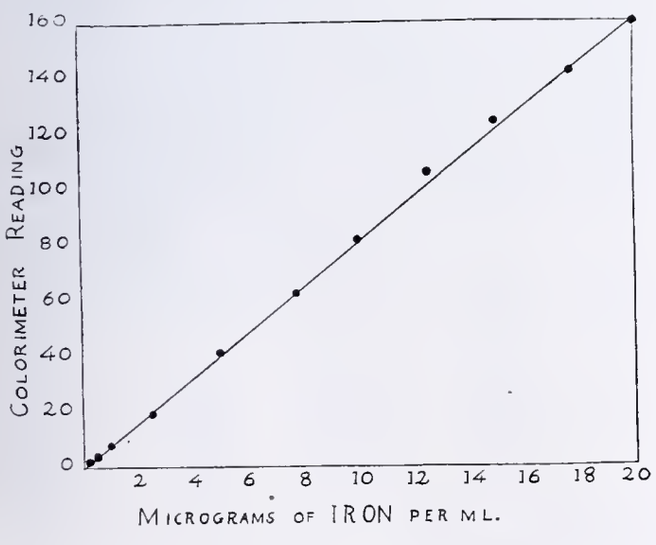

FIGURE 1

With considerably greater concentrations of ferrous iron in the unknown than 20 micrograms the writer recommends dilution and proper adjustment of the volume of nitroso R salt.

Aqueous solutions of nitroso R salt are yellow. In the presence of ferrous iron they form a green color, the amounts of which are directly proportional to the quantities of iron. Because of the existence of a stoichiometric relationship between ferrous iron and nitroso R salt, as shown in Table I, it is desirable to add nitroso R salt to solutions employed for the determination of iron in quantities directly proportional to those of iron.

The stoichiometric relationship is evidenced by the fact that approximately 2.5 and 5.0 mg. of nitroso R salt, contained in 0.5 and 1.0 ml., respectively, of 0.5 per cent solution were used up completely by solutions of ferrous iron corresponding in the former case with 0.1 mg. (10 ml. × 10 micrograms = 0.1 mg.) and in the latter, with 0.2 mg. (10 ml. × 20 micrograms = 0.2 mg.) of iron. The proportions of the reacting amounts of nitroso R salt and iron, based on the above data, were approximately 25 to 1. Comparing this with the ratio of the molecular weights of nitroso R salt ($C_{10}H_5NO_8S_2Na_2$ = 475) and iron (Fe = 55.84) which is 8.5 to 1 (475 ÷ 55.84 = 8.5); the iron salt of 1-nitroso-2-hydroxy-3,6-naphthalene disodium sulfonate has the formula $Fe(C_{10}H_5NO_8S_2Na_2)_3$. The difference of 2 per cent between the observed ratio of 25 to 1 and the calculated ratio of 25.5 to 1 is relatively small and within the limits of an experimental error. Van Klooster (*1*) has suggested the formula $(C_{10}H_5NO_8Na_2)_3Co$ for the corresponding salt of cobalt.

The addition of amounts of nitroso R salt greatly in excess

TABLE I. VALUES ON PHOTOELECTRIC COLORIMETER SCALE

(For different amounts of iron per ml. of solution and nitroso R salt added)

Iron γ	Nitroso R Salt 0.5 ml.	1.0 ml.	2.0 ml.
0.25	2	2	3
0.50	4	4	6
1.00	8	8	9
2.50	20	19	23
5.00	42	41	44
7.50	62	63	66
10.00	78	82	85
12.50	85[a]	106	108
15.00	86[a]	124	127
17.50	87[a]	142	145
20.00	88[a]	160	165
22.50	88[a]	182[a]	184
25.00	86[a]	182[a]	202
30.00	87[a]	184[a]	240

[a] Results obtained with insufficient quantities of nitroso R salt as compared with those of iron.

TABLE II. COLORIMETER READINGS

(Obtained with a Summerson-Klett photoelectric colorimeter and calculated values of per cent deviations on solutions containing 1 ml. of a 0.5 per cent solution of nitroso R salt, 1 ml. of a 10 per cent solution of hydroxylamine sulfate, 1 ml. of 1.5 *N* ammonium hydroxide, and 20 ml. of solution containing different amounts of iron, copper, and nickel and prepared as indicated)

Solution No.	1.5 *N* Ammonium Hydroxide	4 *M* Sodium Acetate	Fe	Cu	Ni	Readings	Deviation Between solutions and checks, No.	%
	Ml.	*Ml.*	*Micrograms per ml.*					
1	1	0	0	0	0	10	Check	..
2	1	0	1	0	0	18	Check	..
3	1	0	5	0	0	52	Check	..
4	1	0	0	1	0	10	1	0
5	1	0	0	5	0	10.5	1	5
6	1	0	0	0	1	10	1	0
7	1	0	0	0	5	11	1	10
8	1	0	1	1	0	19	2	5.5
9	1	0	1	5	0	19.5	2	7.5
10	1	0	5	1	0	53	3	2
11	1	0	5	5	0	52	3	0
12	1	0	1	0	1	20	2	11.1
13	1	0	1	0	5	20.5	2	11.4
14	1	0	5	0	1	53	3	2
15	1	0	5	0	5	53	3	2
16	0	2	1	5	0	19	2	5.5
17	0	2	5	5	0	53	3	2
18	0	2	1	0	1	18.5	2	2
19	0	2	5	0	5	53	3	2
20	0	2	0	0	0	18.5	1	3

is not necessary, as excess amounts interfere with the sensitivity of the method.

The yellow color of the nitroso R salt interferes with the determination of ferrous iron with an ordinary colorimeter without the aid of light filters. This difficulty may be overcome if more than one standard is employed.

The green color formed by the compound iron–nitroso R salt in aqueous solutions is very stable and does not fade in the course of 48 hours under ordinary room conditions.

Interfering Elements

Cobalt, if present, interferes with the determination of iron through the formation of a wine-red color. Copper and nickel form with nitroso R salt a yellowish brown color at concentrations as low as 1 microgram per ml. of copper or nickel. However, the yellowish brown color forms only at pH values lower than 7.0 and almost disappears at pH values most suitable for the development of the green color between nitroso R salt and iron. The per cent of deviation in the colorimeter readings resulting from contaminations of 1 to 5 micrograms of either copper or nickel per milliliter of solution is reported in Table II.

The data in Table II show that the per cent deviation of the colorimeter readings of nitroso R salt and iron solutions, when admixed with copper and nickel as contaminants, ranged from 0 to 7.5, excepting in solutions 12 and 13 where the deviation values reached 11.1 and 11.4, respectively.

The color produced by cobalt cannot be eliminated under the same conditions. However, the writer has not so far encountered cobalt in the tissues of *Ananas comosus* (L.) Merr., in amounts sufficiently high to cause interference in the determination of iron by this method.

The sensitivity of the nitroso R salt method for iron is as good as that of the *o*-phenanthroline or α-α-dipyridyl methods. The green color of the nitroso R salt method is better suited for colorimetric measurements than the pink color of the other two methods.

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The Microanalysis of Gases

Nitric Oxide–Hydrogen, Nitric Oxide–Nitrogen, and Nitrous Oxide–Ammonia Mixtures

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In the microanalysis of mixtures of nitric oxide with hydrogen and nitrogen, the nitric oxide may be removed by addition of oxygen in the presence of potassium hydroxide and determined by difference from analysis of the remaining gas. Ammonia may be separated from nitrous oxide by absorption on monochloroacetic acid. A further improvement in combustion coil construction is described.

PROCEDURES have been devised for the determination of nitric oxide–hydrogen, nitric oxide–nitrogen, and nitrous oxide–ammonia mixtures by the dry method of gas microanalysis described in earlier papers (*2*, *3*).

Nitric Oxide

The method for the determination of mixtures containing nitric oxide is based on the macromethod of Baudisch and Klinger (*1*). When adapted to a micro scale, it becomes indirect and somewhat unorthodox, but if performed correctly it works well.

A moist potassium hydroxide bead is inserted into a measured volume of the sample containing nitric oxide. [Nitric oxide was prepared by allowing solid potassium iodide and sodium nitrite to react with 6 *N* sulfuric acid (with dissolved air removed) in an evacuated vessel. The water present and the resulting iodine were frozen out with dry ice. The nitric oxide was removed from the system by a Toepler pump, transferred to a gas cup, and stored over mercury. To test its purity an excess of oxygen was added; the fact that only oxygen remained after removal of the nitrogen dioxide so formed showed the purity to be 100 per cent.]

The gas cup together with the potassium hydroxide bead and sample is lowered over the buret tip until the latter is just below the mercury surface. Oxygen is then slowly added from the buret. Any nitrogen dioxide or intermediates in its formation are immediately removed by the moist potassium hydroxide bead. If the nitric oxide content of the sample is high, a momentary brown coloration due to nitrogen dioxide is noticed; otherwise there is none. It is highly important to use a moist bead. Unless performed in the presence of the potassium hydroxide bead, this reaction cannot be used because the resulting nitrogen dioxide reacts with mercury.

The amount of oxygen added need not be known, since, contrary to the work of Baudisch and Klinger, there is no quantitative relationship between the amount of oxygen used and the amount of nitric oxide originally present. This is probably due to the formation of unpredictable relative amounts of potassium nitrate and nitrite.

To accommodate both the bead holder and the buret tip, a gas cup with an inside diameter of 9 mm. has been found to be satisfactory. With added care, a cup of 8-mm. inside diameter works equally well. It is immaterial whether oxygen is added to the nitric oxide sample or vice versa, but it is advisable to use a volume of oxygen at least equal to the volume of nitric oxide plus the volume of any hydrogen which may be present.

In the case of nitric oxide–hydrogen mixtures, after addition of oxygen the potassium hydroxide bead is removed and the resulting volume measured. A combustion coil is then introduced and the hydrogen burned to water, the water removed on another potassium hydroxide bead, and the contraction measured.

Table I. Analyses of Nitric Oxide–Hydrogen Mixtures

Determination	Volume of Sample	Nitric Oxide		Difference
		Theoretical	Determined	
	Cu. mm.	%	%	%
1	22.77	0.00	0.66	0.66
2	18.90	13.76	14.79	1.03
3	19.13	25.35	26.61	1.26
4	25.85	38.88	39.75	0.87
5	30.17	49.62	50.28	0.66
6	24.55	58.66	59.02	0.36
7	19.41	74.86	74.45	−0.41
8	15.05	100.00	99.40	−0.60
				Av. 0.73

The hydrogen is calculated from this contraction, and the nitric oxide determined by difference. Results on known mixtures are shown in Table I.

For the nitric oxide–nitrogen samples (Table II) the procedure was the same except that after the removal of the nitric oxide a measured excess of hydrogen was added and combustion carried out. From the resulting contraction, the remaining volume of hydrogen and the amount of oxygen present in the sample after the removal of the nitric oxide were calculated; by difference the amount of nitrogen became known. From this, the volume of nitric oxide originally present was calculated.

A sample containing hydrogen, nitrogen, and nitric oxide was analyzed to determine the reproducibility of this method for such a mixture (Table III). The procedure in this case was the same as for hydrogen–nitric oxide mixtures, except that after determination of the amount of hydrogen present a measured excess of hydrogen was added and a second combustion performed. From the resulting contraction the amount of oxygen present in the sample after removal of the hydrogen and nitric oxide was calculated; by difference the amount of nitrogen became known.

Hydrogen and nitrogen were chosen as diluent gases because these gases occurred with nitric oxide in the problem for which the method was developed. The volumes used are smaller than those generally reported in papers of this nature, but again, this was conditioned by the nature of the problem to which the method was to be applied. As a result of the small volumes, the per cent error is rather large, but the absolute error is about the same as that for other methods using the same type of apparatus.

Mixtures of nitric oxide and nitrous oxide cannot be determined in this manner because nitrous oxide is absorbed by potassium hydroxide.

Table II. Analyses of Nitric Oxide–Nitrogen Mixtures

Determination	Volume of Sample	Nitric Oxide		Difference
		Theoretical	Determined	
	Cu. mm.	%	%	%
1	19.37	13.42	14.66	1.24
2	22.00	24.55	25.41	0.86
3	26.06	37.34	37.53	0.19
4	31.52	48.45	47.97	−0.48
5	25.58	61.69	61.41	−0.28
6	21.60	75.00	74.96	−0.04
7	22.30	100.00	99.69	−0.31
				Av. 0.49

Table III. Analyses of a Sample Containing Hydrogen, Nitrogen, and Nitric Oxide

Volume of Sample	H_2	N_2	NO
Cu. mm.	%	%	%
30.55	33.45	34.70	31.85
30.25	33.59	34.38	32.03
34.43	32.82	34.85	32.32
	Av. 33.29	34.64	32.07

Determination of Nitrous Oxide in the Presence of Ammonia

Combustion of nitrous oxide with hydrogen (*4*) cannot be accomplished in the presence of ammonia because it also takes part to some extent in the reduction. A phosphorus pentoxide bead cannot be used to remove ammonia, as some of the nitrous oxide is also removed. It was found that a fused bead of monochloroacetic acid rapidly, quantitatively, and specifically removes ammonia in the presence of nitrous oxide. To prevent contamination of the mercury surface inside the gas cup, it is necessary to submerge the bead below the mercury surface just before complete crystallization after fusion. Table IV shows the results of analyses performed on known mixtures.

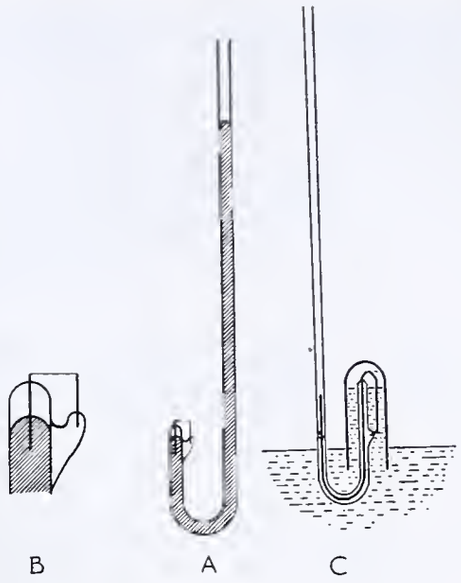

Figure 1. Combustion Coil
A. Complete
B. Enlarged detail of tip
C. Operating position

Table IV. Analyses of Nitrous Oxide–Ammonia Mixtures

Determination	Volume of Sample	Nitrous Oxide		Difference
		Theoretical	Determined	
	Cu. mm.	%	%	%
1	18.40	100.00	100.00	0.00
2	19.42	100.00	99.48	−0.52
3	24.47	78.96	79.28	0.32
4	24.49	77.66	77.79	0.13
5	28.35	64.90	65.43	0.53
6	29.54	63.54	64.22	0.68
7	36.44	50.55	51.04	0.49
8	36.55	49.25	49.44	0.19
9	28.77	40.91	41.01	0.10
10	28.88	23.99	24.40	0.41
11	34.23	16.45	16.51	0.06
				Av. 0.30

An Improved Combustion Coil

The combustion coil mentioned in connection with the analyses for nitric oxide was an improvement over the older type (*3*) and warrants a short description.

A coil holder (Figure 1) was shaped to fit snugly into the customary absorbent holder on the apparatus. A piece of No. 24 (0.5-mm., 0.02-inch) platinum wire was sealed into the end of the holder at the center so as to make contact with the mercury with which the coil holder was filled. A second piece was sealed into a little glass arm at one side and near the end. These two pieces of wire were trimmed off about 1 mm. from the end of the holder and joined by a 2.5-mm. piece of 0.05-mm. (0.002-inch) platinum wire spot-welded to their ends. A small commercial spot welder may be used for this purpose. The coil operates very satisfactorily on 12 volts, and may be suitably controlled if it is used in series with a slide wire resistance.

With reasonable care the coil will not burn out, but if this should happen, it is a matter of but a few minutes to spot-weld another platinum thread into place. It was found that in a gas cup with an inside diameter of 8 mm. the coil could be used for combustion in which the volume remaining at the end of the process was 11 cu. mm. or more.

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Perchloric Acid in Micro-Kjeldahl Digestions

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Data are presented in an effort to discourage the use of perchloric acid as an aid in micro-Kjeldahl digestions, as the authors are convinced that this oxidizer can cause a serious loss of nitrogen.

IN 1921, Mears and Hussey (*14*) suggested that the powerful oxidizer, perchloric acid, might be of value to shorten the time of Kjeldahl digestions, but warned that "an excess" should be avoided. Their figures and curves illustrate strikingly that low results will follow if such surplus be present. Shortly before this publication, Willard and Cake (*24*) had tried 60 to 70 per cent perchloric acid, with and without sulfuric acid, and concluded that "it is practically impossible to avoid loss of nitrogen if the organic matter is completely oxidized". Not long after, Parker and Terrell (*16*) used perchloric acid to aid in the difficult digestion of leather, but it was clear to them also that low results could easily follow. Nevertheless, perchloric acid so readily destroys otherwise resistant organic matter that many workers soon attempted to apply it to all sorts of Kjeldahl digestions.

Lematte, Boinot, and Kahane (*12*) employed a very strong mixture for digesting animal organs, and Frey, Jenkins, and Joslin (*6*) claimed agreement with other methods when they accelerated the digestion of leather with potassium perchlorate. However, Fiszerman and Fiszerman-Garber (*4*) reported that in their Kjeldahl digestions (of liver and kidney meals), those aided by perchlorates revealed loss of nitrogen, and Yoe (*26*) had cautioned earlier that in the case of fluids such as milk and urine, low results would always follow unless a certain minimum of perchloric acid was used. Nearly all the experimenters named above had carried through their digestions with mixed perchloric and sulfuric acids. LeTourneur-Hugon and Chambionnot (*13*) recommended that the perchloric acid be cautiously added dropwise to the boiling sulfuric acid after some preliminary destruction. They also stated that not all methods of using it are suitable and that it should not be applied to milk or flour. Gauduchon-Truchot (*8*), in a doctorate thesis on the Kjeldahl technique, cited some analysts who distrusted perchloric acid, along with those who favored it, but on the whole this author considered it to give generally satisfactory results.

When perchloric acid was applied to microquantities as of biological fluids, etc., one might expect that investigators here and there would mention lower results.

Rose (*18*), however, employing a powerful sulfuric-perchloric acid mixture (supplemented by hydrogen peroxide) for micro quantities, claimed good agreement with the calculated nitrogen of various organic compounds and with a "macromethod" otherwise not identified. Dupray (*3*) suggested a much milder digestion mixture for nonprotein nitrogen determinations on blood filtrate but gave no figures at all. Chiles (*1*) used a digestion mixture containing some perchloric acid but preferred to omit this oxidizer when dealing with urine, mentioning "somewhat low results". He did, nevertheless, employ it for other biological fluids. Kito (*10*) adopted Chiles' formula for flour, milk, etc., but accelerated the digestion by the addition of selenium. Walters (*21*), interested in the nitrogen of brewery products, clearly stated that the digestion methods of Mears and Hussey and of Rose seemed to cause a loss of nitrogen and that the findings were always less than those of regular Kjeldahl digestions. Doneen (*2*) attempted to determine the nitrogen of plant material with what must have been a large excess of perchloric acid. [The micro digestion mixtures of Fuchs and Falkenhausen (*7*), and Zakrzewski and Fuchs (*27*), are so low in perchloric acid that it is scarcely significant.]

One encounters scattered general statements in the literature questioning the advisability of utilizing perchloric acid in Kjeldahl digestions.

Smith (*19*) remarks in passing that "unfortunately the process in certain applications gives low results, presumably due to loss of nitrogen in the digestion period". Myers (*15*) doubts that it is as satisfactory as hydrogen peroxide for urine and blood analyses. The most emphatic pronouncement is in the text of Peters and Van Slyke (*17*), who maintain, "if, however, the organic material present does not reduce all the perchloric acid the latter destroys part of the ammonia formed. For this reason perchloric acid cannot be used as a general reagent in Kjeldahl digestions."

It is to such opinion that the authors hold, but this is not a general conviction. Nitrogen determinations, both macro and micro, that employ the aid of perchloric acid are still being done and still being proposed. Some of the better known digestion mixtures containing perchloric acid which have appeared in the literature are shown in Table I.

TABLE I. INITIAL CONCENTRATIONS OF PERCHLORIC ACID DIGESTION MIXTURES

(Percentages on weight/weight basis)

Digestion Mixture	$HClO_4$	H_2SO_4	H_3PO_4	Na_2SO_4	$CuSO_4$
Mears and Hussey	1.46	87.5	...	...	1.99
Parker and Terrell	1.59	78.2	...	...	2.83
Rose	21.8	27.2	...	...	...
Dupray	1.31	57.0	2.90	...	...
Lematte, Boinot, and Kahane	39.3	35.4	...	...	...
Chiles	2.06	51.6	...	6.89[a]	0.46
Zakrzewski and Fuchs	0.126	61.5	...	...	...

[a] Not all will remain dissolved.

Experimental Work

The authors had often suspected that micro-Kjeldahl determinations gave lower results when done with the aid of perchloric acid. The literature reveals differences of opinion, but no good presentation of comparative analyses of various substances by both perchloric acid and other digestion procedures. No modification of the Kjeldahl method gives nitrogen quantitatively on all compounds, but the technique is suited for a fair approximation of this element as found in such very heterogeneous mixtures as biological materials. One does not generally employ the Kjeldahl method for pure organic compounds.

The authors present here comparable nitrogen analyses of materials digested with perchloric acid techniques and with other methods, and have concerned themselves with micro quantities only, for it is there that the use of this terrific oxidizer is most objectionable.

For the major experiment, they chose samples of four typical biological fluids: serum, cow's milk, human urine, and protein-free blood filtrates. These last were prepared from serum and trichloroacetic acid, or from whole blood and tungstic acid. Determinations were done in triplicate on each sample of suitably diluted fluid, taking aliquots containing 0.2 to 0.5 mg. of nitrogen. Digestions were carried out in Folin-Wu tubes or in 25 × 200 mm. Pyrex test tubes and heated over microburners. The hydrogen peroxide digestion of Koch and McMeekin (*11*) and the selenium-sulfate mixture of West and Brandon (*22*) were selected as reference procedures to be compared with various perchloric acid techniques. All analyses were by direct nesslerization of the diluted digest and reading in a Klett-Bio colorimeter. The Nessler's reagent used was that of Koch and McMeekin but prepared with mercuric oxide as described elsewhere (*23*). Every ordinary precaution was taken. The distilled water was all redistilled from dilute sulfuric acid–permanganate solution to remove traces of ammonia. Blanks were run on every reagent.

TABLE II. MICRO-KJELDAHL DIGESTIONS COMPARING PERCHLORIC ACID METHODS WITH OTHER PROCEDURES

Biological Fluid		Digestion Method	Total Protein	Average	Approximate Difference
			%	%	%
Serum	1	Koch and McMeekin	7.2, 7.7, 7.8	7.6	
		1 drop 60% $HClO_4$	5.4, 5.1, 5.2	5.2	32
	2	Koch and McMeekin	7.5, 7.6, 7.8	7.6	
		Rose	6.9, 5.6, 6.3	6.3	17
	3	West and Brandon	8.1, 7.9, 8.2	8.1	
		2 drops 72% $HClO_4$	4.5, 3.4, 4.2	4.0	51
	4	West and Brandon	7.6, 8.4, 7.6	7.9	
		Rose	6.2, 7.1, 6.1	6.5	18
	5	West and Brandon	7.4, 7.8, 7.5	7.6	
		Chiles	1.7, 5.3, 2.8, 6.4	4.0[a]	47
Milk	1	Koch and McMeekin	3.4, 3.5, 3.3	3.3	
		2 drops 72% $HClO_4$	2.1, 1.9, 2.4	2.1	38
	2	Koch and McMeekin	3.7, 3.7, 3.8	3.7	
		Rose	3.0, 2.7, 2.8, 3.0	2.9	22
	3	West and Brandon	4.4, 4.7, 3.9	4.3	
		1 drop 72% $HClO_4$	3.0, 3.0, 2.9	3.0	30
	4	West and Brandon	3.4, 3.4, 3.7	3.6	
		Rose	3.0, 2.6, 3.2	2.9	19
	5	Koch and McMeekin	3.3, 3.3, 3.2	3.3	
		Chiles	1.5, 1.2, 1.7, 1.3	1.4	57
			Total Nitrogen		
			%		
Urine	1	Koch and McMeekin	2.0, 2.0, 1.9	2.0	
		1 drop 60% $HClO_4$	1.7, 1.8, 1.8	1.8	10
	2	Koch and McMeekin	2.3, 2.5, 2.4	2.4	
		Rose	2.2, 2.2, 2.2	2.2	8
	3	Koch and McMeekin	0.71, 0.71, 0.71	0.71	
		Rose	0.64, 0.64, 0.67, 0.62	0.64	10
	4	West and Brandon	1.4, 1.4, 1.3	1.4	
		1 drop 72% $HClO_4$	1.1, 1.2, 1.2	1.2	14
	5	West and Brandon	2.1, 2.5, 2.3	2.3	
		Rose	1.9, 1.8	1.8	22
			Nonprotein Nitrogen		
			Mg./100 cc.	*Mg./100 cc.*	
Filtrate	1	Koch and McMeekin	54, 52	53	
		Dupray	41, 47, 44	44	17
	2	West and Brandon	43, 45, 48	45	
		Dupray	39, 41	40	11
	3	Koch and McMeekin	36, 37, 36	36	
		Dupray	22, 28, 27	26	28
	4	West and Brandon	44, 47, 43	45	
		Dupray	26, 24, 27	26	42
	5	Folin and Wu (*5*)	31, 29, 28	29	
		Dupray	25, 20, 27	24	17

[a] It, is, of course, almost meaningless to average such scattered results. Chiles' mixture can give very varying figures, depending partly upon the critical heating time.

The standard solutions for the colorimetric comparison were always of similar acidity as the samples, and standard and sample were brought to the same temperature before matching. Six careful readings of the colorimeter were averaged for each single determination.

Typical findings are given in Table II. In every instance, the results from the perchloric acid digests were lower and usually more scattered than those of the other procedures.

As a further experiment, a number of microdigestion techniques were all compared on a single sample of complex nitrogenous content. This was a solution of hemolyzed blood, consisting of oxalated whole blood which had been diluted 1 to 100 with water containing a trace of saponin. The results are shown in Table III. There is good agreement between the reference methods, and lower results with greater scattering where perchloric acid was present. (The authors have found in other work also that the digestion procedures of Koch and McMeekin and West and Brandon, and the persulfate oxidation of Wong, *25*, always give essentially the same figures.)

Whatever the reasons for low results with perchloric acid, at least part of the loss can occur in the final digest after charring has quite cleared. If an ammonium sulfate–sulfuric acid solution be heated in the presence of perchloric acid, a disappearance of nitrogen can result. To illustrate this, the following experiment was performed:

Five-cc. portions of an ammonium sulfate solution (≈ 0.5 mg. of nitrogen) containing 0.05 per cent dextrose were pipetted into ignition tubes together with 1 cc. of 1 to 1 sulfuric acid and two small quartz pebbles. (The dextrose served to mark the charring point at which some workers drip in the perchloric acid.) The tubes were heated (electrically, for uniformity) until the dextrose charred and sulfur trioxide fumes escaped. They were then lifted from the heater coils and let cool slightly to avoid splatter when the 72 per cent perchloric acid was introduced. This was added in varying numbers of small drops, and the tubes were covered with 30-mm. watch glasses, returned to the coils, and heated for an additional 5 minutes. They were then diluted, nesslerized, and read in the usual manner.

TABLE III. COMPARISON OF MICRO-KJELDAHL DIGESTIONS ON HEMOLYZED BLOOD

Digestion Method	Nitrogen Found	Average	Significant	Approximate Difference from Above, %
	Mg. per 100 cc.			
Koch and McMeekin	33.6, 35.7, 34.3	34.5	34	
West and Brandon	34.4, 33.4, 34.1	34.0	34	
Wong	33.8, 34.0, 33.6	33.8	34	
Rose	26.7, 22.6, 24.9, 24.3	24.6	25	26
Dupray	29.6, 27.9, 28.4, 26.5	28.1	28	18
1 to 2 drops of 72% $HClO_4$ added	27.2, 24.2, 26.8, 25.6	26.0	26	24

The results are illustrated in Figure 1, each point representing the average of three to four runs. The curve is necessarily a ragged one, but it does show progressive loss of nitrogen with increased perchloric acid. Heating time was a little longer and perchloric acid concentration somewhat higher than would have been required by most microdigestions. The curve illustrates simply that perchloric acid can remove nitrogen from a heated ammonium sulfate–sulfuric acid solution.

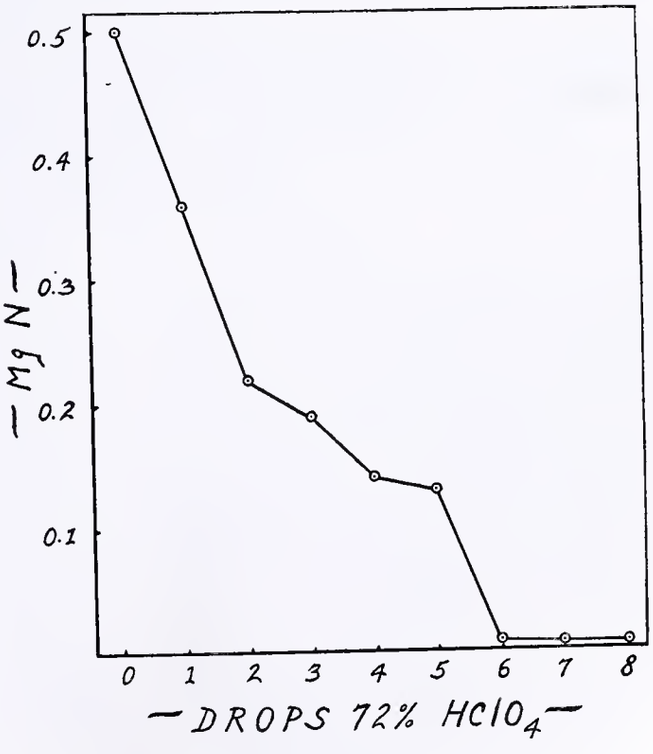

FIGURE 1. DESTRUCTION OF NITROGEN IN AMMONIUM SULFATE–DEXTROSE–SULFURIC ACID SOLUTION BY ADDED PERCHLORIC ACID

Discussion

The mechanism of the apparent loss of nitrogen is not clear. Mears and Hussey suggested that possibly an excess of perchloric acid formed ammonium perchlorate which then decomposed. Walters, Smith, Willard and Cake, and Peters and Van Slyke evidently believed that part of the ammonia (as ammonium sulfate) could be oxidized to free nitrogen. Myers regarded the lower results in colorimetric micro determinations as probably due to the formation of amines which, he stated, would not give full color development with Nessler's reagent. This does not seem to be sufficient explanation, although Gortner and Hoffman (*9*) pointed out that about 7 per cent of amine nitrogen results with ordinary Kjeldahl digestion, and Villiers and Moreau-Talon (*20*) earlier claimed that the presence of strong oxidizers promotes amine formation. (Their paper was prior to the introduction of perchloric acid.)

Certain analysts may protest that, granting all the above, if an excess of perchloric acid be avoided, the error will still be negligible. This may be true for sizable samples, and where just enough perchloric acid is added to clear the solution after preliminary digestion by sulfuric acid. But for microquantities, even a small excess may release a considerable portion of the nitrogen—for example, 2 small drops may be inadequate and 3 drops excessive.

Temperature

Apparently temperature has an important influence on the oxidizing power of perchloric acid. The acid does not seem to have much effect on the organic matter until sufficient water has been driven off from the digest to elevate the boiling point considerably, after which the combustion is vigorous and rapid. A little perchloric acid added then is much more potent than a great deal present in the original digestion mixture. Certain experiments suggest that, with very careful temperature regulation, a zone might be found where one could complete the oxidation of the carbon without loss of nitrogen. The authors wonder, however, whether such procedure would prove either practical or trustworthy.

Acknowledgment

The authors are grateful to G. F. Smith of the University of Illinois for the loan of a monograph (*8*) otherwise difficult to obtain.

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Improvements in the Colorimetric Microdetermination of Phosphorus

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TWO fundamentally distinct methods have been in use for almost two decades for the colorimetric determination of phosphorus—the Bell-Doisy (*1*) and Copaux (*4*) methods. The former, depending on the estimation of a blue color formed by the yellow phosphomolybdate compound reacting with some reducing agent, has undergone many modifications in the hands of various workers. A very excellent account of this method and modifications has been presented by Snell (*6*) and Peters and Van Slyke (*5*). Zinzadze's studies (*7*, *8*) pertaining to amounts of reducing reagent, time, temperature, etc., are interesting.

A great improvement in the colorimetric determination of phosphorus was made with the introduction of the Berenblum-Chain (*2*, *3*) method. Incorporating the features of both the Bell-Doisy and Copaux methods, this proved more successful than either. In the Berenblum-Chain method the yellow phosphomolybdate compound is extracted with isobutyl alcohol instead of ether as in the method of Copaux, and then changed into the blue phosphomolybdate compound by means of a reducing agent (stannous chloride in 37 per cent hydrochloric acid). Also, the ether phosphomolybdate extract obtained by the method of Copaux can be easily converted into a blue color by addition of the tin–hydrochloric acid reagent used by the writer. However, this procedure is not recommended, owing to the high degree of volatility of the ether and rapid changes in the volume of the extract. *n*-Butyl alcohol is preferred to ether as a solvent.

The main points of divergence of the writer's technique from that of Berenblum-Chain are in the use of metallic tin instead of stannous chloride in 37 per cent hydrochloric acid, elimination of ethyl alcohol for washing the separatory funnel and dilution of the blue phosphomolybdate compound, heating the mixture containing the unknown, 2 *N* sulfuric acid, and ammonium molybdate, and the use of normal instead of isobutyl alcohol.

A reducing agent prepared by mixing stannous chloride and 37 per cent hydrochloric acid sometimes loses its reducing power and fails to give an intense indigo blue color during an

Table I. Readings on Summerson-Klett Photoelectric Colorimeter

Phosphorus Mg./l.	Scale Readings[a]	Scale Readings[b]	% Loss of Color[b]	Scale Readings[c]	% of Original Color[c]
0	15	..	..	..	..
0.5	50	26	48	47	94
1.0	74	50	32	68	92
1.5	100	64	36	90	90
2.0	130	110	15	127	98
2.5	150	130	13	146	97
3.0	180	152	15	175	97
3.5	220	171	22	200	91
4.0	250	206	17	234	94
4.5	280	223	20	265	95
5.0	320	245	23	300	94
6.0	370	302	19	340	92
7.0	440	370	16	420	96
8.0	470	420	11	455	97
9.0	540	480	11	530	98

[a] 2 hours after extraction.
[b] 72 hours after extraction.
[c] After addition of tin–HCl reagent to 72-hour standing samples.

entire day's work and often the color of samples has a greenish blue tinge. The metallic tin–hydrochloric acid reducing agent, as recommended by the writer, is considerably more effective than the stannous chloride–hydrochloric acid reagent of Berenblum-Chain, so that reduced samples seldom possess a greenish blue tinge.

The use of *n*-butyl alcohol instead of ethyl alcohol retains the water-immiscible properties of the phosphomolybdate extract, an advantage for samples which have failed to undergo complete reduction to the indigo blue color so desirable for an accurate determination of phosphorus. Such samples can be treated again, at any time, with the metallic tin–hydrochloric acid reagent in a separatory funnel until the indigo blue color is obtained, without appreciable loss of the phosphomolybdate extract.

The choice of *n*-butyl alcohol over isobutyl alcohol is of no great importance and either form of butyl alcohol will serve the purpose very well.

Reagents

Tin–Hydrochloric Acid. Place 0.5 gram of metallic tin (mossy tin) in a 50-ml. volumetric flask containing 40 ml. of 37.5 per cent hydrochloric acid, and allow to stand for 1 or 2 hours, agitating the mixture gently at intervals until complete solution.

Ammonium Molybdate. Dissolve 40 grams of ammonium molybdate in 1000 ml. of water and filter.

n-Butyl Alcohol.

Sulfuric Acid, 5.6 Per Cent. Prepare a 5.6 per cent (2 *N*) solution of sulfuric acid by pouring 56 ml. of concentrated sulfuric acid (density 1.84) into a 1000-ml. volumetric flask containing 500 ml. of water. Cool and complete the volume with water.

Sulfuric Acid (1.4 Per Cent 0.5 *N*) and Tin. Transfer 50 ml. of the 5.6 per cent solution of sulfuric acid to a 200-ml. flask, add 10 ml. of the tin–hydrochloric acid reagent, and make to the mark with water.

Method of Procedure

Ash 1 or 2 grams of dry plant tissue in a platinum dish, dissolve the ash with 10 ml. of 5 *N* hydrochloric acid, then add 20 ml. of water and heat on a hot plate (90° C.). Pour the solution into a 100-ml. volumetric flask, add water to complete the volume, and filter.

Transfer an aliquot of 10 ml. of the filtrate into a Pyrex ignition tube (25 × 200 mm.), neutralize with 5 *N* sodium hydroxide, using a small piece of litmus paper as indicator, then add 2 ml. of 2 *N* sulfuric acid and 5 ml. of ammonium molybdate, and heat in a water bath for 10 minutes. (The heating of the mixture was found to facilitate the extraction with butyl alcohol, besides speeding the formation of the phosphomolybdate compound.) Pour the mixture into a 250-ml. Squibb separatory funnel, rinse the tube with 5 ml. of hot 0.5 *N* sulfuric acid, and transfer the washings to the funnel. Add 10 ml. of *n*-butyl alcohol, close with a glass stopper, and agitate vigorously for 30 seconds. Allow the upper yellowish phosphomolybdate layer to separate from the lower aqueous acidified layer. Often an emulsion forms between butyl alcohol and water, requiring a long time for the separation of the two layers. The emulsion can be easily broken up by the addition of 5 ml. or more of 0.5 *N* sulfuric acid. Discard the aqueous layer in all cases; add 10 ml. of the reagent of tin–hydrochloric acid in 0.5 *N* sulfuric acid to the butyl alcohol layer containing the yellow phosphomolybdate compound, and agitate the mixture vigorously.

After allowing sufficient time for the separation of the two layers, discard the lower aqueous layer. If the phosphomolybdate compound shows a greenish blue tinge, add more of the tin–hydrochloric acid reagent until the blue color is entirely free from a greenish tinge. After the removal of the aqueous layer transfer the blue butyl alcohol layer to a 15-ml. or any other convenient size graduated centrifuge tube, and, if necessary, add more butyl alcohol to the separatory funnel to wash away all traces of the blue phosphomolybdate compound which is added to the extract in the centrifuge tube. Finally adjust the volume of the butyl alcohol–phosphomolybdate extract in the centrifuge tube by the addition of more butyl alcohol.

The addition of 2 to 5 ml. of the tin–hydrochloric acid reagent to the butyl alcohol extract in the centrifuge tube was found to stabilize and retard the fading of the blue color. However, the addition of the tin–hydrochloric acid reagent often causes a slight emulsification of the extract which can be easily broken up by centrifugation in the course of 5 to 10 minutes. After centrifugation leave the tin–hydrochloric acid in the tube to occupy the lower layer while a volume of approximately 8 ml. of the butyl alcohol layer containing the phosphomolybdate compound is transferred by means of a pipet to a glass cell or a tube and phosphorus is determined by means of a visual colorimeter, a photoelectric colorimeter, or a step photometer with appropriate light filters.

Experimental Results

The data reported in Table I were obtained by the above method with known amounts of phosphorus. The scale readings for known amounts of phosphorus were obtained with a Summerson-Klett photoelectric colorimeter employing a No. 59 light filter with transmission limits of 565 to 630 millimicrons and a 2.5-mm. cell. The 2.5-mm. thickness of the cell was obtained by inserting a 7.5-mm. thick plunger into a 10-mm. cell. Three sets of readings are presented in Table I, obtained (1) 2 hours after the extraction of the butyl alcohol–phosphomolybdate compound; (2) after 72 hours of standing in ordinary light and at room temperature (25° to 29° C.); and (3) after the addition of 5 ml. of the tin–hydrochloric acid reagent to the samples which had stood 72 hours.

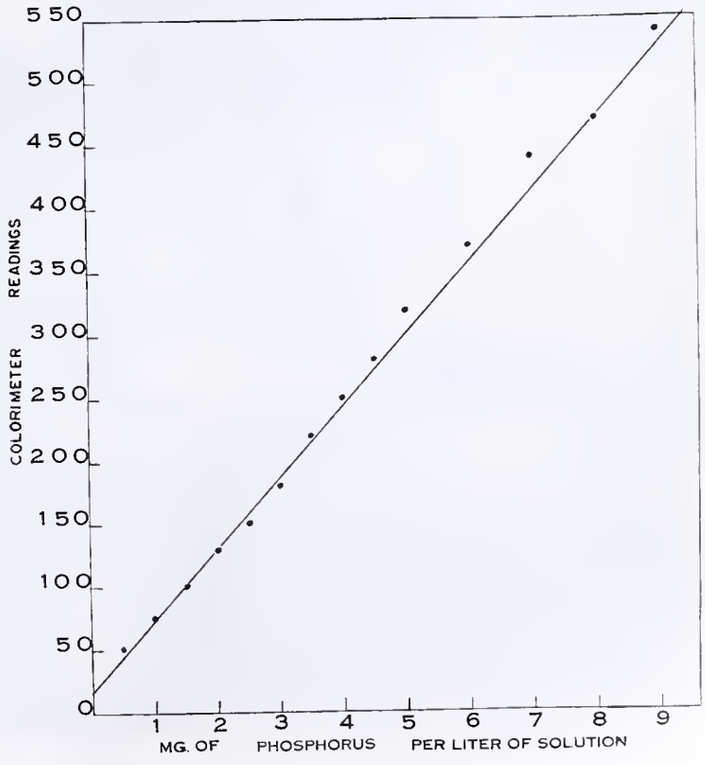

Figure 1. Determination of Phosphorus

The reagents showed a color reading of 15 scale units, representing a phosphorus contamination of approximately 0.2 microgram of phosphorus per ml. of solution.

The curve drawn in Figure 1 was set for its zero phosphorus value at 15 of the colorimeter scale, because of the phosphorus contamination of the reagents, and for its 9 micrograms of phosphorus value at 525 (540 − 15 = 525). The deviation of the various experimental points ranges from 0 to 7 per cent.

The writer wishes to warn against the use of greater concentrations of sulfuric acid than those recommended and the adding of the tin–hydrochloric acid reagent to hot butyl alcohol extracts. Check always with one or more standard solutions the values of unknown samples if obtained from curves based on readings from a photoelectric colorimeter scale.

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Gas-Fired Furnace for Semimicrodetermination of Carbon and Hydrogen

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THE successful combustion of the sample is a very important factor in the determination of carbon and hydrogen. The author has long felt that certain more or less serious difficulties encountered in the combustion operation could be reduced considerably. Among these difficulties are (1) the combustion of graphitic carbon residues, (2) the uncertainty of complete oxidation of the organic vapor, and (3) prevention of premature vaporization or decomposition of the sample.

When the conventional electric furnace type of apparatus is used it becomes necessary to slide the furnace over the carbon residue in order to complete the combustion. Because of the creeping tendency of many types of organic compounds, carbon residues are particularly apt to be deposited on the upper side of the bore of the combustion tube for a considerable distance, especially when large samples are used. It then becomes necessary to slide the furnace a corresponding distance, whereby an equal length of the copper oxide charge becomes exposed to the air and rapidly cools down and becomes ineffective.

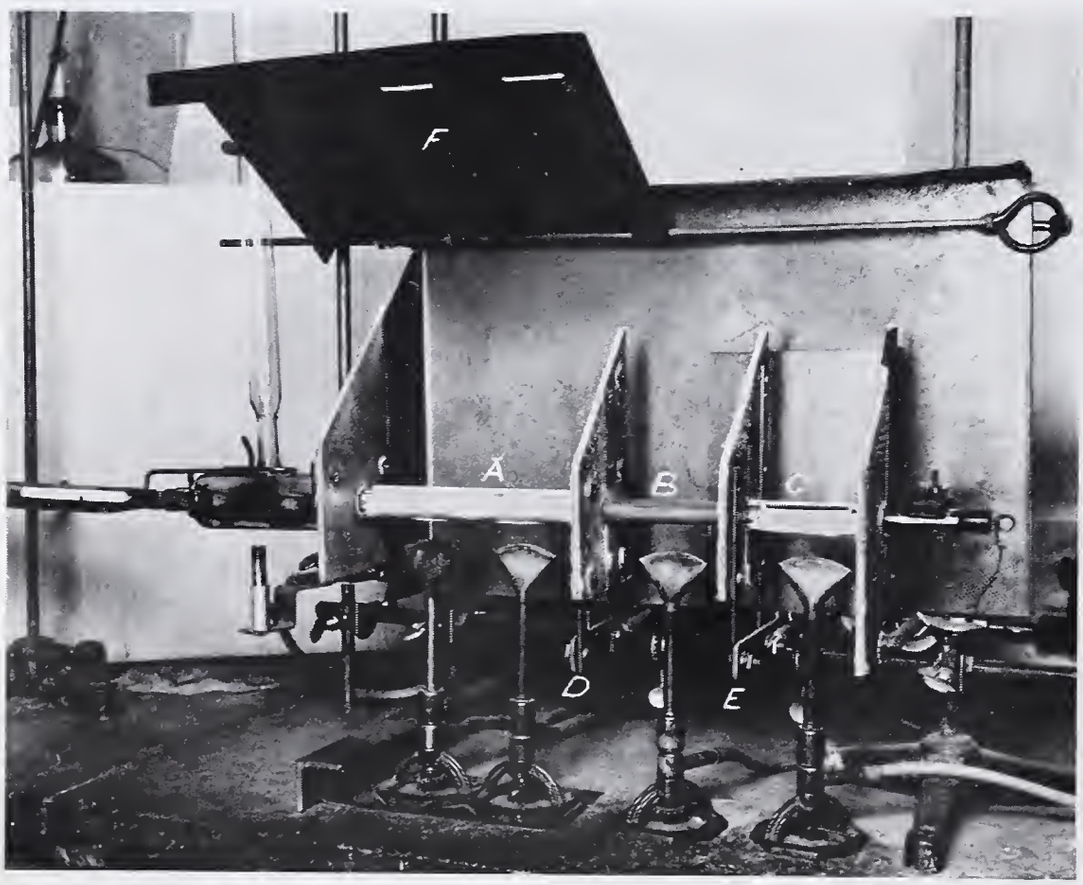

Figure 1. Assembled Furnace

With the conventional gas-fired furnace it is often very difficult to burn carbon residues deposited on the upper side of the combustion tube without overheating the lower side and causing the tube to sag. The use of wire gauze rolls surrounding the tube, in conjunction with heat-reflecting tunnels, does aid considerably in providing a fairly uniform cross-section temperature in the small-diameter microtubes. However, when larger diameter tubes are used in order to accommodate the much larger semimicro-samples, this arrangement becomes increasingly ineffective. This same difficulty may well cause concern regarding the complete oxidation of organic vapors if they pass too

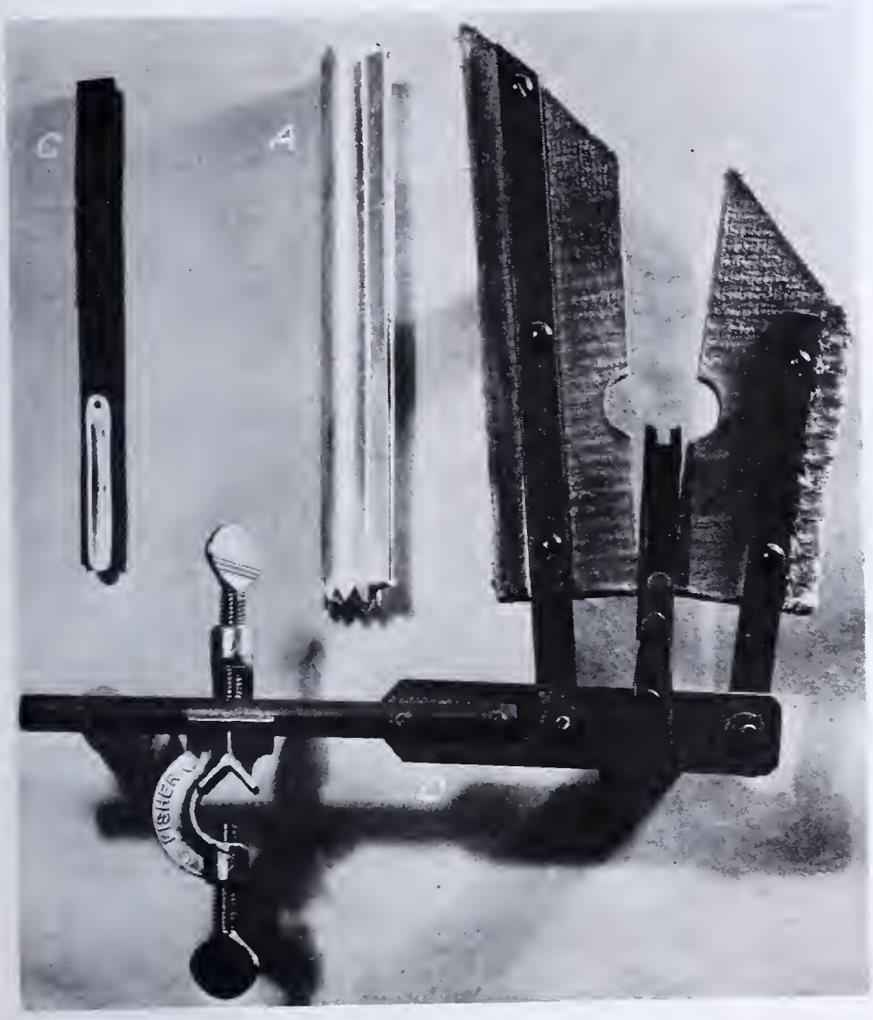

FIGURE 2. DETAILS OF SLEEVE

rapidly through the copper oxide oxidizing section. Any settling of the copper oxide charge will be downward, leaving the colder, top side as the path of least resistance for the vapors to escape unburned. In this respect the electric furnace type is superior, yet the unavoidable heat loss on both ends decreases the effective length of the oxidizing charge. The gas-fired furnace described below is fitted with silver "sleeves" which serve as very efficient heat conductors to provide a practically uniform cross section and linear combustion tube temperature. The design also includes a center combustion tube support which in no way interferes with the burning of the carbon residue. Furthermore, the sample is protected against premature vaporization or decomposition from radiant heat.

Figure 1 shows the assembled furnace. The combustion zone, *A*, is 19 cm. long fitted with a stationary silver sleeve shown in detail in Figure 2, *A*. This sleeve is of pure sheet silver, No. 19 B. and S. gage (0.9 mm., 0.036 inch thick), made by bending the proper sized rectangular sheet of silver around an iron shaft 1.9 cm. (0.75 inch) in diameter. Sufficient extra width was allowed so that the edges of the seam could be turned up at right angles to form a ridge about 4 mm. high and a slot about 3 mm. wide between the edges. On each end the turned-up edges were cut back with a jeweler's saw tangent to the surface for a distance of about 8 mm. and then bent over each other to prevent the tube from either expanding or contracting out of shape. The slot permits the operator to observe the temperature of the copper oxide within the combustion tube. The purpose of the saw-toothed ends is to produce an irregular hot-cold junction. Otherwise, the combustion tube might crack at these places at the ends of the sleeves. As a further protection against possible tube breakage two small holes about 1 cm. apart were made on the top side of each end of the sleeve and a staple of Nichrome wire (22 or 24 gage) was passed through from the inside and clinched on the outside to prevent the silver from touching the glass. The silver sleeve 8 cm. long in the safety gauze section (Figure 1, *C*), is made in the same manner, with a saw-toothed edge like that of the long sleeve, so that the two edges will interlock when the sleeves are pushed together. The inside diameter should allow about 1-mm. clearance on all sides between the sleeves and the combustion tube. The bending and shaping of the sheet silver are rather difficult and tedious. More recently the author has obtained seamless silver tubing made to his specifications, at a very reasonable cost; hence, the making of such sleeves becomes a simple matter.

D and *E* (Figure 1) and *D* (Figure 2) split asbestos board shields, 9 cm. wide and 9 and 18 cm. high on the front and back, respectively. Small round-headed bolts attach the asbestos board to 0.31 × 1.25 cm. (0.125 × 0.5 inch) iron bars, which are in turn loosely bolted to a plate as shown. Lock washers placed under the nuts provide sufficient tension to hold the bars firmly when the split shields are open or closed. The end plates which serve as the main supports of the combustion tube are the same shape, but 10.5 cm. wide. This extra width allows the split shields to be opened while the metal covers (described below) are in place. In order to keep the shields in alignment with the end plates they are fastened with clamp holders onto a horizontal 1.25-cm. (0.5-inch) octagonal bar.

Shield *D* (shown in Figure 2, *D*) is fitted with an auxiliary support to prevent possible sagging of the combustion tube. A sheet of Nichrome metal folded over on each edge (to give added rigidity) is riveted to the center bar as shown. A slot is filed in the end and the resulting tips are filed to the approximate curvature of the combustion tube. The exact curvature is then easily obtained by milling with a piece of fine emery cloth wrapped around a combustion tube. The center bar, being fastened to the side opposite the asbestos boards, is thus offset sufficiently so that two holes may be made through the under side of the silver sleeve to permit the Nichrome supporting tips to pass through the sleeve and contact the combustion tube. This construction could be simplified by drilling two properly spaced vertical holes in the end of the center bar and driving in short sections of heavy Nichrome wire to serve as supporting tips.

The so-called "boat-carrier" (Figure 2, *C*) made of No. 22 B. and S. gage (0.625 mm., 0.025-inch) sheet Nichrome, 17.5-cm. (7 inch) over-all length, contains the copper oxide safety gauze and the combustion boat. When placed in the combustion tube (with the aid of a long nickel tweezer) the safety gauze lies in section *C* (Figure 1) and the combustion boat in *B*.

F (shown in raised position) is a cover for the oxidizing zone. This is made of ordinary black stovepipe iron, hinged and supported by a horizontal rod 0.625 cm. (0.25 inch) in diameter. When lowered it extends down about 2.5 cm. (1 inch) below the lower level of the combustion tube. Narrow slots cut parallel to the combustion tube enable the operator to observe the furnace temperature within. A similar detachable cover is provided for section *C*. A sheet of the same metal is hung the entire length of the back of the furnace.

The fixed burners under section *A* are fastened to a metal plate which is then rigidly bolted to the improvised angle-iron stand. Extension arms are soldered to the adjustment screws of the burners. Sufficiently accurate temperatures are obtained by setting the extension arms at predetermined positions.

COMBUSTION SEQUENCE. Both split shields, *D* and *E*, are closed, the oxidizing zone, *A*, is heated to the working temperature, and the boat carrier is removed. The combustion boat containing the sample is placed in the open trough of the boat carrier and immediately introduced into the combustion tube. The oxygen flow is then started and the burner under the short silver sleeve, *C*, is turned on. Seven to 10 minutes are required to heat the safety gauze to redness. (In the meantime the sample has been protected from radiant heat on either side by the asbestos shields. Furthermore, Nichrome metal is a relatively poor conductor of heat; hence not enough heat is transmitted by the boat carrier to cause premature volatilization of easily volatile compounds.) The sample is then cautiously vaporized or charred by directly heating the bare combustion tube. When only carbon remains, the split shield, *E*, is opened and the short silver sleeve is gradually moved along toward the boat at the rate of

about 1 cm. per minute. The burner is moved with the silver sleeve. During this time the burner under *B* is slowly moved back and forth to keep the boat section hot. When the short silver sleeve has reached the shield, *D*, the latter is opened and the two sleeves are pushed together so that the serrated edges interlock, and the heating is continued until all carbon residues are burned. While the safety gauze is being heated, and again later when the carbon residue is being burned, the room is darkened considerably by drawing the curtain and turning off the ceiling lights. In this way temperatures can be readily observed. A droplight hanging above the furnace is used whenever more light may be needed.

Discussion

The high efficiency of the silver sleeves as uniform heat conductors is well shown by the fact that the two Tirrill type burners (using natural gas, 1000 B. t. u.) fitted with wing tops will heat the entire combustion zone to working temperature in 15 minutes. The flames are set at a total height of about 1.25 cm. (0.5 inch), while the height of the burners themselves is so adjusted that only the uppermost fringe of the flames strikes the silver. In total darkness, no difference in temperature color of the top and the bottom of the sleeve can be detected visually. A very slight gradient toward the ends is noticeable. The saw-toothed edges are uniformly red to the very tips.

Since silver is a "white" metal, it, unfortunately, does not distinctly show temperature colors in the 650° to 700° C. range in a well-lighted room. The black metal covers and back are necessary to provide partially dark surroundings in order to observe the temperature. However, once the proper burner adjustments have been established, by means of a thermocouple, it is sufficient merely to check the temperature visually a few minutes before the sample is ignited.

After long service the silver sleeves may gradually become dull and slightly rough, particularly if heated directly in a reducing flame. If heated unnecessarily hot, bad blisters will form. Two furnaces as described have been in use in the author's laboratory as standard equipment for class work and research for over a year. A total of nearly 300 analyses have been carried out on the one shown here. The long sleeve still retains its original luster, except for a slight roughness which has developed where the flames strike. The short sleeve was accidentally overheated and badly blistered shortly after the furnace was made, but it continues to function efficiently. The other furnace, which has been used somewhat less, is heated with a micro longburner. This long sleeve has shown no deterioration whatsoever. The silver sleeves therefore can be expected to give very long service.

Samples of 25 to 30 mg. are usually used. However, where large amounts of sample are available, 40- to 50-mg. samples have been used, particularly for low hydrogen compounds. For this reason a 13 × 17 × 650 mm. combustion tube (Pyrex brand glass No. 172) is used.

The combustion tube is filled with the conventional copper oxide, metallic silver, and lead dioxide charge. Thus far, catalysts such as platinum black or cerium dioxide have not been found necessary. In addition to a large number of student analyses of "knowns" such as sucrose, benzoic acid, acetanilide, and *p*-nitrobenzoic acid, over 200 analyses of research preparations have been carried out. The following list includes some of the types of compounds analyzed: quinoline and benzoquinoline derivatives; piperidino-, morpholino-, tetrahydroquinolino-, tetrahydroisoquinolino-, and *N*-methylamino- ketones. The corresponding derivatives were also analyzed: amino ketone hydrohalides, amino bromoketones, amino alcohols, amino alcohol hydrochloride, and amino ketoximes; as well as 4-piperidino- and 4-morpholino- 4-phenyl-2-aminobutanes and their benzamides, and benzoates of piperidino and morpholino alcohols.

In general, where the physical properties of the compounds indicated a high degree of purity, the carbon found rarely deviated as much as ±0.15 per cent from the theoretical—namely, 2.5 parts per 1000, on the basis of a 60 per cent carbon compound. The hydrogen values found were also very satisfactory. During periods of high humidity these values tend to run somewhat too high, although still well within the permissible tolerance.

Pyrex Brand Glass Wool as a Filtering Medium

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THE description of Pyrex brand glass wool (*1*) states that the glass wool "made from 'Pyrex' brand glass No. 719 of low alkali content can be used as a filtering medium where solubility or pH are critical factors". Unfortunately, when this glass wool was used as a filtering medium in semimicroanalysis (*2*) aluminum ion was reported incorrectly by a large per cent of the students. An investigation showed that a solution of a strong base or acid, used to extract the glass wool, gives a precipitate of aluminum hydroxide when neutralized and treated with a slight excess of ammonia. The presence of aluminum in the precipitate was confirmed by the aluminon test. Excellent tests for aluminum were obtained when approximately 0.1 gram of the glass wool was extracted with 1 ml. of 0.1 *N* hydrochloric acid. The aluminum calculated as the oxide, in 25 ml. of 2.5 *N* hydrochloric acid used to extract 0.5315- and 0.8005-gram samples of the glass wool, was 0.21 per cent of the weight of the sample.

After the glass wool had been extracted several times with dilute hydrochloric acid and thoroughly washed, additional acid used to extract the glass wool gave no precipitate of the aluminum lake of aluminon, but a comparison of the solution with a blank showed that a small amount of aluminum ion was present. The use of the glass wool as an inert filtering medium is open to question. For all except the most exacting uses, this glass wool will be satisfactory after it has been thoroughly extracted with a hot acid and washed.

Recently a small sample of a high-silica glass wool submitted by the Corning Research Department was extracted with hot hydrochloric acid (5 *N*) and sodium hydroxide (2 *N*). The liquid did not give a precipitate with the aluminon reagent. The sample was made in a small experimental unit and is not yet available commercially.

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INDUSTRIAL AND ENGINEERING CHEMISTRY

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Determining the Oil Content of Paraffin Waxes

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A NUMBER of laboratory methods have been proposed for the analysis of oil-wax mixtures (*1–7, 10–14*). Some of these methods were developed for use on waxy stocks containing large amounts of oil and are employed chiefly for controlling commercial dewaxing operations. These are not sufficiently accurate for the analysis of waxes containing small amounts of oil. Of the methods which have been proposed for the analysis of both scale and refined grades of paraffin wax, only the press method (*2*) has been used extensively in the petroleum industry. This is generally recognized to be lacking in accuracy and was abandoned as a tentative standard by the A. S. T. M. in 1939. The other published methods for the analysis of paraffin waxes involve complicated procedures or are limited in applicability to waxes of known origin.

The analytical problem is complicated by the absence of a sharp line of demarcation between "oil" and "wax", owing to the existence of intermediate compounds which may be defined as either "oil" or "wax" (*8, 9*). An arbitrary definition of the boundary line between oil and wax is, thus, necessary in analytical methods of this type.

This paper describes a simple and reliable method for the determination of oil in scale and refined paraffin waxes containing not over 10 per cent by weight of oil. The applicability of the method to waxes of higher oil content has not yet been fully investigated. The method is based on the selective separation of oil and wax by solvent extraction under carefully specified conditions. "Oil" is defined in this test as the percentage of solvent-free extract minus a correction of 0.15 per cent. The 0.15 per cent correction factor was developed empirically on waxes containing known quantities of oil and having melting points between 115° F. (46° C.) and 145° F. (63° C.). For waxes melting outside this range different factors may be necessary. The results obtained by this method are usually accurate to about ±0.05 per cent on oil-wax mixtures containing less than 1 per cent oil.

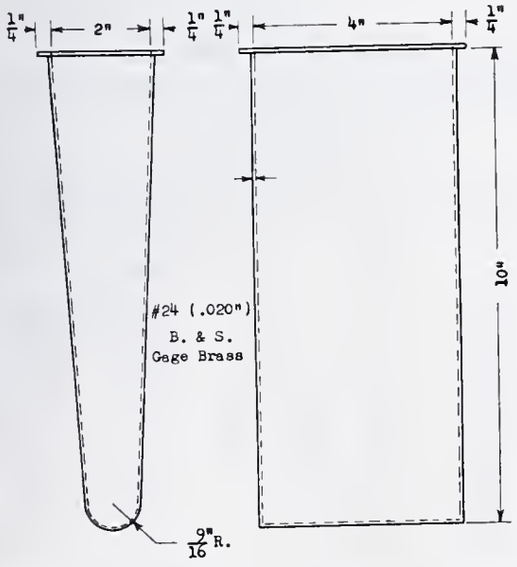

FIGURE 1. COOLING VESSEL

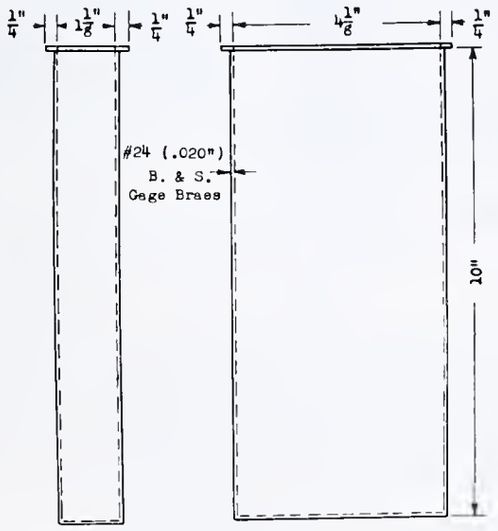

FIGURE 2. PRECOOLING CHAMBER

The method involves dissolving a 25-gram sample of wax in 375 ml. of methyl ethyl ketone and chilling the mixture to −25° F. (−31.7° C.). A portion of the chilled mixture is filtered by means of an immersion-type filter leaf using vacuum. The amounts of oil and solvent in the filtrate are determined by evaporation of the ketone. As the proportion of solvent and oil in the filtrate is the same as that in the chilled mixture, the quantity of oil in the wax sample can be calculated by simple arithmetical rules. The filtration of only a portion of the chilled mixture eliminates the need of washing the wax cake with chilled solvent, thereby obviating errors due to occlusion of oil by the wax and permitting the use of relatively simple apparatus and procedure.

Apparatus

The apparatus consists of the following items:

Cooling vessels, V-shaped, of about 1-liter capacity and having the dimensions shown in Figure 1.

Precooling chamber for filter leaf having the dimensions shown in Figure 2.

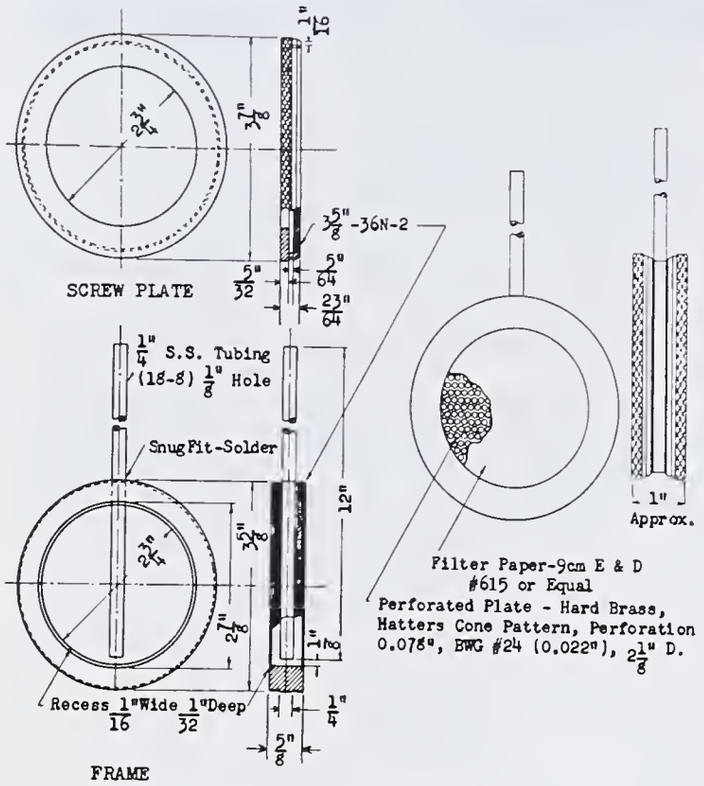

FIGURE 3. FILTER LEAF

All parts nickel-plated

Filter leaves of the type shown in Figure 3. The filter medium is No. 8-0 filter canvas tightly fitted to the leaf. A qualitative grade of filter paper may be used on the circular leaf, provided it is supported by a perforated plate having a free area of not less than 40 per cent.

Insulating cooling bath large enough to accommodate the desired number of cooling vessels and precooling chambers. The bath is filled with a suitable liquid such as methyl ethyl ketone which is refrigerated by solid carbon dioxide or brine circulation. The temperature of the bath is maintained at $-35 \pm 5°$ F. ($-37 \pm 3°$ C.) throughout the test.

Erlenmeyer flasks of 500-ml. capacity for preparation of the wax-solvent mixture.

Erlenmeyer flasks of 250-ml. capacity for collecting the filtrate and evaporating the solvent therefrom.

Evaporation assembly as shown in Figure 4. Jets are provided for delivering a stream of clean, dry air vertically downward into the evaporation flasks. The inside diameter of the jet is 3 ± 0.2 mm. and the tip of the jet is 25 ± 5 mm. above the surface of the evaporating liquid at the start of evaporation. Air is supplied at the rate of 2 to 3 liters per minute per jet. The air is purified by passing it first through a commercial air filter and then through a 30-cm. column of 30- to 60-mesh fuller's earth. The cleanliness of the air is checked periodically by evaporating 100 ml. of methyl ethyl ketone in a water bath which is maintained at 95 ± 2° F. (35 ± 1° C.). The residue left on evaporation should not exceed 0.002 gram.

Thermometers of 0° to 300° F. range to measure the temperature of the wax-solvent solution and thermometers of $-70°$ to $+70°$ F. range to measure the temperatures of the cooling bath and cooling vessel.

Solvent

Methyl ethyl ketone is used as the solvent in this test. The commercial grade is satisfactory, provided its refractive index at 68° F. (20° C.) is 1.378 ± 0.002 and the residue left on evaporating a 100-ml. sample by the procedure outlined below for evaporating solvent from the filtrate is not more than 0.002 gram.

Procedure

A representative portion of the wax sample is melted in a suitable container, using a water bath or oven maintained at 160° F. (71° C.) to 180° F. (82° C.). The wax sample should not be held in the molten condition longer than is necessary for its complete liquefaction. Twenty-five grams (±1 gram) of the molten wax are weighed into a tared 500-ml. Erlenmeyer flask, making weighings to ±0.2 gram, and 375 ± 5 ml. of methyl ethyl ketone are added to the flask. A water-cooled condenser is attached to the flask and the mixture is heated on an electric hot plate or steam bath until the wax is completely dissolved, taking precautions to prevent surging of the solution into the condenser. The flask is then removed from the source of heat and, after boiling has ceased, the condenser is disconnected. The flask is then weighed to ±0.5 gram and quickly stoppered with a cork equipped with a thermometer.

The solution is allowed to cool to about 130° F. (54° C.) but before any wax precipitates it is poured into the V-shaped vessel in the cooling bath which is maintained at $-35 \pm 5°$ F. ($-37 \pm 3°$ C.). The wax solution is stirred with a thermometer at frequent intervals until the temperature reaches $-20°$ F. ($-29°$ C.) and then continuously until the temperature reaches $-25 \pm 0.5°$ F. ($-31.7 \pm 0.3°$ C.).

The filter leaf is removed from the precooling chamber in the cooling bath and immersed quickly in the chilled mixture. The filter leaf is connected to a 250-ml. Erlenmeyer flask tared to ±0.001 gram and to the vacuum system as shown in Figure 5. The vacuum should be maintained between 250 and 600 mm. of mercury gage. After about 100 ml. of filtrate have been collected in the flask, the suction is discontinued and the flask dis-

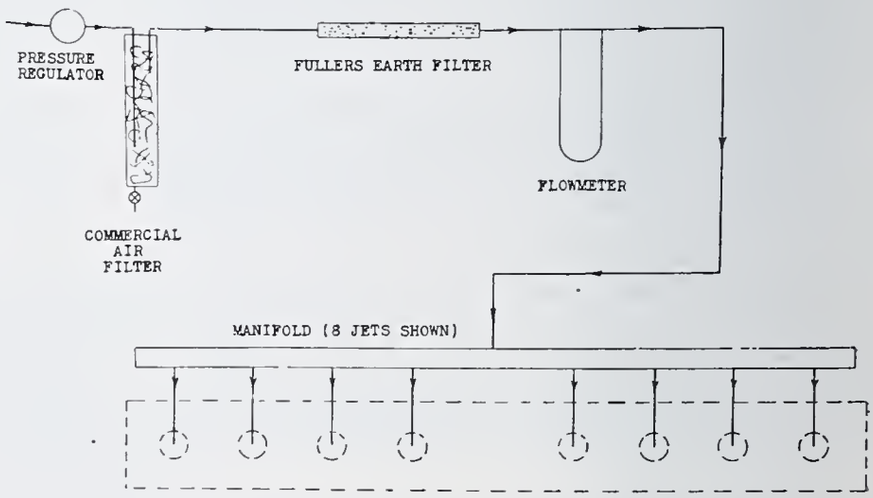

FIGURE 4. EVAPORATION ASSEMBLY

TABLE I. ANALYSIS OF KNOWN OIL-WAX MIXTURES BY PROPOSED METHOD

	Wax Component		Oil Component				Oil Content	
No.	Description	Melting point ° F.	Pour point ° F.	S. U. V. at 100° F.	Flash point ° F.[a]	% by wt. of blend	Found by proposed method	Calculated
							% by wt.	
1	Deoiled refined wax	132.2	..	...	...	0.00	0.00	0.00
2		131.4	..	...	...	0.00	0.00	0.00
3		124.0	..	...	...	0.00	0.05	0.00
4		123.2	..	...	...	0.00	0.00	0.00
5		123.7	..	...	...	0.00	0.08	0.00
5		123.7	15	94.3	320	0.19	0.18	0.19
5		123.7	15	94.3	320	0.38	0.34	0.38
5		123.7	15	94.3	320	0.99	0.89	0.99
5		123.7	15	94.3	320	2.09	1.72	2.09
6	Refined wax	131.1	..	...	...	0.00	0.19	..
6		131.1	0	90.7	...	0.49	0.75	0.68
6		131.1	15	86.8	...	0.41	0.56	0.60
7		123.2	..	...	...	0.00	0.41	..
7		123.2	15	86.8	...	3.84	4.03	4.25
7		123.2	20	196.0	...	0.99	1.39	1.40

[a] Pensky-Martens.

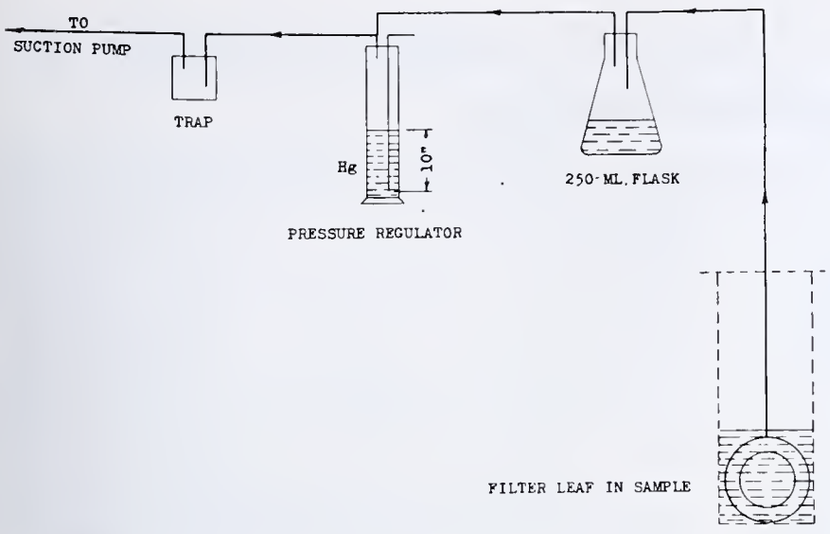

FIGURE 5. FILTRATION ASSEMBLY

connected from the filter leaf and vacuum system. The outside of the flask is washed with acetone, wiped off with a cloth, and weighed to ±0.2 gram.

The methyl ethyl ketone is evaporated from the filtrate, using the evaporation assembly described above. The flask is weighed to ±0.001 gram after 2 hours' evaporation and then after 0.5-hour evaporation periods until the loss between successive weighings is not over 0.002 gram. The flask should be rinsed with acetone to remove water, placed on a paper towel or other blotter to dry for one minute, then placed in a desiccator containing anhydrous calcium chloride at balance room temperature for about 15 minutes prior to weighing. The time required for evaporation is usually 2.5 hours.

The oil content of the wax sample is computed by the following equation:

$$\text{Oil, \% by wt.} = 100 \times \frac{\text{(weight of oil in filtrate)} \times \text{(weight of solvent in charge mixture)}}{\text{(weight of wax sample)} \times \text{(weight of solvent in filtrate)}} - 0.15$$

In performing this test, special care should be exercised to keep the equipment clean. This applies particularly to the filter leaf. The cleanliness of the filter leaf can be checked by filtering through it about 100 ml. of methyl ethyl ketone at ordinary temperatures and evaporating the filtrate as previously outlined. The residue should not exceed 0.002 gram.

TABLE II. INTERLABORATORY REPRODUCIBILITY OF PROPOSED METHOD

Wax Sample		Oil Content: Laboratory A	Laboratory B	Laboratory C	Average of three laboratories	Maximum Deviation from Average
		Per cent by weight				
130–132° F. m. p., refined wax		0.17	0.19	0.18		
		0.17	0.21	0.11		
	Av.	0.17	0.20	0.15	0.17	0.03
128–130° F. m. p., refined wax		0.29	0.24	0.17		
		0.25	0.22	0.24		
	Av.	0.27	0.23	0.21	0.24	0.03
126–128° F. m. p., refined wax		0.30	0.35	0.40		
		0.28	0.38	0.40		
	Av.	0.29	0.37	0.40	0.35	0.06
122–124° F. m. p., refined wax		0.34	0.41	0.47		
		0.36	0.41	0.39		
	Av.	0.35	0.41	0.43	0.40	0.05
126–128° F. m. p., scale wax		0.95	0.89	1.25		
		0.91	0.90	1.15		
	Av.	0.93	0.90	1.20	1.01	0.19
121–123° F. m. p., scale wax		1.83	1.72	1.83		
		1.85	1.76	1.89		
	Av.	1.84	1.74	1.86	1.81	0.07

Results

The accuracy of the results obtained in determining the oil content of paraffin waxes by the described method is illustrated by Table I. These results were obtained on deoiled refined waxes and known blends of dewaxed oils with refined waxes before and after deoiling. For waxes containing less than 1 per cent oil, the method gives results which are usually accurate to ±0.05 per cent.

The interlaboratory reproducibility of the method is demonstrated by the data in Table II. Except in one instance, the results from different laboratories differ from the average by less than 0.1 per cent.

A comparison of results obtained by the proposed method and the press method is shown by Table III. These data show that scale and refined paraffin waxes can be readily differentiated on the basis of oil contents as determined by the proposed method but not by the press method.

TABLE III. COMPARISON OF PRESS AND PROPOSED METHODS

Wax Sample	Oil Content: Press method[a] (Duretta cloth)	Proposed method
	% by weight	
Refined wax		
119.6° F. m. p.	1.2	0.45
123.6° F. m. p.	1.3	0.35
126.4° F. m. p.	1.0	0.27
131.0° F. m. p.	1.4	0.15
Scale wax		
118.8° F. m. p.	2.3	3.78
123.0° F. m. p.	0.8	2.98
126.4° F. m. p.	1.0	0.89
128.2° F. m. p.	1.1	0.52

[a] A. S. T. M. D-308-29T (abandoned in 1939).

Acknowledgment

The authors wish to express their appreciation to J. B. Rather for permission to publish this article, and to thank L. C. Drake, T. A. Petry, T. G. Roehner, and E. L. Smith of the Socony-Vacuum Oil Co., Inc., and E. L. Ruh and A. Williams of the Standard Oil Co. of New Jersey for many helpful suggestions in the development of this method.

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Location of the End Point on Certain Graphical Titration Curves

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The equations for certain types of titration curves are derived and possible methods for graphically finding the end point described. The practical aspects resulting from the analysis are discussed.

IN TITRATIONS such as polarometric (amperometric) (*1,5*), conductometric, radiometric (*4*), and others, in which the readings of the end-point indicator are proportional to the concentration of ions or molecules in the solution, graphical methods are usually most convenient for determining the equivalence or end point. Various constructions have been suggested for finding the equivalence point from the titration curve. Some of these methods, that of Majer (*5*), for example, are difficult to apply because they require the accurate knowledge of certain constants which are in most cases unknown. Others, such as the tangent method (*1*), are uncertain to apply when the solubility of the product is high or the solutions to be titrated are dilute and therefore the shape of the titration curve starts to deviate strongly from straight lines.

In this paper an attempt is made to derive the equations for the ideal titration curves under idealized conditions. An analysis of the curve obtained for the end point in a precipitation reaction indicated the correlation to Majer's solubility method, the approximation of the tangent method, and led to a new graphical construction for determining the end point which should suffer from none of the aforementioned difficulties if the specified conditions can be realized.

Theoretical

We will first consider the simple precipitation reaction

$$A^{n+} + B^{n-} = AB_{(solid)} \tag{1}$$

In order to keep the formulas reasonably simple, we will make the following assumptions:

The solubility of AB is not changed by either the varying nature of the solution during the titration or aging effects. Furthermore, AB is completely dissociated in solution.

The titration is performed at constant volume, V, or the indicator reading has been corrected for volume increases, and for the background which is always present.

B^{n-} is added to A^{n+}. It will be clear from the symmetry of the equations that the results will apply equally well to the opposite assumption.

The concentrations are such that the activity coefficients may be taken equal to one.

We let the corrected indicator reading be i, the initial amount of A^{n+} in the solution be A_0, the amount of B^{n-} added be B_x, and the quantity of AB thus formed be p. If S is the solubility product of AB, the condition on the concentrations of A^{n+} and B^{n-} is

$$(A^{n+}) \times (B^{n-}) \leq S \tag{2}$$

The indicator reading will be

$$i = k_a (A^{n+}) + k_b (B^{n-}) \tag{3}$$

in which the k's are proportionality constants.

When

$$B_x \leq S/A_0, \; p = 0$$

and we have

$$i = k_a A_0 + k_b B_x \tag{4}$$

When

$$B_x \geq S/A_0$$

we have

$$(A_0 - p)(B_x - p) = S \tag{5}$$

and

$$i = k_a (A_0 - p) + k_b (B_x - p) \tag{6}$$

Solving Equation 5 for p and substituting in Equation 6, we obtain for i as a function of B_x

$$i = \frac{k_a - k_b}{2}(A_0 - B_x) + \frac{k_a + k_b}{2}\sqrt{(A_0 - B_x)^2 + 4S} \tag{7}$$

This is the analytic expression for the titration curve (for examples, see Figures 1 and 2). By definition, the equivalence point is given by $B_x = A_0$ or

$$i = (k_a + k_b)\sqrt{S} \tag{8}$$

Majer (*5*) obtained the special case for one of the k's equal to zero.

If we transpose the first member of the right-hand side of Equation 7 and square both sides, we obtain

$$[i - k_a (A_0 - B_x)]\,[i + k_b (A_0 - B_x)] = (k_a + k_b)^2 S \tag{9}$$

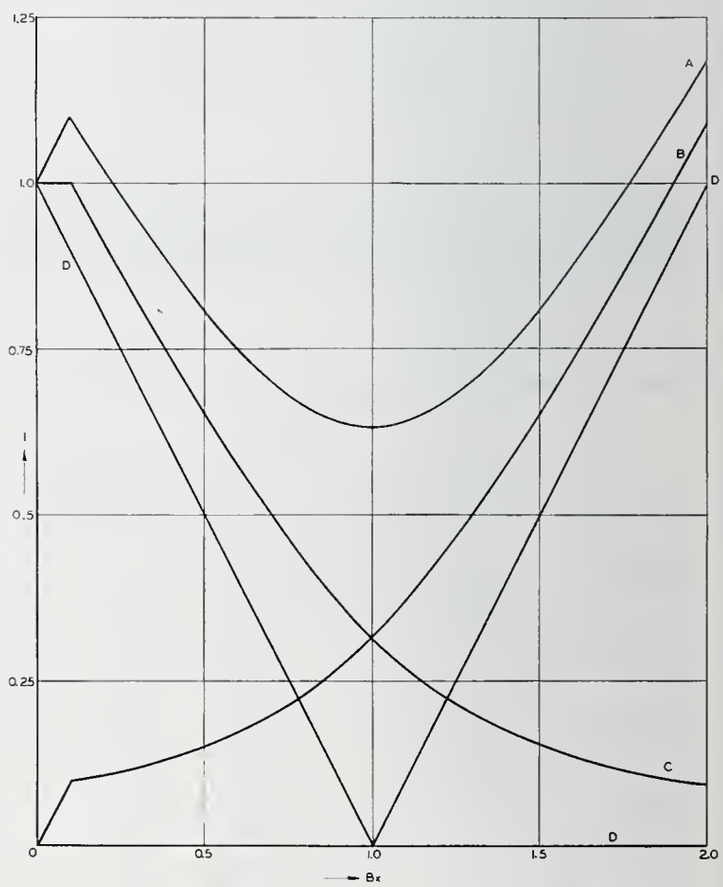

FIGURE 1. TITRATION CURVES
$S/A_0 = 0.1$ for all curves
A. $k_a = k_b = 1$
B. $k_a = 0, k_b = 1$
C. $k_a = 1, k_b = 0$
D. Asymptotes

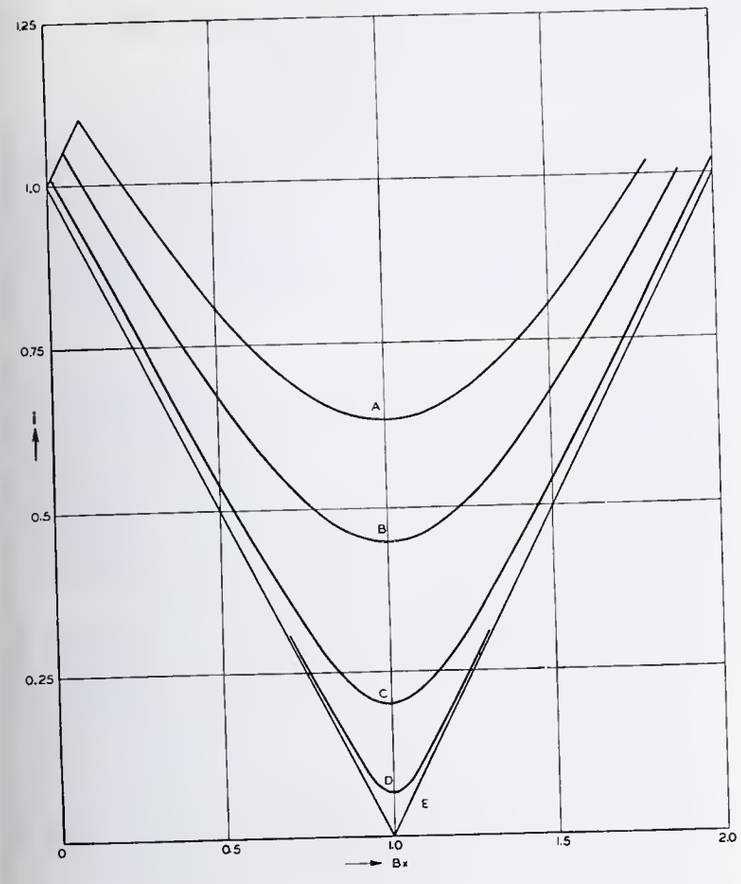

FIGURE 2. TITRATION CURVES

$k_a = k_b = 1$ for all curves

A. $S/A_0 = 0.10$
B. $S/A_0 = 0.05$
C. $S/A_0 = 0.01$
D. $S/A_0 = 0.001$
E. $S/A_0 = 0.000$

which is a hyperbola with the asymptotes

$$i = k_a (A_0 - B_x)$$
$$i = -k_b (A_0 - B_x) \qquad (10)$$

Solving Equations 10 we find that the intersection of the asymptotes of the titration curve occurs at the end-point condition.

This result should make it possible to use very simple geometric constructions to find the end point (*6*). From the theorem (I) the conjugate diameters of a hyperbola are also conjugate diameters of the asymptotes; it may be shown that the bisector of any pair of parallel chords of the hyperbola goes through the point defined by the intersection of the asymptotes. Thus the problem of determining the end point from the titration curve is just that of finding the point of intersection of the bisectors of two pairs of parallel chords which are not mutually parallel. This procedure is illustrated in Figure 3. It is clear from Equations 10 that the method is independent of the values of k_a and k_b. Or the theorem (II) that on any chord the parts between the hyperbola and the two asymptotes are of equal length, could also be used for finding the end point if the titration curve and one asymptote are known, since the second one can easily be constructed. This procedure is illustrated in Figure 5.

In Figures 1 and 2 are shown the various forms that the titration curve, Equations 4 and 7, may assume for particular values of k_a, k_b, and S/A_0. An examination of Figure 2 shows that for the ratio $S/A_0 < 0.001$, the tangents and the asymptotes coincide well. When $S/A_0 > 0.001$, it would be necessary to extend the titration curve to fairly large values of B_x to draw a tangent that would at all closely approximate the asymptote. In many cases, the titration of metallic ions with organic reagents, for instance, this would not be possible because of the limited solubility of the reagent itself. The

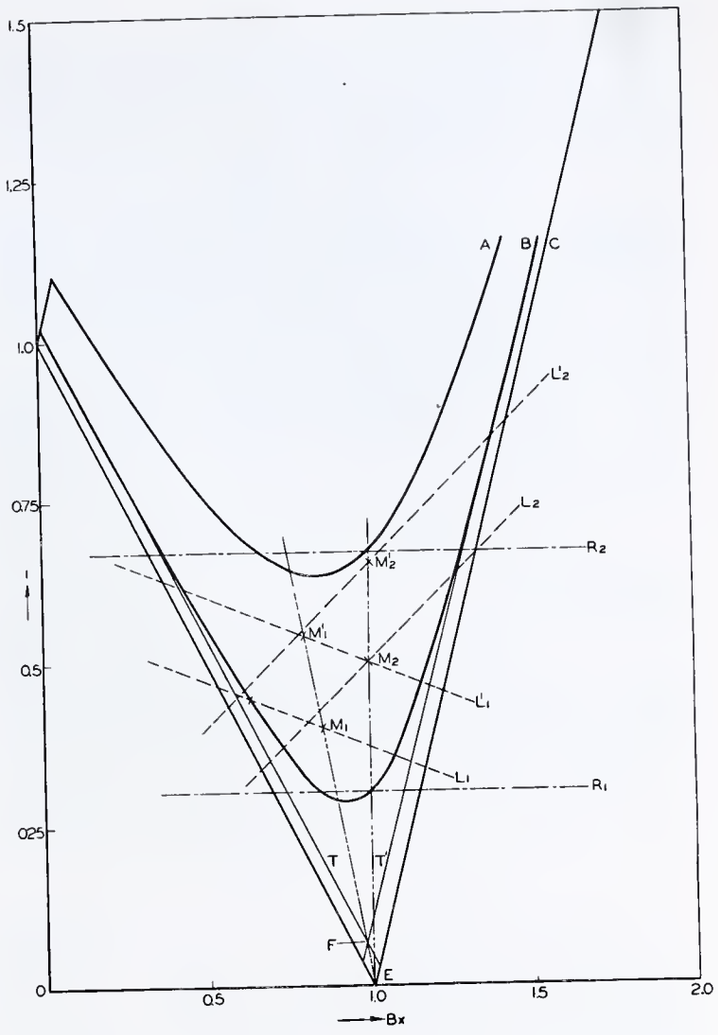

FIGURE 3. TITRATION CURVES

$k_a = 1$, $k_b = 2$, for all curves

A. $S/A_0 = 0.05$
B. $S/A_0 = 0.01$
C. $S/A_0 = 0.000$

L. Parallel chords. *M*. Midpoints of chords. *E*. Equivalence point. R_1 and R_2 correspond to Equation 8. *T* and *T'* are tangents of *B*, intersecting at *F*, which is about 2 per cent different from *E*.

initial tangent becomes different from the asymptote at $B_x = 0$ when $S/A_0 < 0.001$. From the construction illustrated in Figure 3 it is clear that only the easily found portion of the titration curve is necessary to enable one to construct the true end point, the intersection of the asymptotes.

The horizontal lines *R* in Figure 3 which illustrate Majer's (*5*) method of obtaining the end point (Equation 8) will be observed to intersect the titration curve twice. Such am-

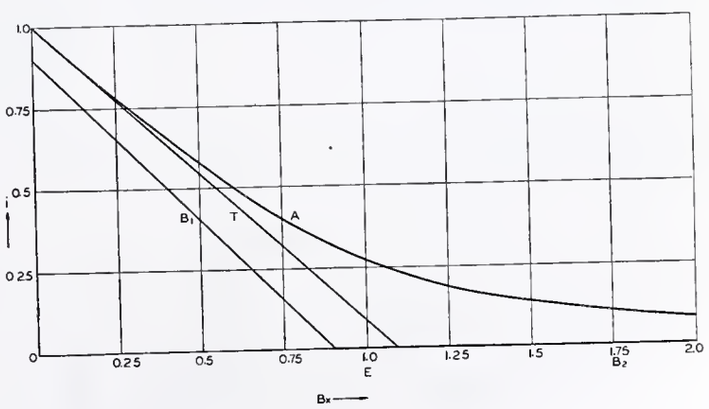

FIGURE 4. TITRATION CURVES

$K = 0.1$, $A_0 = 1$, $k_a = 1$, for *A*. B_1, B_2, asymptotes of *A*. *T* is the tangent of *A* at $B_x = 0$. *E* is the equivalence point.

biguity will always arise in this method when $k_a \neq k_b$, both k's not equal to zero.

The method given for constructing the end point is applicable only if the reaction is of the simple type (Equation 1). For the more general reaction

$$nA^{n+} + mB^{n-} = AnBm \qquad (11)$$

the titration curve is of higher order and absolute methods for constructing the end point are not known to us. If the reaction is of the type of Equation 11 even greater care must be exercised in applying the tangent method to the titration curve. If the solutions are very dilute the error in the end-point determination may easily exceed several per cent.

If AB is a soluble, slightly dissociated substance rather than an insoluble one, the discussion given above does not apply. Instead of the condition of Equation 2 on the concentration, we have

$$K = \frac{(A^{n+})\ (B^{n-})}{(AB)} = \frac{(A_0 - p)\ (B_x - p)}{p} \qquad (12)$$

We will let

$$i = k_a\ (A^{n+}) = k_a\ (A_0 - p) \qquad (13)$$

rather than the more general Equation 3 in order to keep the algebra within bounds. Solving Equation 12 and substituting in 13, we get the titration curve

$$i = k_a/2\ [A_0 - B_x - K + \sqrt{(A_0 + B_x + K)^2 - 4A_0B_x}] \qquad (14)$$

This is again a hyperbola with asymptotes

$$i = 0$$

$$i = k_a\ (A_0 - B_x - K) \qquad (15)$$

Their intersection is $B_x = A_0 - K$. Thus the asymptote method of finding the end point would be in error by exactly the value of the dissociation constant of AB. The intersection of the initial tangent

$$i = \left(\frac{di}{dB_x}\right)_{B_x = 0} \times B_x + k_aA_0 \qquad (16)$$

with $i = 0$ is the point $B_x = A_0 + K$. This follows from the value of $\left(\frac{di}{dB_x}\right)_{B_x = 0}$ obtained by differentiating Equation 14.

$$\left(\frac{di}{dB_x}\right)_{B_x = 0} = \frac{-k_aA_0}{A_0 + K} \qquad (17)$$

Thus the end point may be obtained as the mean of the two points of intersection, since they have the same error with opposite sign.

From symmetry considerations it is clear that the results would be the same if we took

$$i = k_b\ (B_x - p) \qquad (18)$$

in place of Equation 13.

If AB is soluble, there is not an equation analogous to Equation 8, since for $B_x = A_0$

$$i = k_a/2\ (-K + \sqrt{4A_0K + K^2}) \qquad (19)$$

which contains the unknown quantity, A_0, which is to be determined from the titration.

The form of the titration curve when AB is soluble but only slightly dissociated is shown in Figure 4. The slow approach of the titration curve to the asymptotes when $K/A_0 > 10^{-3}$ would make determination from such reactions unsatisfactory, unless K itself were accurately known, so that Equation 15 could be used in conjunction with the construction of the asymptote intersection.

Experimental

Merely as an illustration that the theoretically predicted shapes of the titration curves can be realized and that the proposed construction for finding the end point can be used, two examples, polarometric (amperometric) titration of sulfate (*5*) and chromate (*1*) with lead nitrate under conditions where the titration curve deviates from straight lines, will be discussed.

The procedure for such titrations has already been described in detail (*1*, *2*). A Leeds & Northrup Type K potentiometer was used in a compensation type of apparatus and an external saturated calomel electrode as a reference electrode. Oxygen was expelled by nitrogen. Titration was at room temperature, around 28° C. The volume of the supporting electrolyte in which the substance was determined was large in comparison with the volume of the reagent added and therefore the errors in the indicator readings due to the volume change were neglected. All values were corrected for the background current.

Figure 5 represents the titration of 5 ml. of 0.10 N potassium sulfate with 0.10 M lead nitrate diluted to 250 ml. with 0.1 M potassium nitrate containing 10 per cent (curve *a*) and 20 per cent (curve *b*) alcohol. The tangents drawn to curve *a* give too high a result, but in such a case, since the volume axis is one of the asymptotes to the curve, the second asymptote can be easily constructed, using theorem (II). On any chords ch_1 and ch_2 if one makes the lengths $AB = A'B'$ and $CD = C'D'$, by joining points $B'D'$ we will obtain the second asymptote (S) which intersects the volume at the end point, E. By this procedure the end point is reached within the limits of the graphical errors.

In curve *b* the tangent already coincides well with the asymptotes and in such a case a practically correct end point is obtained with the tangent method.

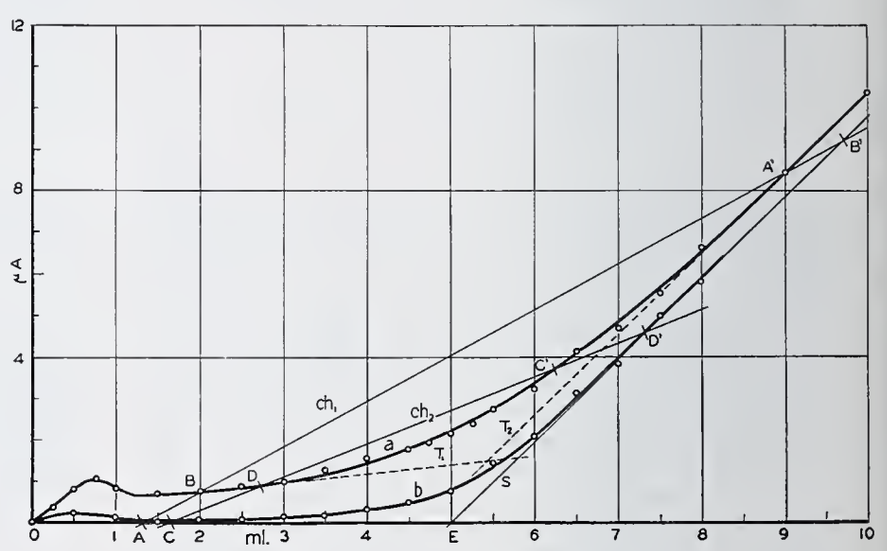

FIGURE 5. TITRATION CURVES

Titration of 50 ml. of 0.1 M potassium sulfate in 250 ml. of 0.1 M sodium nitrate with traces of gelation and (*a*) 10 per cent alcohol, (*b*) 20 per cent alcohol. Titrated with 0.1 M lead nitrate at applied potential of −0.9 volt.

Figure 6 represents the titration of potassium chromate in a mixture of acetic acid and hydrochloric acid, at zero potential with 0.01 M lead nitrate. It is obvious that such a curve can hardly be used to find the end point by the tangent method, since such a procedure would give us values completely misleading, whereas a construction derived from theorem (I) and described in Figure 3, or the construction mentioned previously, still gives reasonable values.

These examples lead to the conclusion that the titration curves under certain specified conditions follow closely the theoretically derived shape and that the end point can be found by determining the intersection of the asymptotes. It can be seen in Figure 5 that the sharpness of the transition

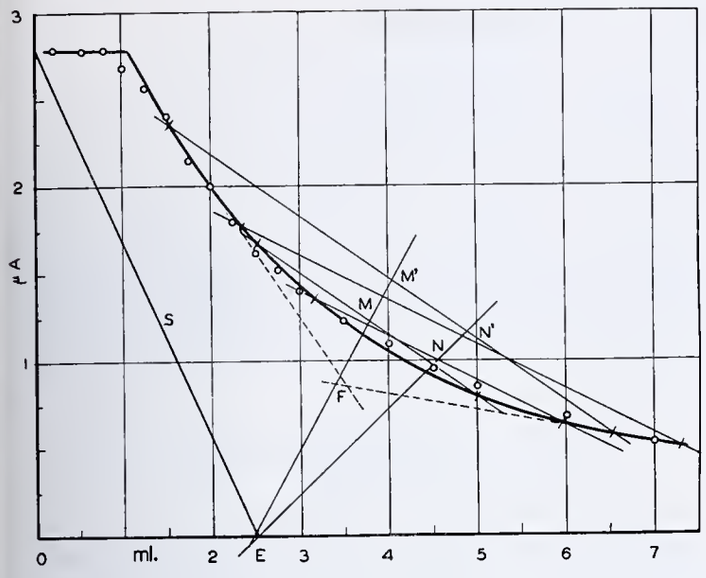

FIGURE 6. TITRATION CURVES

Titration of 25 ml. of 0.001 *M* potassium chromate diluted with 70 ml. of 0.2 *M* acetic acid and 5 ml. of 0.2 *M* hydrochloric acid. Titrated with 0.01 *M* lead nitrate at zero potential.

from the initial linear portion of the titration curve to the hyperbolic portion is not realized experimentally, probably because of peculiar solubility relations caused by the known dependence of solubility on particle size. Probably this relation makes it also necessary that after each addition of the reagent sufficient time (from 1 to 5 minutes, depending on the precipitate and nature of the mother liquid) has to be given for the precipitate to mature enough so that a definite solubility equilibrium is established in the solution before the indicator reading is taken again. If the titration is performed too quickly, the curve sometimes departs markedly from that obtained when a longer time is taken after the addition of the reagent. The points on such a rapidly taken curve are often scattered and because of the solubility change during the titration, it deviates from an ideal curve and gives no exact result for the end point with any method.

In order to obtain a predictable titration curve which can be solved for the end point, besides the particle size–solubility consideration, the solubility must not change because of the varying composition of the solution during the titration. For example, in the titration of copper with benzoinoxime in a strong ammonia solution (*3*), the solubility was changing because some ammonia was expelled by each mixing with the nitrogen during the titration. In some other cases, the solubility can be affected during the titration by the addition of an alcoholic solution of the reagent, a reagent dissolved in a strong acid or alkali, and so on. The volume change during the titration has to be either negligible or corrected. The correction factor is a complicated function. The indicated simple graphical constructions are limited to a reaction $A^{n+} + B^{n-} = AB$ solid only. However, the statement that the intersection of the asymptotes to any titration curve in a precipitation reaction gives the correct end point is general. In many constructions the possible graphical errors must also be considered.

The theoretical analysis has shown that the shape of the titration curve depends on the ratio S/A_0 and that the tangents can replace the asymptotes as a good approximation if this ratio becomes smaller than approximately 10^{-3}. Obviously, for practical analytical purposes such conditions will be sought that the tangent method can be used for finding the end point.

The indicated analysis shows that there are two ways to obtain such conditions, keeping the solubility of precipitate as low and the concentration of the solution as high as possible.

The first condition is given by the precipitate itself and the supporting electrolyte. Only the supporting electrolyte can be varied to give the lowest solubility, as, for example, by keeping the pH of the solution in a limited range, as was found in the titration of zinc with *o*-oxychinoline, by cooling the solution as in the titration of potassium with dipicrylamine, by adding alcohol as in the titration of molybdate with lead nitrate, or by other means. In case of low solubility the volume correction factor is also of a simpler form.

If the amount of the given substance is small and the solubility unfavorable, it is necessary to increase the concentration by reducing the volume. Experiments showed that the mercury dropping electrode is very convenient for microtitrations even in volumes less than 0.5 ml.

From other investigations it can be said that the given analysis of the end point on certain titration curves has some practical application, besides a theoretical interest. The described method of finding the end point can be used with advantage for solubility determinations from titration curves; however, only curves can be practically evaluated which depart considerably from straight lines.

Acknowledgment

The authors wish to thank E. U. Condon for helpful discussion of several aspects in this work.

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Alkalimetric Standardization of Iodine Solutions

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THE reaction:

$$SO_3^{--} + H_2O + I_2 = SO_4^{--} + 2HI$$

may be used to check a volumetric iodine solution by means of a known solution of sodium hydroxide.

A solution of pure sodium or potassium sulfite is prepared, which is slightly alkaline to thymolphthalein. With this solution a measured volume of the iodine solution is decolorized; an excess of 1 or 2 drops of sulfite does not matter. Then the acid is titrated with sodium hydroxide and phenolphthalein. Because of the very small sulfite content, the solution is not buffered and the change is very sharp at the end point; no difference was observed in the volume of acid required in several trials.

To test the procedure, an approximately 0.1 *N* solution of oxalic acid was titrated with the sodium hydroxide solution; on the other hand, the same oxalic acid solution was titrated with permanganate, the permanganate was used to check, iodometrically, a thiosulfate solution, and the latter was used to titrate the iodine.

RESULTS. 10 ml. of iodine = 9.70 ml. of sodium hydroxide directly, and 9.62, 9.69, and 9.67 ml. average = 9.66 ml. by the indirect way.

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Lower Aliphatic Alcohols

Application of the Zerewitinoff Determination

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THE problem of the application of the Zerewitinoff determination to the lower aliphatic alcohols aroused the authors' interest when they attempted to use a Kohler Grignard machine (*6*, *7*) for a Zerewitinoff determination on a low boiling alcohol. Kohler's directions did not involve the use of a solvent for the unknown but because of the much greater volatility of their unknown, as compared with the examples reported by Kohler, they elected to use isoamyl ether as a solvent for the unknown as well as for the Grignard reagent. Discordant results followed and a literature study revealed that Hibbert (*4*) had obtained "rather startling results" with methanol, ethanol, and 1-propanol, dissolved in isoamyl ether and in phenetol, although his values for α-naphthol were satisfactory. Oddo (10) also reported that isoamyl ether, anisole, toluene, and ligroin sometimes gave low values, but he and other workers (*2*, *12*) obtained good results by dissolving the alcohols in pyridine and in ethyl ether (*1*). With the exception of Ciusa, all the workers mentioned worked with the Grignard reagent in isoamyl ether.

Relation between Concentration and Gas Evolution

The use of isoamyl ether might have been suspected as the source of the troubles of Hibbert and the authors, except that Kohler seldom had any solvent but isoamyl ether present in his application of the method to substances of higher molecular weight. A systematic investigation involving isoamyl ether soon demonstrated that the concentration of the alcohol in the reaction mixture was the disturbing factor. With methanol at 0.245 mole per liter of reaction mixture, 89 per cent of the theoretical amount of gas was obtained, at 0.100 molar, 94 per cent, and at 0.0647 molar, 99.9 per cent.

The amount of gas produced appeared to depend inversely on the amount of methoxymagnesium iodide that precipitated, because at the higher concentration a large amount of precipitate, sticky and gelatinous in appearance, was present, whereas at 0.0647 molar the amount was very slight. When methanol, ethanol, and 1-propanol are treated in concentrations of about 0.23 molar the results are lowest for methanol, intermediate for ethanol, and theoretical for 1-propanol; the precipitates are, respectively, heavy, intermediate, and light.

Kohler (*7*) observed that the formation of insoluble products not infrequently makes it impossible to complete the reaction. Hibbert (*8*) recently suspected a precipitate of interfering with the determination of active hydrogen and carbonyl addition in vanillin, which was dissolved in isoamyl ether, and Haurowitz (*3*) suggested that benzoic acid gave only 66 per cent of the ethane expected on treatment with diethyl zinc because the benzoic acid formed a complex with the resulting zinc benzoate.

The data submitted by Zerewitinoff (*11*, *12*) and Flaschentrager indicate that concentrations of their alcohols were all below 0.1 molar and with the additional advantage of the greater solubility of polar compounds in pyridine their satisfactory results are readily understood. The present work indicates that the critical concentration in isoamyl ether reaction mixtures for methanol is about 0.07 molar, for ethanol is about 0.1 molar, and for 1-propanol may be above 0.24 molar.

In the present work a concentration effect was also noticed on the amount of gas given by acetophenone. Whereas Kohler (*7*) recorded 15 per cent of the theoretical for one active hydrogen at a probable molarity of 0.56, the authors obtained 13 per cent at 0.221 molar, 15 per cent at 0.133, 22 per cent at 0.0753, and 23 per cent at 0.0653. In similar machines others (*8*) have obtained values of 12 per cent for no solvent, 3 per cent for xylene, and 78 per cent for dioxane and pyridine without noting the concentrations.

Loss of Alcohol on Sweeping Apparatus with Nitrogen

At corresponding concentrations Hibbert's values were much lower than the authors', for methanol 46 per cent against their 89 per cent, for ethanol 73 per cent against 101 per cent, and for 1-propanol 83 per cent against 100 per cent. Obviously the formation of a precipitate cannot account for all of Hibbert's error, especially in the case of 1-propanol where, by the authors' experience, the formation of a precipitate was slight. An explanation was found in a detail of Hibbert's experimental procedure (*4*, *5*) in which the sample in solution was placed in the flask and "the air in the flask was displaced by dry nitrogen" which was led into the flask by a tube that reached nearly to the surface of the solution. This procedure undoubtedly swept some of the alcohol vapors out of the apparatus, inasmuch as in a similar procedure the authors found that 5 ml. of isoamyl ether lost 21.4 mg.; after addition of 39.3 mg. of methanol, and sweeping at the same rate for the same length of time, the loss was 50.7 mg. If it is assumed that the loss of isoamyl ether was the same in both instances, approximately 75 per cent of the methanol was lost during the sweeping. Hibbert's losses could not have been as great, but these results indicate a probable source of error. The lower values were to be expected from the more volatile alcohols, a prediction that is supported by Hibbert's data.

Effect of Heating on Yield of Gas

A study was also made of the effect of heating and time of reaction on the amount of gas produced. Ten minutes' im-

TABLE I. EFFECT OF HEATING

	Molarity of Methanol in Reaction Mixture	% of Theory of Methane	Time of Standing before Heating *Min.*	Time of Heating *Min.*	Total Time *Min.*
2	0.245	89.0	30	10	60
1	0.226	86.4	30	None	30
1	0.226	95.6	30	10	60
3	0.167	92.8	30	18	60
5	0.162	81.7	10	None	10
5	0.162	89.0	360	None	360
5	0.162	89.7	480	None	480
5	0.162	93.2	480	10	510
4	0.142	93.1	630	None	630
6	0.114	96.3	13	17	60
9	0.104	94.2	55	15	120
10	0.103	95.4	5	17	60
8	0.102	97.0	600	None	600
11	0.100	94.1	3	10	60
11	0.100	99.8	3	10	1260
11	0.100	103.0	3	10	4080
13	0.0647	99.9	10	10	60
15	0.0618	100.8	10	17	60
12	0.0591	101.9	10	10	60
16	0.0578	97.9	11	15	60
14	0.0575	100.8	7	6	60
18	0.0532	102.2	8	10	60
19	0.0528	99.4	10	10	60
17	0.0519	98.4	13	25	120

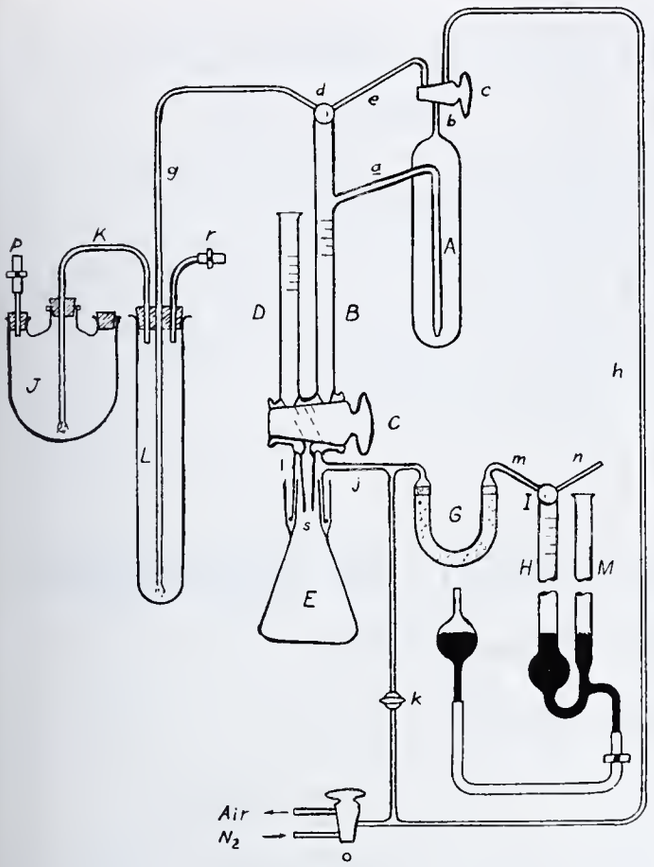

FIGURE 1. APPARATUS

mersion of the reaction flask in a beaker at 70° to 100° C. was sufficient when the concentration of the alcohol was correct. Long periods of standing, up to 10.5 hours in the case of methanol, did not produce a theoretical yield of gas when the concentration of the alcohol was incorrect (Table I). Preliminary experiments, in which the temperature was raised to 130° by means of an oil bath, also did not serve in lieu of a proper concentration.

Absorption of Oxygen by Reagent

If air is present within the apparatus, oxygen will be absorbed by the Grignard reagent and low results will be obtained. In all the methods in the literature, this difficulty has been eliminated (*a*) by replacement of the air with some indifferent gas such as nitrogen (*5*, *10*, *11*), methane, or ethyl ether vapor (*1*), or (*b*) by allowing the oxygen of the air within the apparatus to be absorbed before proceeding with the reaction liberating the hydrocarbon (*2*, *9*, *11*).

Experience during this work indicated that different preparations of methylmagnesium iodide absorbed oxygen at different rates and that a simplified procedure wherein air is used directly is not practical. Tank nitrogen, without further purification of any kind, provided a satisfactory atmosphere. However, the authors have had insufficient experience with tank nitrogen to justify extending its use to cover the original purpose of the "machine", in which Kohler used tank nitrogen purified by passage through a train consisting of ten parts in order to preserve a standardized Grignard solution for months.

Experimental

MODIFICATION AND USE OF MACHINE. The apparatus used is similar to Kohler's, with the exception that (see Figure 1) *I* is now a 3-way 120° cock, *c* is a simple 2-way cock, the drying tube contains calcium chloride, an auxiliary mercury column is attached to the gas buret, and none of the parts are jacketed. *E*, *i*, *m*, and *G* enclose approximately 105 ml. of gas, depending on which particular flask, *E*, is attached. A simple calculation indicates that the temperature of the ordinary laboratory is not likely to change sufficiently during the usual analysis to affect the results seriously.

The particular advantage of an unjacketed apparatus is that it may be mounted on a single large ring stand and thus made extremely portable and less fragile. The obvious disadvantages of changes in temperature and in pressure in operating over long periods of time were overcome by measuring the volume of gas enclosed in the reaction chamber, *E*, the lead tubes, *i* and *m*, the drying tube, *G*, and down to the zero mark on the gas buret. This volume was designated as *X* and determined with the aid of the mercury auxiliary tube, *M*, as follows:

With a flask attached to the machine and with *H* connected to *n* the mercury level was lowered until near the 20-ml. mark of the buret. The machine was left to come to room temperature for a few minutes and stopcock *I* was turned to connect first *m* and *n* and then *m* and *H*. Again the machine was allowed to stand several minutes and the mercury level observed, to make certain the whole was at room temperature. The barometric pressure was observed. A pressure greater or less than atmospheric was then exerted on the enclosed gases by allowing mercury to flow in or out of the auxiliary leveling tube, *M*. A cathetometer was used to determine the difference in heights of the levels of mercury in the two columns.

The volume, *X*, may then be calculated using the equation:

$$X = \frac{P_1(z - y)}{P_2 - P_1} - z$$

where P_1 is the initial and atmospheric pressure, P_2 is the final pressure imposed by the leveling bulb, z is the final buret reading, and y is the buret reading made at the beginning of the determination.

The per cent of the theoretical volume of gas evolved was determined with the aid of the following equation:

$$\% = \frac{100\,M\,P_2(z - y) + (X + y)[P_2 - P_1 - 2.6(T_2 - T_1)]}{RT_2W}$$

where *M* is the molecular weight of the alcohol, T_2 is the temperature at the beginning of the experiment, *W* is the weight of the alcohol in grams, and *R* is 62,400 (in mm. of mercury times ml. per mole per degree). The meaning of the other symbols is the same as above.

The procedure adopted after the discovery that tank nitrogen could be used as an inert atmosphere was to weigh the alcohol in about 40 ml. of isoamyl ether and dilute it to 50 ml., using a volumetric flask. After sweeping the apparatus with tank nitrogen, including the empty reaction chamber, *E*, an aliquot part of the alcohol solution was added from buret *D*, followed by the methylmagnesium iodide, allowance being made for the 0.1-ml. portion of alcohol solution which always remained in the bore of stopcock *C*. After addition of the reagent, the flask was heated by a 600-ml. beaker of hot water, heated to boiling in another part of the room. After 10 minutes' heating, *E* was cooled to room temperature with a beaker of cool water, the beaker removed, *E* wiped dry, and the apparatus allowed to stand until the mercury column in the gas buret indicated that thermal equilibrium had been reached—generally 5 to 10 minutes. Reference is made to the original articles on the machine (*1*) for additional details.

OTHER ALCOHOL ANALYSES. In seven determinations with ethanol, commercial absolute reagent, at molarities running from 0.222 to 0.201 the average was 98.5 ± 1.7 per cent; four determinations at molarities from 0.0810 to 0.0754 gave 102.1 ± 1.4 per cent.

1-Propanol from Eastman, fractionally redistilled (b. p. 96.8–97.2° at 767 mm., n_D^{20} 1.386), gave in experiments 2 to 11 at molarities from 0.238 to 0.225 an average of 100.3 ± 1.4 per cent. Experiment 1 for an unknown reason gave only 92.8 per cent.

Allyl alcohol from Eastman was fractionally distilled (b. p. 96.5–96.7° at 760.6 mm., n_D^{20} 1.413) and in five determinations gave an average of 100.0 ± 0.6 per cent.

tert-Butyl alcohol from Eastman was fractionally recrystallized, m. p. 25.3–25.7°, and gave in experiments 2 to 4 an average of 101.4 ± 0.9 per cent with experiment 1 giving 105.3 per cent.

The methanol used in obtaining the data in Table I was fractionally redistilled Baker and Adamson grade, absolute (b. p. 64.8–64.9° at 762.4 mm., n_D^{20} 1.329).

Summary

The lower aliphatic alcohols react quantitatively with methylmagnesium iodide, isoamyl ether being used as the

solvent for both the substance being investigated and the Grignard reagent.

From a practical standpoint the complete reaction must take place in a reasonable length of time, which requires that the reaction mixture be so dilute that a precipitate does not interfere by keeping the reactants from each other. Heating at a high temperature or very long standing is not a satisfactory substitute for a proper concentration of the alcohol in the reaction mixture.

The apparatus described by Kohler and co-workers has been modified as to stopcocks and jacketing and made much more portable. The use of tank nitrogen without further purification is suggested as an inert atmosphere for the Zerewitinoff determination.

Results are given for analyses using methanol, ethanol, 1-propanol, *tert*-butyl alcohol, allyl alcohol, and acetophenone.

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Determination of Soybean Flour in Sausages and Other Meat Products

A Protein Separation Method

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SAUSAGE and similar meat food products are prepared from meats and meat by-products that are usually pickled and then cooked and smoked. They may contain a little spice, cereals, starch, milk powder, and eggs (*16*, *68*), and also salt, nitrates, nitrites, sugars, and sodium benzoate (*67*). The regulations provide that the combined amount of cereal, starch, flour, and milk powder in sausages shall not exceed 3.5 per cent (*66*), and in certain meat products it is also necessary to know, for label requirements, whether more or less than 5 per cent has been added (*65*). Soybean flour is now finding a use in these products, and food control officials and members of the industry realize that it will eventually be necessary to develop a method of analysis for determining the percentage in these products.

Available Methods

To analyze such a complex mixture for the amount of soybean flour, some particular constituent of the soybean must be found which will differentiate the soybean from all the other ingredients. This constituent or a definite part of it must withstand the manufacturing process of the sausage, as the cooking and smoking, and also its own manufacturing process in being converted into flour, as the oil expression or extraction.

La Wall and Harrisson presented a test based on the enzyme urease, which may be detected by the liberation of ammonia from urea (*36*). This is limited to sausage samples where the urease has not been destroyed or inactivated, and later the authors advised confirming the test by identifying the characteristic cell structures (*37*). On the other hand, it has been recommended that the enzymes of soybean flour be inactivated by heating to a sufficiently high temperature for better processing (*18*). Kerr recommended these tests as qualitative, pointed out some limitations, and concluded that a quantitative method is urgent (*33*). In locating the hourglass or I-shaped cells with the microscope, it is best to examine with polarized light, under which they take on a certain sheen. The present-day methods of preparing flour make it almost impossible to locate a single cell even with polarized light.

Assuming that there is little or no nitrogen-free extract in meat, excepting liver products, but a considerable amount in soybean, Hayward reported on a calculating method (*14*), and also on an immunological method developed by Glynn which is based on a quantitative precipitin test (*11*). On the former test no further report was made by the A. O. A. C., while on the latter a detailed study was recently published (*7*). The method appears to be specific for soybean but it requires time for the production of a satisfactory serum and the zone of optimal precipitation may shift because of the presence of other ingredients.

A method based on the determination of insoluble nonfermentable sugars was proposed by Hendrey (*15*). After the sausage is given a preliminary treatment to remove moisture and fat, the soluble sugars are removed with 50 per cent alcohol, and the remaining carbohydrates are hydrolyzed with hydrochloric acid and then fermented with yeast. The sugars remaining after the fermentation are determined and multiplied by a factor. This method was tried out by Lythgoe *et al.* (*38*), who concluded that it is sufficiently accurate for law-enforcement purposes but not specific for soybean.

For some time the present author has been working on the chemical separation of the soybean protein and has rendered several reports, but has withheld publication because the method was not previously adaptable to liver sausage or to meat products that contained liver.

Theory

Soybean or soy flour is prepared by finely grinding soybeans, removing the hulls, and debittering, usually by a steaming process. The flour may contain all the fat of the soybean but is generally low in fat, seldom containing above 6 or 7 per cent. Soybean flour may be considered an offspring of the soybean oil meal. Commercial flours in 1915 gave an average protein content of 42.5 per cent (*63*), while oil meals prepared by present-day manufacturing methods give slightly higher figures (*71*). The chief protein of soybean is a globulin, glycinin, so named by Osborne and Campbell (*53*). A great part of this protein dissolves in a water solution of the ground seed, probably owing to the presence of phosphates, and is precipitated by the addition of a little acid in the form of white spheroids. Some other properties of glycinin are given in Table I.

The principle of the method is based on the separation of glycinin or a definite part of it from all the other proteins found in meats and in other binders and vegetable substances included in meat products. Glycinin can be boiled in a water solution with little coagulation and the remaining sol-

uble portion will still be precipitable at its isoelectric point. On the other hand, the albumins and the animal globulins in general are coagulable by heat, and the uncoagulable water-soluble proteins and the nitrogenous and nonnitrogenous extractives can be filtered through. The glycinin can then be dissolved in a 10 per cent sodium chloride solution, in which it will withstand almost a boiling temperature and a saturation with magnesium sulfate. Any additional gelatin dissolved (*4*) and perhaps other soluble meat proteins, as well as any casein present (*39*), are expected to precipitate with this saturation. On saturating further with sodium sulfate the glycinin will finally precipitate, possibly with other very soluble proteinate substances, as the solution is strongly concentrated with salts. To obtain this glycinin in a purer state it can again be dissolved and its property of precipitating at half ammonium sulfate saturation can be utilized. Those proteoses and nitrogenous extractives, which previously had been precipitated along with the glycinin, remain in solution at this relatively low concentration of salt. The precipitated glycinin can again be redissolved and then precipitated as an insoluble metal proteinate that will lend itself to thorough washing and drying for a final weighing.

The vegetable proteins can also be eliminated in the same manner. Among the cereal grains the chief proteins are the prolamins, which are insoluble in salt solutions, and the glutelins, which are also insoluble and coagulable. As may be seen from Table I, there exists only a small amount of globulin in the cereals, wheat, corn, rye, barley, oats, rice, and buckwheat. Flours of tuber origin, like the potato, sweet potato, and tapioca, contain a small amount of protein. The predominating proteins in the oilseeds and legumes are globulins. Cottonseed flour is high in protein, and although its main globulin requires more than half ammonium sulfate saturation for precipitation, it will probably be eliminated earlier in the procedure because of being coagulable. The globulins of the beans and tomato seed are similar. Part of the globulins of peanut and peas have two properties in common with glycinin, uncoagulability and low ammonium sulfate precipitation limits. Because their isoelectric points are higher than that of glycinin (*5*), they can be expected to filter through when glycinin is precipitated with acid and filtered. Part of the casein can also be eliminated in the same way. In egg white there are the glycoproteins which along with the nucleoproteins may be entirely neglected because of their presence in insignificant quantities. Likewise, the pumpkin or squash and malt of malted milk contain small amounts of protein. A scheme of analysis can then be worked out to eliminate all the proteins and the like save a definite part of the glycinin, which would be a factor of the amount of soybean flour.

Table I. Removal of Globulins and Similar Proteins

Source	Protein Present %		Globulin, etc.	Coagulation ° C.	Precipitates on Saturation	Isoelectric Point	Citation
Soybean	...	.....	Glycinin	Uncoagulable	Na_2SO_4	..	(*53*)
					0.5 $(NH_4)_2SO_4$	4.7	(*5, 26*)
Wheat flour	0	Globulin	.....	.....	.....	..	(*17*)
Embryo	5	Globulin	.....	.....	.....	..	(*54*)
Bran	2	Globulin	.....	.....	.....	..	(*28*)
Endosperm	Trace	Globulin	Edestin	Part coagulable	$MgSO_4$	..	(*49, 56*)
Corn	0.4	Globulin	Maysan	70	.....	..	(*46*)
	...	.....	Unnamed	62	.....	..	.....
	...	.....	Edestin	Part coagulable	$MgSO_4$	..	(*49*)
Rye	Trace	Globulin	.....	.....	.....	..	(*44*)
Barley	Trace	Globulin	.....	.....	.....	..	(*45*)
Oats	Trace	Globulin	.....	.....	.....	..	(*41*)
Rice	Trace	Globulin	.....	74–90	.....	..	(*29, 61*)
Buckwheat	2	Globulin	.....	.....	.....	..	(*20*)
Potato	Trace	Protein	Tuberin	60–80	$MgSO_4$	..	(*48*)
Sweet potato	2	Protein	.....	.....	.....	..	(*10*)
Tapioca	3 to 7	Protein	.....	.....	.....	..	(*2*)
Cottonseed	2.6	.....	α-Globulin	95–97	.....	..	.....
	16	.....	β-Globulin	92–93	0.7 $(NH_4)_2SO_4$	..	(*25*)
	16	.....	Edestin	Part coagulable	$MgSO_4$	..	(*57*)
Adsuki bean	Small amount		α-Globulin	88	.....	..	.....
	...	.....	β-Globulin	97	0.7 $(NH_4)_2SO_4$	..	(*27*)
	...	.....	Phaseolin	.....	1.0 $(NH_4)_2SO_4$	..	(*51*)
Lima bean	...	.....	α-Globulin	68	.....	..	.....
	...	.....	β-Globulin	95	.....	5.2	(*5, 30*)
Navy bean	...	.....	Phaseolin	.....	1.0 $(NH_4)_2SO_4$	..	.....
	Small amount		Conphaseolin	Coagulable	.....	..	(*43, 70*)
Mung bean	Small amount		α-Globulin	95–100	.....	..	.....
	...	.....	β-Globulin	68–71	+0.5 $(NH_4)_2SO_4$	..	(*24*)
Velvet bean	...	.....	α-Globulin	70–78	.....	..	.....
	...	.....	β-Globulin	90–100	+0.5 $(NH_4)_2SO_4$	..	(*23*)
Jack bean	...	.....	Canavalin	.....	1.0 $(NH_4)_2SO_4$	..	.....
	Small amount		Concanavalin	.....	0.6 $(NH_4)_2SO_4$	5.5	(*5, 32*)
Tomato seed	...	.....	α-Globulin	74	.....	..	.....
	...	.....	β-Globulin	96	1.0 $(NH_4)_2SO_4$	..	(*21*)
Peanut meal	25	.....	Arachin	Uncoagulable	.....	5.35	(*5, 22, 31*)
	8	.....	Conarachin	80	.....	..	.....
Pea	...	.....	Legumelin	Coagulable	.....	..	(*52*)
	...	.....	Legumin	.....	.....	5.35	(*5*)
	...	.....	Vicilin	95–100	.....	5.2	.....
Cow pea	...	.....	Vignin	Similar to legumin	.....	..	.....
	...	.....	Phaseolin	.....	1.0 $(NH_4)_2SO_4$	..	(*50*)
Milk solids	32	.....	Casein	.....	$MgSO_4$	4.85	(*5, 39*)
Egg white	Small amount		Ovomucin	.....	+0.5 $(NH_4)_2SO_4$	..	.....
	...	.....	Ovomucoid	.....	+0.5 $(NH_4)_2SO_4$	..	(*6, 55*)
Squash	1.3	Protein	Curcurbitin	73–95	$MgSO_4$	..	(*1, 42, 49*)
Malt	2.8	Globulin	.....	Part coagulable	.....	..	(*47*)

Solutions and Materials

Iodine Indicator. Dissolve 0.1 gram of iodine and 0.4 gram of potassium iodide in 30 cc. of water.

Ethyl Orange Indicator (Eastman Kodak No. 122). Dissolve 0.015 gram in 30 cc. of 50 per cent alcohol.

Methyl Red Indicator. Dissolve 0.03 gram in 30 cc. of 60 per cent alcohol.

Phenolphthalein Indicator. Dissolve 0.3 gram in 30 cc. of 50 per cent alcohol.

Phenol Red Indicator. Dissolve 0.03 gram in 30 cc. of water and add 0.1 cc. of *N* sodium hydroxide. This solution should be just yellow.

Malt Diastase Solution. Digest 2 grams of U. S. P. IX malt diastase powder in 100 cc. of water at room temperature for 1 or 2 hours and decant the clear solution through a filter, refiltering the first 20 to 40 cc. of filtrate through the same filter.

Solvents. Acetone and carbon tetrachloride (technical grades).

Salts. Sodium chloride, magnesium sulfate crystals, sodium sulfate anhydrous powder, ammonium sulfate, and cupric sulfate crystals (all C. P. grades).

Filter Paper. An open texture crepe paper, preferably 15 cm., also 12.5 and 11 cm.

Standard Solutions. Approximately *N* hydrochloric acid and *N* sodium hydroxide.

Method of Analysis

Samples must be fresh and free from slime and any discoloration. If it is necessary to preserve samples for a length of time, run through step 1.

Step 1. Solvent Extractions. Remove the skin, cut off the dried outer layer, and grind the meat finely by passing it from one to four times through a meat chopper of a cutting type. Weigh a 50-gram sample rapidly to avoid loss of moisture, put into a 400-cc. beaker, and add 75 cc. of acetone. Let stand at room temperature for 3 to 4 hours with an occasional brisk stirring to break up meat clusters, decant through a dry 15-cm. filter, and press out as much of the acetone as possible. A 120-ml. (4-ounce) oil sample bottle is very convenient for this pressing because it can be set on the table in an inverted position without loss of particles. If there are a lot of meat particles on the filter, squeeze

out the liquid with a flexible spatula and return the solid mass to the beaker. Discard the acetone extract.

Add 75 cc. of carbon tetrachloride to the beaker, place in a water bath, and bring the carbon tetrachloride slowly to a boil. Let stand at room temperature for 3 to 4 hours with occasional stirring, decant through the same filter, and press out as much of the carbon tetrachloride as possible, using the same oil sample bottle. [The final 15 to 25 cc. usually filter very slowly. Set a 7.5-cm. (3-inch) wire triangle, which has been bent inward a little, on the funnel and set the filter in the triangle. The filter paper is sufficiently strong to support liquid almost three-quarters filled. Although this device is not absolutely necessary, it is a great convenience and one can hardly do without it in the succeeding filtrations.] Discard the carbon tetrachloride extract, scrape off whatever particles are on the filter paper and bottle, stir up the meat, and drive off the carbon tetrachloride and acetone by heating in the water bath. Stir occasionally to prevent caking.

(May be left overnight or longer.)

Step 2. Water Leaching. Add 250 cc. of cold distilled water and mark the beaker at the water surface. Stir and let soak for about 5 minutes, then add a drop or two of phenolphthalein and with *N* sodium hydroxide, make pink and then back to just colorless with *N* hydrochloric acid, and note the amount of *N* sodium hydroxide required. Heat slowly with stirring to expel the remaining acetone and carbon tetrachloride, being very careful to avoid loss due to foaming. After danger of foaming is over, boil gently for 5 minutes and scrape down any crust formed on the sides of the beaker. Cool to room temperature, dilute to the 250-cc. mark, and add the equivalent amount of *N* hydrochloric acid exactly to neutralize the *N* sodium hydroxide added before the boiling. Add a drop or two of iodine indicator, and if it does not give a blue color or just a trace omit step 2*a* and proceed with 2*b*.

(*a*) Add about 5 cc. of freshly prepared malt diastase solution, place in a water bath, and keep with occasional stirring at 40° to 45° C. for about 1 to 5 minutes or until there is no color or just a trace of a blue color with iodine. Cool to room temperature and proceed with 2*b*.

(*b*) Add *N* hydrochloric acid in small portions until orange or just faintly pink to ethyl orange added internally. To get the color change let the meat settle and add another drop of indicator. It is necessary to do this after every hydrochloric acid addition and after every stirring; 1.5 to 5 cc. of *N* hydrochloric acid are generally required. Let settle and filter through a dry 15-cm. filter into a 250-cc. beaker. In filtering try to retain the meat in the beaker and use the oil sample bottle to press out as much water as possible. Reject filtrate. The filtrate generally comes out yellow or faintly orange.

(May be left overnight if kept in a cool place. Fold filter paper so that contents will not dry too much.)

Step 3. Sodium Chloride Extraction. Using a small wash bottle with 200 cc. of 10 per cent sodium chloride solution, wash the residue on the filter paper back into the original beaker, and mark the beaker at the solution surface. Avoid throwing in the filter paper. Add a drop or two of phenolphthalein and then make pink with *N* sodium hydroxide. Keep at room temperature with occasional stirring for 30 minutes and if the phenolphthalein becomes colorless add more *N* sodium hydroxide, 0.5 to 1 cc. at a time. (There is no harm done if it is accidentally kept 1 hour longer.) Place in a water bath and in a period of 15 to 20 minutes raise its temperature 85° to 90° C., and then hold for exactly 30 minutes further at that temperature with occasional stirring. During heating add a drop of phenolphthalein and around 0.5 cc. of *N* sodium hydroxide occasionally to maintain the red color. Remove from water bath, allow to cool a little, replace water lost by evaporation, stir up, let settle, and filter as before, finally pressing out as much solution as possible. Reject residue.

(May be left overnight but not in an alkaline state. Make just acid with *N* hydrochloric acid, and upon resumption of work again make alkaline with *N* sodium hydroxide.)

Step 4. Magnesium Sulfate Salting-Out. With *N* hydrochloric acid make just acid to phenolphthalein and measure volume. Add slowly in 3- to 4-gram portions and with gentle stirring 30 grams of magnesium sulfate heptahydrate per 100 cc. of solution. After all the magnesium sulfate has been added and all or nearly all has dissolved, place the beaker in a water bath and in exactly 30 minutes raise the solution gradually to 50° C., with an occasional slow stirring. (No harm is done if it is accidentally kept 30 minutes longer at a lower temperature.) Avoid going above 55° C. at any time. Let settle and filter, preferably while warm. Reject residue if any.

(May be left overnight.)

Step 5. Sodium Sulfate Salting-Out. While the solution is kept at 35° to 40° C. in the water bath, add slowly in 1.5- to 2-gram portions and with gentle stirring 11 grams of pulverized anhydrous sodium sulfate per 100 cc. of sodium chloride solution used in step 4, disregarding the increase in volume due to the magnesium sulfate and the slight loss due to filtering. After nearly all has dissolved hold at 35° to 40° C. for 75 minutes longer, and after the precipitate has agglomerated do not stir. (No harm is done if it reaches 45° C. for a while or is kept for 1 to 2 hours longer at room temperature.) Filter while warm with as little breaking of the agglomeration as possible. It is sometimes better to divide the filtering over two separate filter papers, and it is not absolutely necessary for this to filter to complete dryness. Reject filtrate.

(Proceed with the next step immediately.)

Step 6. Sodium Hydroxide Dissolving. Using a wash bottle, with a strong stream wash the filter with warm water (30° to 40° C.) made pink to phenolphthalein with *N* sodium hydroxide (0.15 to 0.30 cc. of *N* sodium hydroxide to 500 cc. of water) into the previous beaker to a volume of 150 to 200 cc.; where there is no or hardly any precipitate, 100 cc. will do. If it is necessary to scrape the filter or the volume reaches 250 to 260 cc., no harm is done. Keep warm and stir a little till all dissolves. Wash sides of beaker down with water.

(May be left overnight but not in an alkaline state. Make just acid with *N* hydrochloric acid, and upon resumption of work again make alkaline with *N* sodium hydroxide.)

Step 7. Ammonium Sulfate Salting-Out. With *N* hydrochloric acid make just acid to phenolphthalein and measure volume. Add slowly in 2- to 3-gram portions with gentle stirring 22 grams of ammonium sulfate per 100 cc. of solution. After all has been added raise temperature and keep for 15 minutes at 40° C., towards the end stirring very little and very slowly. (There is no harm done if it is accidentally kept for 1 to 2 hours longer at room temperature.) Let settle and filter while warm with as little breaking of the agglomeration as possible. It is not absolutely necessary for this to filter to complete dryness. Reject filtrate.

(Proceed with the next step immediately.)

Step 8. Sodium Hydroxide Dissolving. Repeat step 6. Cool to room temperature, add a few drops of phenol red, and make just yellow with *N* hydrochloric acid. (If necessary may be left overnight.) Measure volume and add slowly in 3-gram portions with gentle stirring 10 grams of magnesium sulfate heptahydrate per 100 cc. of solution. After all has been added and dissolved, add 1 to 3 drops of methyl red and if solution is not yellow to methyl red also, make it so with *N* sodium hydroxide. The solution must be distinctly yellow to both indicators and sometimes it may be necessary to use 0.1 *N* sodium hydroxide and 0.1 *N* hydrochloric acid. Filter off the filter paper fuzz and scum if any.

(May be left overnight.)

Step 9. Cupric Sulfate Precipitation. Add 20 cc. of filtered 10 per cent cupric sulfate pentahydrate and keep at 40° to 45° C. for 60 minutes with occasional slow stirring for the first 10 to 20 minutes. Do not stir when it becomes noticeable that the flocculent precipitate starts to agglomerate and settle. (No harm is done if it is kept for 1 to 2 hours longer at room temperature.) Without stirring the precipitate, filter on a moistened tared filter paper, preferably 11 cm., previously dried and weighed in a weighing bottle. In transferring the precipitate from the beaker to the filter use warm water, not above 45° C., or it will stick tenaciously to the glass. If it becomes slow in filtering owing to a clogged filter, wash the precipitate loose from the filter with a stream of hot water, 60° to 75° C. Finally, wash the precipitate 2 or 3 times with hot water, dry, and weigh in a weighing bottle. Calculate, using the factor:

0.035 gram of ppt. = 1.0 per cent of soy flour

Discussion

Step 1. Most sausages are exposed to the smoke of burning woods, which deposit on the surface certain creosotic substances that give a distinctive flavor and an antiseptic property. Instead of smoking, it is also common practice to treat the meat with crude pyroligneous acid, obtained from the destructive distillation of wood. The small amounts of formaldehyde and tannic acid introduced are both capable of precipitating proteins under certain conditions. Most of these two substances can be removed by cutting off the harder surface, and they can also be extracted from the ground meat with acetone. The carbon tetrachloride is used because the acetone does not remove all the fats, which would interfere in the succeeding two steps.

STEP 2. When commercial soybean flour extracted with acetone–carbon tetrachloride is boiled with water the glycinin is apparently not coagulated. If the solution is cooled and then acid is added to pH 4.1 to 4.2 the glycinin can be seen precipitating. This is the same isoelectric point as that of petroleum ether–extracted soybean flour (*40*, *62*). This phenomenon has the appearance of falling snow. If the pH is at the higher limits the snow will be heavy, while at the lower limits the flakes will be fine. If the proper pH has not yet been reached, the solution will be cloudy after the glycinin or snow has stopped falling, at the exact pH the solution will be clear, and when the isoelectric point has been passed the solution will again be cloudy. In the presence of the skeletal meats the phenomenon will be the same. After stirring, the meats will settle first and then the snow can be seen. If a little salt, some sugars, or starches are present the behavior will still be the same. With starch the snow is heavier unless removed with malt diastase.

With hashed or minced liver the picture is different. When the soybean liver mixture is warmed, a break in the cloudiness occurs. Soon the break spreads, a coagulation occurs, and the surrounding liquid becomes brilliantly clear, much before the boiling point is reached. On cooling it becomes evident that the glycinin is not in solution. On long standing in the cold the same thing occurs. If acid is then added to a wide pH range of 4.0 to 4.4 no precipitation occurs. On continuing further with the procedure, a sodium chloride extraction is also brilliantly clear and later there is no precipitation with sodium sulfate.

It was found that kidneys also cause the same difficulty but to a lesser degree. Because these two organs are rich in various minerals an attempt was made to precipitate them with dithizone, 8-hydroxyquinoline, cupferron, carbonates, etc. Only the carbonates gave promising signs, yet this idea, too, had to be abandoned. Because choline has strong basic properties and its presence may be expected, an exhaustive leaching with alcohol and the addition of potassium triiodide to the water extraction failed to overcome the difficulty.

Since the addition of carbonates was somewhat helpful, it occurred to the author that the iron of the hematin may form some positive colloids. A thorough investigation into the hemoglobins was then made.

To the hashed liver was added a little sodium nitrite, thoroughly stirred in, and then treated with large quantities of acetone. This produced bright red solutions with acetone, carbon tetrachloride, or alcohol. Exhaustive leaching with acetone was made until the dried liver was almost white and powdery. It was taken for granted that the red color was due to nitrosohemochromogen, and that in this way all the iron was removed. Again the same difficulty occurred and at times it appeared more pronounced.

Long ago Haldane showed that on boiling in solution nitrosohemoglobin is broken down into nitrosohemochromogen and globin (*13*). Globin is classified as a histone because of its basic character. It is coagulated by heat. Anson and Mirsky precipitated denatured globin with acetone and then converted a large part of it into a soluble globin which still had the power to combine with heme and form hemoglobin (*3*). This denatured globin has an isoelectric point of around pH 8.0. At pH 7 the soluble globin may attach itself to acid proteins like glycinin and cause a mutual precipitation. If this is true and it is coagulated by heat, it apparently takes the glycinin down with it forever. If, while the globin is still in the soluble state, alkali is added to pH 8.0 the globin should be discharged and precipitated, and if alkali is added beyond pH 8.0 the globin should take on acidic properties and leave the glycinin alone. Apparently that is what happens, for after this pH adjustment neither liver nor kidney had any effect on the glycinin.

STEP 2*a*. In the analytical procedure the solution is not adjusted to the optimum pH 4.5 to 5.5 for malt diastase digestion (*12*), because the hydrolysis is accelerated at a higher pH by the presence of soluble proteins (*8*). The starch must be removed as completely as possible, because globulins enter into combinations with the polysaccharides at varying experimental conditions (*58*). The author checked this and found, on not removing the starch in a sample of sausage, two and a half times as much soybean as was actually present.

STEP 2*b*. This step is to remove all soluble substances, such as proteins, amino acids, starch, and salts. The ethyl orange just changes color at the isoelectric point of glycinin. Incidentally, very soluble globulins of different isoelectric points might also be removed at this point. In avoiding loss of glycinin the pH of the solution at filtration is of cardinal importance and is not hard to adjust because of the presence of such buffering substances as gelatin, peptides, amino acids, and sugars.

STEP 3. Although the pH of the extracting solution is not uniform, concordant results can be obtained, and this is in accord with the literature (*19*, *59*).

STEP 4. In order to have uniformity in the salting-out, Moir's method of precipitating casein was followed, in which the solution is made just acid to phenolphthalein before adding the saturating salt (*39*). The salts lower the pH and bring it within the range of pH 6, 7, or 8, where the same amount of protein is precipitated (*60*).

When the solution is raised to 50° C. the precipitated casein will form a sticky mass that may cling to the beaker or stirring rod or float on top of the solution, while at a lower temperature it would be almost impossible to filter.

STEP 5. Since the precipitation of colloids depends on whether the precipitating salt is added in one or successive portions (*9*), the optimum conditions were determined and the same technique followed for both the magnesium and ammonium sulfates.

STEP 6. This is a convenient way of bringing the precipitated proteins back into solution.

STEP 7. This step was introduced to retain in solution proteins which are more soluble than glycinin and yet were forced out by the double concentration of magnesium and sodium sulfates.

STEP 8. In order to avoid the danger of reaching the "tolerance zone" in the next step (*34*, *64*), a desirable pH range and a volume of precipitant were determined. Methyl red was selected so as to be on the alkaline side of the glycinin isoelectric point. Phenol red then keeps the solution at a pH not high enough to form a metal hydroxide, does not interfere with the color of the methyl red, and yet allows a sufficient pH range to be easily adjusted, since the solution might not always be adequately buffered. Phenolphthalein was entirely unsuitable here, on account of the presence of small amounts of ammonium sulfate.

Practically all the gelatin is washed out in the water step, but more will be extracted with the sodium chloride. This small amount can then be salted out with magnesium sulfate at room temperature (*4*), while at a higher temperature some will be retained in solution. This gelatin will then be precipitated with the sodium sulfate and again with the ammonium sulfate. Fortunately, it is not precipitated with the heavy metals, yet it is undesirable in being a very powerful protective colloid. Protective or reversible colloids like gelatin may completely inhibit the precipitating action of electrolytes. Even if the gelatin is present in a minute quantity, it will prevent somewhat, if not entirely, the precipitation of the glycinin by the heavy metal. To prevent this from happening it is necessary to decrease the two stability factors, electrical charge and hydration (*35*). The addition of the magnesium sulfate will decrease both. A little heat may sometimes be needed to help the dehydration. Although this course may not always be necessary, yet after taking these precautions the precipitations were much better.

STEP 9. Precipitation with a heavy metal appeared best for forming an insoluble glycininate so that it could be washed free from salts, dried, and weighed. Copper is best because other metals may form insoluble sulfates and chlorides.

The cupric sulfate solution is sufficiently dilute to avoid reaching the "tolerance zone" if an excess is added and not too dilute to cause large volumes. The amount of copper in the precipitate should not vary much under such a control, for in similar work, in precipitating milk proteins with copper sulfate, the amount of copper in the precipitate varied from 2.4 to 4.1 per cent (*69*). Such a variance would not be noticeable.

The factor was obtained by analyzing carefully prepared samples and is shown to be proportional to the amount of soybean.

Experimental

It was not deemed necessary to present data on the development of the method. The data shown were obtained by using the method as it is presented. Table II shows the flours used in the succeeding determinations. The work in Table III was performed to show that the factor for a particular brand of soy flour is not materially different with the varying percentages, the different kinds of meat, and the different treatments the meat may undergo alone or with the soy flour. The factor was derived by dividing the copper glycininate precipitate by the percentage of soy flour.

The meat of samples 1 to 4 was finely ground cured meat for frankfurters, obtained from a small sausage maker. This was mixed with salt and definite amounts of soy flour, stuffed tight into a 5-cm. (2-inch) casing, and then cooked for about 25 minutes at 170° F. Samples 3 and 4 were smoked by the sausage maker in his usual way. In samples 5 to 7 U. S. inspected smoked Kosher bologna was ground and mixed with the soy flour in a beaker. In samples 8 to 11, 5 cc. of a 1 per cent sodium nitrite and 2 grams of sodium chloride in 10 to 15 cc. of water were added to 50 grams of ground meat in beakers, which were placed in the refrigerator overnight. The next day the required amount of soy flour was stirred in.

For samples 12 to 14 ground raw meat was weighed and mixed with soy flour. In samples 15 to 18, 50 grams of ground raw meat in a beaker were stirred with 5 cc. of 1 per cent sodium nitrite for 4 to 5 minutes, the soy flour was added with a little water, and then the acetone was added. The acetone extract in this case was as red as that of samples 1 to 11. In the next step 2 grams of sodium chloride were added. This same procedure was followed with the other samples that were pickled in the beaker, except that the amount of sodium chloride added in the water-leaching step was varied all the way down to nothing. For sample 21 a chunk of liver was baked for a few minutes until dry, then ground for a sample. The liver sausage of samples 23 and 24 bore the U. S. inspection stamp. Samples 25 to 28 were run because these meats may be present in some meat products, especially dog foods. The method is normal with these present, as shown by the results.

In samples 29 and 30, Table IV, 43 grams of lean hamburger were pickled in the beaker, and 5 grams of cereal and 2 grams of soy flour were next stirred in. These two and the next three samples show that starchy materials, even in large amounts, cause no interference when calculated with the factor 0.0251, the known factor for this brand. The meat loaf, obtained from a sausage maker, contained eggs, the usual spices, starch, and no soy flour. The blood sausage was from the same source and contained tongue, starch, and no soy flour. Samples 34 to 38 were prepared similarly, using skim-milk powder in varying proportions. Part of the casein filtered through in step 2, as the filtrates were very cloudy, a large part salted out in step 4 as a large sticky precipitate that fused into a film, and then in step 5 some additional casein precipitated. Although the results for soy flour are not very gratifying with milk solids present, they are somewhat lower than the sum of the milk powder and the soy flour which together make up the total binder used. They appear to be the sum of the soy flour and three fourths of the milk solids. If the amount of milk solids is known, the results can be corrected for their presence.

All the meats of Table V were pickled in beakers as before and the factors for other brands were derived. The factor 0.0350 was adopted because it is almost the average. This factor can be changed from time to time in the light of additional experimental data, and if the source of the soy flour is known an absolute accurate factor can be used. The results calculated with this factor are also shown, and, similarly, in Table IV the presence of milk solids is less of an interference. It is possible to make up other combinations, such as tamales, foreign specialties, etc., for trial, but as this work had to be brought to a finish, the presence of peas, beans, etc., had to be left with the theoretical explanation.

TABLE II. ANALYSIS OF COMMERCIAL SOY FLOURS

Manufacturer	Brand	Moisture %	Fat %	Protein %	Process
Allied Mills	Kreemko	5.0	5.5	45.0	Expeller
Archer-Daniels-Midland	Cherry Blossom	7.5	4.0	46.0	Solvent
Central Soya	No. 500	3.0	6.6	52.3	Expeller
	No. 103	8.5	4.0	49.9	Solvent
Glidden	Soyalose	4.5	6.8	50.5	Expeller
	Soyagrain	6.5	1.3	52.5	Solvent
Spencer Kellogg	Special X	5.0	7.4	49.6	Expeller
	Soyaflake	4.9	22.1	43.5	Whole fat

TABLE III. ANALYSIS OF MEATS WITH KREEMKO

No.	Kind of Meat	Treatment	Soy Flour Added, %	Factor
1	Frankfurter meat	Pickled, cooked	1	0.0255
2			4	0.0250
3		Pickled, cooked, smoked	3	0.0248
4			5	0.0245
5	Bologna	.....	0	....
6			4	0.0305
7			9	0.0263
8	Meat	Pickled overnight	0	....
9			2	0.0249
10	Veal	.....	0	....
11			2	0.0245
12	Meat	Raw	0	....
13			3	0.0252
14	Veal	.....	4	0.0258
15	Meat	Pickled in beaker	3	0.0246
16			6	0.0255
17			8	0.0260
18	Veal	.....	7	0.0255
19	Pork liver	Raw	5	0.0245
20		Pickled in beaker	3	0.0195
21		Baked	4	0.0254
22	Beef liver	Raw	4	0.0248
23	Liver sausage		0	....
24			4	0.0261
25	Pork, beef, lamb kidneys	Raw	4	0.0250
26	Pork heart, brains, veal	Pickled in beaker	4	0.0253
27	Chicken and beef lungs, beef spleen, cartilage	Raw	4	0.0232
28	Lamb cheeks, tongue, tripe, ground bone	Pickled in beaker	4	0.0245
			Av.	0.0251

Summary

A gravimetric method for the determination of soybean flour in meat products is based on determining a certain fraction of glycinin, the chief soybean protein. The meat sample is subjected to a series of operations in which the other proteins and interfering substances are removed by solvent extractions, different salt concentrations, coagulation temperature, and an isoelectric and heavy metal precipitation. Provision is made for removing starch. The method is adaptable to the various meats and meat by-products, whether cooked, smoked, or pickled, and whether the meat product contains flours, starches, cereals, a known amount of milk powder, or tomatoes. Theoretically the method should also be adaptable to meat specialties that contain beans, peas, etc.

The analytical procedure is divided into steps, each step eliminating some substances or else bringing the glycinin back into solution. All conditions, such as pH, time, temperatures, concentrations, reagents, etc., were worked out and standardized. Although the method is time-consuming,

TABLE IV. ANALYSIS OF MEATS WITH SEVERAL BINDERS

No.	Kind of Meat	Other Binders Added		Soy Flour Added (Kreemko)	Soy Flour Found Factor 0.0251	Soy Flour Found Factor 0.0350
		%		%	%	%
29	Hamburger	10	Barley, buckwheat, oatmeal, and corn	4	3.71	2.67
30		10	Noodles, wheat flour, corn and potato starches	4	4.05	2.91
31		7	Rye bread and rice			
		3	Dried tomatoes	0	0.01	0.01
32	Meat loaf		Starch present	0	0.00	0.00
33	Blood sausage		Starch present	0	0.00	0.00
34	Meat	3	Milk powder	0	2.93	2.10
35		2	Milk powder	3	4.70	3.37
36		6	Milk powder	4	8.27	5.93
37		2	Milk powder	3	4.00	2.87
38		3	Milk powder	0	2.39	1.73

TABLE V. ANALYSIS OF MEATS WITH SOY FLOURS

No.	Kind of Meat	Brand of Soy Flour	Soy Flour Added	Factor	Soy Flour Found (Factor 0.0350)
			%		%
39	Meat	No. 500	4	0.0345	3.93
40		No. 103	4	0.0355	4.05
41		Cherry Blossom	4	0.0406	4.63
42		Soyalose	4	0.0401	4.59
43		Soyagrain	4	0.0363	4.14
44		Special X	4	0.0333	3.81
45		Soyaflake	4	0.0336	3.84
1–28		Kreemko (calculated on 4)		0.0251	2.87
			Av.	0.0349	

it is very simple and requires no special skill nor special apparatus. The determination is made by multiplying a copper proteinate precipitate by an empirical factor.

Acknowledgment

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CORRECTION. In the article entitled "Fractionation of Colloidal Systems", by Fancher, Oliphant, and Houssiere [IND. ENG. CHEM., ANAL. ED., **14**, 552 (1942)], the last term in Equation 1 should read

$$\frac{X_0^2 - R_2^2}{4}$$

Determination of Olefins in Gaseous Hydrocarbons

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A method is described for determining olefin content of gaseous hydrocarbons by direct titration of the sample dissolved in cold chloroform. Bromine in glacial acetic acid is used for titration. The method has given satisfactory results on synthetic gaseous blends comprising the complete range of unsaturation for C_3 and C_4 hydrocarbons as well as C_5 olefins in small amounts. The higher gaseous olefins (C_3, C_4, and C_5) can be determined to the exclusion of ethylene if not more than 10 per cent of it be present. The method is suitable for routine plant control purposes because of its simplicity and rapidity.

Hydrogen sulfide, mercaptans, and 1,3-butadiene interfere and must be removed before analysis.

THE importance of determining unsaturation in hydrocarbon gases is evident from the comparatively numerous publications on the subject in recent years. Some of these have dealt with improvement of existing procedures (*5*, *7*) and others with new methods of attack (*4*, *6*). The present paper describes a method developed to satisfy the need for one that is rapid and suitable for routine plant control purposes.

The methods commonly used for determining unsaturation in gases involve absorption in sulfuric acid solutions of various concentrations, absorption in bromine solutions of various concentrations, or catalytic hydrogenation. Methods such as bromination in the vapor state (*6*), fractional desorption (*3*), and desorption–thermal conductivity (*2*) are not suitable for routine use.

Matuszak (*5*) extensively reviews and presents the principles and limitations governing the selection of conditions for determining gaseous olefins by absorption in sulfuric acid. By developing an apparatus which requires very small portions (approximately 1 ml.) of reagent, he minimizes such factors as reversibility of absorption, solubility of gaseous paraffins, increase in solubility of hydrocarbons because of acid-soluble absorption products, solubility of hydrocarbons in precipitated polymerization products, and liberation of unabsorbable gas. Matuszak reports good results with olefins up to and including the C_4 hydrocarbons. The time required for analysis may be slightly longer than that required for other absorption methods or catalytic hydrogenation.

Both McMillan *et al.* (*4*) and Savelli *et al.* (*7*) show that conventional bromine water methods for determining olefin give high results. The latter had considerable success in using quarter-saturated bromine water, both with and without excess potassium bromide. This solution is weaker than usually employed and its superiority over the more concentrated solutions is attributed to its inactivity to butanes. Failure to obtain exact reproducibility was attributed to absorption of small quantities of saturated hydrocarbons in the liquid dibromides formed by reaction of bromine with unsaturated constituents.

McMillan's catalytic hydrogenation method (*4*) for determining unsaturation of gases has been used successfully by this laboratory and others; it is accurate but has certain disadvantages for routine use. Many plant gases contain impurities that poison the catalyst, necessitating frequent regeneration of it or delaying hydrogenation. Deviations from perfect gas laws and adsorption of sample on catalyst cause erroneous results unless proper corrections are applied.

The method of Uhrig and Levin (*8*) for determining bromine addition number of liquid hydrocarbons prompted the investigation of a similar procedure for gaseous hydrocarbons. The principle (bromination by a solution of bromine in glacial acetic acid, under conditions of titration) remained the same but modifications were made to adapt the procedure to gases. This was easily done for gaseous hydrocarbon mixtures containing C_3, C_4, and C_5 olefins. The method was also extended to determine these olefins to the exclusion of ethylene if the latter did not exceed 10 per cent of the sample.

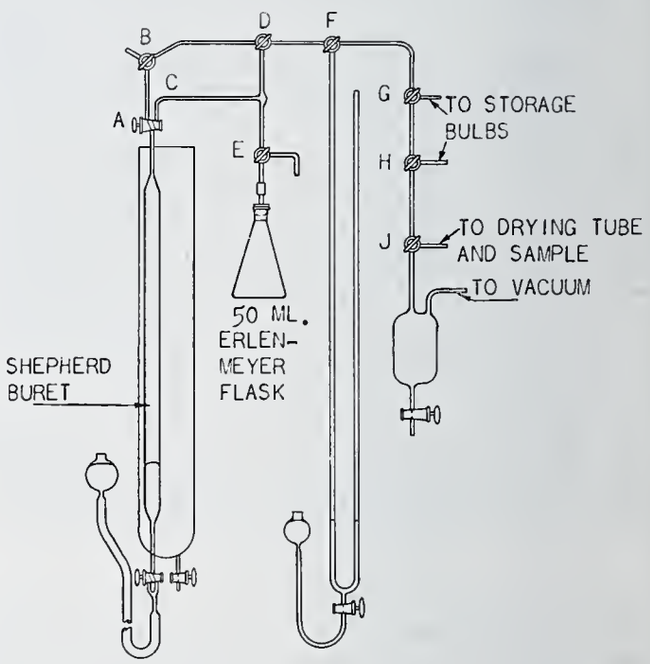

FIGURE 1. APPARATUS

An adaptation of the method of Uhrig and Levin (*8*) has recently been published by Benson (*1*), who used it on a micro scale for determining olefins and obtained very good results, particularly when the end point of the direct bromine titration was illuminated with a blue-white lamp against a white background.

Materials Used

The butanes, propane, propene, ethylene, and methane used in the experimental work were all purchased as C. P. materials and tested 99.5 per cent pure or better. A commercial grade of propene, stated to be 95 per cent pure, was used in a few of the preliminary experiments on this compound. The amylenes and butenes were prepared in this laboratory by conventional methods and were 99+ per cent pure. The ethane used was not purchased or prepared in the purified state but added in the form of certain plant gases.

Reagents and Apparatus

Bromine (1 per cent by volume) in C. P. glacial acetic acid, 0.1 *N* sodium thiosulfate, C. P. chloroform, 10 per cent potassium iodide solution, starch indicator solution, kerosene, and dry ice.

Standardize the bromine solution by adding 5 ml. to 25 ml. of 10 per cent potassium iodide solution and 5 ml. of chloroform in an iodine flask. Titrate this mixture immediately with 0.1 *N* standardized sodium thiosulfate solution using starch indicator. It is advisable to standardize the bromine solution daily until its stability is established under the conditions of use.

In addition to the apparatus shown diagrammatically in Figure 1 it is desirable to have a 10-ml. self-filling buret for the bromine solution.

Table I. Typical Results on C_4 Blends Using Method A[a]

Sample	1	2	3	4	5	6	7	8	9	10	11	12
Composition, Volume Per Cent												
Isobutene	12.8	2.3	24.2	9.0	1.6	12.9	4.0	0.8	6.1	1.8	0.3	12.45
1-Butene	..	..	2.7	13.2	15.9	1.5	5.9	6.7	0.7	2.7	2.5	8.25
2-Butene	..	..	3.4	16.5	16.1	1.8	7.4	6.8	0.8	3.4	2.5	11.45
Isobutane	42.6	55.0	24.5	30.0	37.4	13.1	13.4	15.9	59.1	60.3	69.0	37.80
n-Butane	44.6	42.7	45.2	31.3	29.0	70.7	69.3	69.8	33.3	31.8	25.7	30.05
Unsaturation, Volume Per Cent												
Theory	12.8	2.3	30.3	38.7	33.6	16.2	17.3	14.3	7.6	7.9	5.3	32.15
Found	13.1	2.6	29.7	39.5	33.8	16.8	17.5	14.2	7.8	8.0	5.2	32.10
Error, absolute	0.3	0.3	0.6	0.8	0.2	0.6	0.2	0.1	0.2	0.1	0.1	0.05

[a] Except that a 30-second end point was used.

Table II. Typical Results on Blends Containing Propene

(Method A[a] Used on Blends 1 to 6 and Method B on Blends 7 to 10)

Sample	1	2	3	4	5	6	7	8	9	10
Composition, Volume Per Cent										
n-Butane	90.3	83.45	75.55	71.1	58.8	56.8	...	...	...	...
Isobutane	..	...	...	21.3	29.6	24.8	57.55	80.0	88.4	43.7
Propene	9.7	16.55	24.45	7.6	11.6	18.4	42.45	20.0	11.6	56.3
Unsaturation, Volume Per Cent										
Theory	9.7	16.55	24.45	7.6	11.6	18.4	42.45	20.0	11.6	56.3
Found	8.7	15.3	22.60	6.6	10.7	17.1	42.30	20.1	11.6	55.5
Error, absolute	1.0	1.25	1.85	1.0	0.9	1.3	0.15	0.1	0.0	0.8
Apparent purity of propene, %	90.0	92.5	92.3	88.0	92.5	93.0	99.5	100.5	100	99.0

[a] 30-second end point used.

Procedure

Method A. Introduce approximately 5 ml. of chloroform into a 50-cc. Erlenmeyer flask and attach it to the apparatus below *E* (Figure 1). Place a dry ice–kerosene bath around this flask, so that it is at least half submerged, and keep the bath at −55° F. (−48° C.) or lower. After a minute or two in the cold bath evacuate the flask for 1 or 2 minutes by opening it to the vacuum through stopcocks *E*, *D*, *F*, and *J*. After closing *D* to the flask, evacuate the Shepherd buret (1 mm. of mercury or less pressure) through stopcocks *A*, *B*, *D*, *F*, and *J*. Collect 100 ml. of gas sample in the buret by making the appropriate connections with a sample storage bulb or the original sample container. After recording the temperature of the water in the jacket as that of the sample and the pressure from manometer *F*, transfer the sample into the Erlenmeyer flask by mercury displacement to tube *C*. Agitate the flask to hasten solution and condensation of the gas in the cold chloroform. Turn stopcock *A*, so that air will be drawn into the flask through the hole in the end of the stopcock (Figure 2), thus displacing the sample from tube *C*.

After the sample has condensed and dissolved in the chloroform, remove the flask and promptly titrate its contents with a 1 per cent solution of bromine in glacial acetic acid. The solution is not cooled during titration. The end point is that at which a faint color of bromine persists for at least 60 seconds. The color of very dilute bromine water (made by diluting a saturated solution with 100 volumes of water) is used as standard.

Method B. This alternative procedure is different from Method A only in the manner of titration. After the sample has been condensed, remove the flask from the apparatus and titrate rapidly with the bromine solution until 0.5 to 1.0 ml. excess of solution has been added. Allow the flask to stand a short time (30 seconds are sufficient but longer periods up to 30 minutes are unobjectionable) in order to complete the bromination, add 10 ml. of 10 per cent potassium iodide solution, and titrate the liberated iodine with standard sodium thiosulfate solution.

This method is more accurate than Method A and should be used in all cases except on samples containing ethylene. The excess bromine reacts with some ethylene, but not quantitatively. Under the conditions o fMethod A ethylene does not react appreciably.

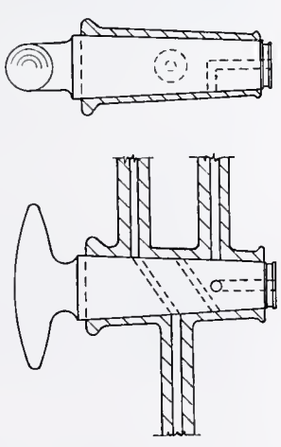

Figure 2. Stopcock

Calculations. Calculate unsaturation as follows:

Per cent unsaturation

$$\text{(gas volume basis)} = \frac{A \times B \times 100}{C \times 7.14}$$

where A = ml. of bromine solution reacting with olefins

B = mg. of bromine per ml. bromine solution

C = ml. of gas condensed

One milliliter of gaseous olefin, measured or calculated to standard temperature and pressure, is equivalent to 7.14 mg. of bromine on the basis of one mole of bromine reacting with one mole of olefin.

Precision and Accuracy

The precision and accuracy of the proposed method are indicated by the tables. In Table I are results obtained by Method A on blends containing all three C_4 olefins. The average deviation from the theoretical is ±0.3 per cent, absolute.

Table II shows the results obtained with propene as the olefin. The first six blends were analyzed by Method A and contained (95 per cent pure) propene from one source. The remaining four samples were analyzed by Method B and contained propene from another source having a purity of 99.5 per cent.

A further comparison of the methods is given in Table III. Method B gave slightly higher results in those cases where propene and 1-butene are present. These two olefins are slower to react with bromine and by using Method A it is possible that small amounts of sample escape during the period of slow additions of bromine.

Table III. Comparison of Results by Methods A[a] and B

Sample	1	2	3	4	5	6
Composition, Volume Per Cent						
1-Butene	17.1	11.2	20.05	10.0	19.8	27.6
2-Butene	..	34.2	...	..	...	26.8
Isobutane	...	...	...	..	80.2	45.6
Plant gas A[b]	82.9	54.6	...	...	..	..
Plant gas B[b]	..	..	79.95	90.0	..	..
Unsaturation, Volume Per Cent						
Theory	...	...	...	..	19.8	54.4
Found, Method A	37.2	59.0	32.8	24.0	20.5	53.8
	36.8	59.0	32.8	23.9	19.2	52.9
Found, Method B, back-titration after:						
30 seconds	..	..	33.3	..	19.9	..
	...	...	33.5	...	19.8	..
2 minutes	38.6	60.1	...	24.7	..	54.6
	38.8	60.8	...	24.6	..	54.5
10 minutes	38.9	60.8	...	..	..	54.7
	38.8	61.0	...	...	...	54.6
30 minutes	..	..	33.4	24.8	19.8	..
	..	..	33.7	24.8	19.9	..

[a] 30-second end point used.

[b] Plant gases A and B were samples of polymerization charge stock and off gas containing C_3 and C_4 unsaturates but neglible amounts of ethylene.

The effect of allowing the small excess of bromine (added according to the directions of Method B) to stand in contact with sample for varying lengths of time up to 30 minutes, is shown in Table III. It is evident that bromination of paraffins is not significant.

The proposed method has been used on very concentrated, as well as very dilute, blends of olefins in paraffins. The results of typical experiments are shown in Table IV. On

Table IV. Analysis of Blends Containing Very High and Very Low Concentrations of Unsaturates

Sample	1	2	3	4	5	6	7	8	9	10	11
Composition, Volume Per Cent											
Isobutane	13.1	24.3	37.0	45.4	..	..	..	..	3.7	2.6	11.8
n-Butane	85.6	73.3	59.4	53.8	..	..	..	..	6.4	17.2	18.3
Isobutene	..	..	..	..	..	..	100	..	89.9	80.2	69.9
1-Butene	1.3	2.4	3.6	0.8	100	..	..	..	..	..	..
2-Butene	..	..	..	..	..	100	..	..	..	..	..
Propene	..	..	..	..	..	..	..	100	..	..	..
Method used	B	B	B	B	A[a]	A[a]	A[a]	B	A[a]	A[a]	A[a]
Unsaturation, Volume Per Cent											
Theory	1.3	2.4	3.6	0.8	100	100	100	100	89.9	80.2	69.9
Found	1.3	2.3	3.0	0.8	98.8	97.7	99.8	97.8	88.7	81.9	68.9
	1.3	2.3	3.5	0.8	100.4	101.4	99.9	98.0	90.8	81.6	69.5
	..	..	..	..	99.7	100.5	..	98.3	88.7	..	69.8
	..	..	..	..	100.7	..	..	..	..	..	..
Average error, absolute	0.0	0.1	0.3	0.0	0.1	0.1	0.2	2.0	0.5	1.6	0.4

[a] 30-second end point used.

blends containing not more than 5 per cent of olefin, an absolute error greater than 0.1 per cent is not common; on pure olefins the results are not as good.

In some of the preliminary work listed in Tables I to IV a 30-second end point was used for all direct titration. For blends containing propene and 1-butene more accurate results were obtained with a 60-second end point as specified in Method A. This was used in the analysis of the complex samples shown in Table V. Here also is shown the utility of combining the method of bromine titration with that of a method for total olefins (bromine water absorption or catalytic hydrogenation).

Discussion

The two variations of the method are given because of their utility in the analysis of plant gases of different composition. Method A may be used on all types of samples except those containing ethylene in excess of 10 per cent. The method finds all the higher olefins but not ethylene. This type of test is particularly valuable on plant gas charge stocks that consistently contain ethylene below the specified limit and in which the higher olefins are the only unsaturated compounds reactive in the commercial operation. This is true in certain polymerization processes. The unsaturation test can therefore be applied to the gas samples, thus avoiding the necessity of fractionation by distillation to eliminate the less reactive ethylene.

Method B is more accurate and more precise than method A and should be used when ethylene is not present. There is very little difference in the time required for analysis by these methods.

Since rapidity of analysis was desired every effort was made to develop such a method, even at the expense of extreme accuracy. It is believed that the accuracy of the method is better than certain routine tests (conventional sulfuric acid and bromine water absorption) and equal to that of catalytic hydrogenation within certain ranges of olefin concentration. The proposed method is faster than the others, duplicate analysis being completed in 15 to 20 minutes after the sample has been introduced into the apparatus.

Hydrogen sulfide, mercaptans, and 1, 3-butadiene interfere and must be removed by scrubbing with doctor solution and melted maleic anhydride. The presence of hydrogen sulfide has an apparent inhibiting effect on the bromination of olefins conducted in the analytical method proposed—for example, a sample containing 21.5 per cent isobutene, 3 per cent hydrogen sulfide, and the balance butanes gives a value of approximately 7 per cent olefins by bromination. The presence of hydrogen sulfide causes an early appearance of an apparent end point, which renders a sample containing it inapplicable to Method A and makes it impracticable to use Method B. This limitation on the method detracts practically nothing from its utility, since hydrogen sulfide is easily eliminated by scrubbing the sample with doctor solution. Methyl mercaptan has little effect, but results tend to be higher than theoretical when it is present in high concentrations (5 to 10 per cent), and butadiene brominates very slowly, giving erratic results by either Method A or B.

The method can be used for the determination of unsaturation of C_3 and C_4 cuts obtained by low-temperature fractional distillation as well as for the numerous plant gases that contain less than 10 per cent of ethylene. It has been used in this laboratory with considerable success, new operators experiencing very little difficulty in acquiring the necessary technique.

Table V. Results Obtained

[Bromine titration (Methods A and B), bromine water absorption (quarter-saturated solution), and catalytic hydrogenation.]

Sample	1	2	3	4	5	6	7	8	9
Composition, Volume Per Cent									
Amylenes	0.6	2.0	2.4	7.0	1.5	1.4	0.4	..	..
Isobutene	1.4	4.8	5.9	3.3	3.6	3.5	1.0	2.2	1.9
1-Butene	1.2	4.0	4.9	2.2	3.0	2.9	0.9	..	..
2-Butene	1.3	4.7	5.7	3.2	3.4	3.3	19.5	..	..
Propene	1.2	4.3	5.2	2.7	3.1	3.1	1.0	8.6	7.3
Ethylene	..	..	..	..	3.4	9.0	6.8	14.7	27.1
Isobutane	84.1	73.2	54.7	56.1	53.8	32.2	62.8	52.8	45.2
n-Butane	..	..	..	..	23.1	32.1	..	..	..
Propane	..	..	..	25.5	..	..	..	21.7	18.5
Methane	10.2	7.0	21.2	..	5.1	12.5	7.6	..	..
Unsaturation, Volume Per Cent									
Theory, total	5.7	19.8	24.1	18.4	18.0	23.2	29.6	25.5	36.3
Theory, less C_2H_4	..	..	..	..	14.6	14.2	22.8	10.8	9.2
Found, Method A	5.8	19.2	23.4	17.5	13.9	13.7	23.0	11.4	10.3
	5.5	19.2	23.2	17.5	13.9	13.8	23.0	..	..
Found, Method B	5.8	19.6	23.7	18.2	..	..	..	..	..
	5.7	19.7	23.7	18.4	..	..	..	..	..
Found, bromine	..	..	..	15.7	18.7	24.0	30.0	..	..
Water absorption (1/4 saturated solution)	..	..	..	16.3	18.4	23.7	29.8	..	..
Found, catalytic hydrogenation	..	..	..	..	..	23.2	29.3	..	..
	..	..	..	..	..	23.4	29.2	..	..

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Photometric Determination of Acetone-Insoluble Material in Soybean Oil

Relation of Acetone-Insoluble Material to Break

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THE quality of soybean oil commonly has been determined by one of two analytical procedures—namely, the modified Gardner break test (*1*) and the official A. O. A. C. foots test (*2*). The American Oil Chemists' Society adopted the modified Gardner break test as an official procedure in 1940, and this test is in general use throughout the soybean industry.

The test consists essentially in heating oil in the presence of hydrochloric acid to 288° C., and separating and weighing the precipitate which appears in the oil at this temperature. The test yields highly reproducible results when run with careful technique, but requires considerable time and measures a decomposition product of constituents of the oil rather than the constituents themselves.

Prior to the introduction of the modified Gardner break test, the foots test (*2*) was used to determine the quality of soybean oil. The foots test measures the acetone-insoluble material in soybean oil by a volumetric procedure. This test was not entirely satisfactory because of the difficulty of obtaining reproducible results and the time required.

Gravimetric methods of determining acetone-insoluble material by actual separation and weighing of the material are time-consuming and are complicated by instability of the acetone-insoluble material in oxygen and heat after separation from the oil phase. This makes the obtaining of reproducible results very difficult. In this investigation a photometric procedure has been developed for the measurement of acetone-insoluble material in solvent-extracted soybean oil without exposing the material to air or heat. The method yields highly reproducible results and is simple and rapid in operation.

The photometric procedure is standardized against gravimetrically determined values and depends upon the preparation of stable colloid sols of acetone-insoluble material from solvent-extracted soybean oil in acetone. These sols remain stable for several hours when prepared in proper concentrations and in the absence of electrolytes. The turbidities of the sols thus prepared were examined in a filter photometer and were found to conform to Beer's law over the concentration range investigated. A correction for the interference of natural color in the oil was made by determining the light extinction coefficient of a solution of oil in hexane. Subtracting this value from the light extinction coefficient of the acetone sol yielded a corrected extinction coefficient which was a measurement of the turbidity of the acetone-insoluble material.

Experimental

APPARATUS AND REAGENTS. Light absorption was measured with a Klett-Summerson No. 800-3 photoelectric colorimeter. This instrument is a filter photometer of the two-cell type. The dial is marked with a logarithmic scale from 0 to 900, and reads in terms proportional to the light extinction coefficient (log I/I_0). A blue filter was used having the rather narrow transmission range of 380 to 430 mμ. Calibrated and carefully matched test tubes were used as absorption cells. Acetone and hexane used in the investigation were dried over anhydrous sodium sulfate and carefully redistilled.

PHOTOMETRIC PROCEDURE. For preparing acetone sols of acetone-insoluble material and determining light extinction coefficients, a sample of solvent-extracted soybean oil was prepared by heating to 70° C., until all of the "foots" had been brought into solution. The sample was then cooled quickly to 25° C. in the water bath, 50 ml. of redistilled acetone were pipetted into a clean 125-ml. Erlenmeyer flask and quickly stoppered, and 50 ml. of redistilled hexane were pipetted into a second 125-ml. Erlenmeyer flask in a similar manner. Exactly 1 ml. of the previously prepared solvent-extracted soybean oil was pipetted into each of the solvents, with removal of the stoppers for only sufficient time to allow for the transfer. The addition to the acetone was made with as little agitation as possible. The acetone-insoluble sol was then formed with a single vigorous swirl of the flask. Four additional swirls were used to mix the sol thoroughly. A sample of the sol was removed for photometric analysis 20 seconds after formation. The hexane solution was mixed thoroughly and a sample taken for photometric analysis.

The photoelectric instrument was set to zero with pure solvent in the absorption cells before each series of determinations and the setting was checked frequently during operation. The zero setting was found to change very little after the instrument had warmed up.

GRAVIMETRIC DETERMINATION OF ACETONE-INSOLUBLE MATERIAL. Acetone-insoluble material was determined gravimetrically by the following procedure which has been used in this laboratory for a number of years: A 10-gram sample of solvent-extracted soybean oil was weighed into a 250-ml. beaker and 100 ml. of acetone saturated with potassium iodide were added. The solution was stirred vigorously until the colloidal precipitate at first formed coagulated and upon settling left the supernatant liquid crystal clear. Approximately 1.5 grams of filter aid were added and the precipitate was transferred to the asbestos filter mat of a Gooch crucible with a vacuum of about 2.5 cm. (1 inch) of mercury. The precipitate was then washed with six 20-ml. portions of pure acetone. After washing, the precipitate was dissolved and transferred into a tared Soxhlet extraction flask with 100 ml. of pure hexane added in 20-ml. portions. The hexane was evaporated from the acetone-insoluble material on the steam bath and final traces were removed in the vacuum oven at 105° C. The flask was then cooled in a desiccator and weighed back to determine the acetone-insoluble material.

GRAVIMETRIC DETERMINATION OF BREAK. Break was determined by the modified Gardner break test (*1*).

TABLE I. RELATION OF CORRECTED LIGHT EXTINCTION COEFFICIENTS AND ACETONE-INSOLUBLE MATERIAL AND BREAK

Oil No.	Colorimeter Readings: Acetone Sol	Hexane Solution	Corrected Reading	A. I. %	Break %
1	125	113	12	0.41	0.123
2	135	90	45	0.68	0.207
3	111	79	32	0.69	0.182
4	122	81	41	0.72	0.204
5	126	76	50	0.81	0.219
6	140	81	59	0.87	0.243
7	169	83	86	1.09	0.292
8	175	85	90	1.09	0.314
9	198	92	106	1.25	0.337
10	187	77	110	1.39	0.357
11	218	82	136	1.54	0.404
12	236	84	152	1.60	0.457
13	235	94	141	1.66	0.437
14	260	80	180	1.93	0.506
15	285	100	185	2.00	0.533
16	266	83	183	2.04	0.513
17	375	135	240	2.45	0.683
18	370	93	277	2.84	0.757

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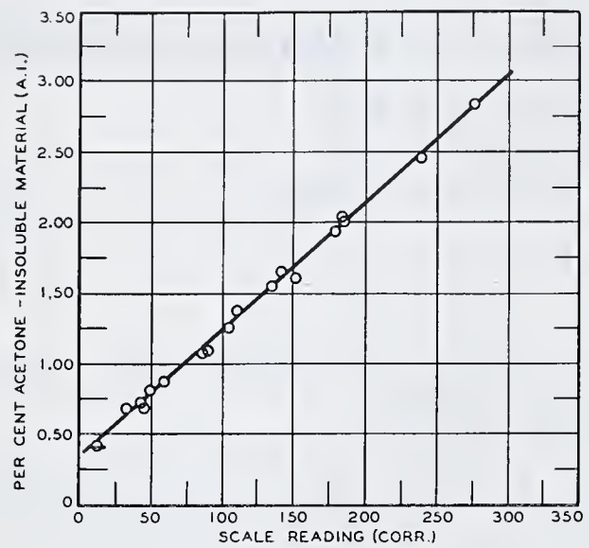

FIGURE 1. PHOTOMETRIC DETERMINATION OF ACETONE-INSOLUBLE MATERIAL IN SOLVENT-EXTRACTED SOYBEAN OIL

Calibration curve

Discussion

In this investigation data were obtained showing (1) the relative light extinction coefficients of stable sols of acetone-insoluble material in acetone, (2) the gravimetrically determined acetone-insoluble contents, and (3) the break contents of 18 different samples of solvent-extracted soybean oil. The samples were selected so that a wide range of acetone-insoluble contents were covered. The collected data are shown in Table I. Each value is the average of two or more checking determinations.

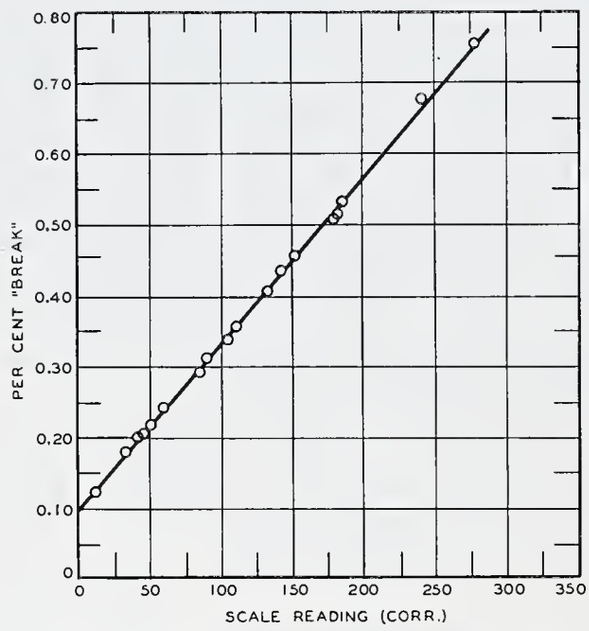

FIGURE 2. PHOTOMETRIC DETERMINATION OF BREAK IN SOLVENT-EXTRACTED SOYBEAN OIL

Calibration curve

Column 2 shows relative light extinction coefficients for the acetone sols, column 3 relative extinction coefficients for hexane solutions. Corrected relative light extinction coefficients are listed in column 4. Acetone-insoluble and break contents in per cent are recorded in columns 5 and 6, respectively. Acetone-insoluble contents ranged from 0.41 to 2.84 per cent, corresponding to 0.123 and 0.757 per cent break.

Relative extinction of light by the acetone sols of acetone-insoluble material was found to be directly proportional to the acetone-insoluble content of the oil. When relative light extinction coefficients were plotted on the abscissa and per cent acetone-insoluble material on the ordinate, a straight-line curve resulted which indicated conformity with Beer's law over the concentration range investigated and served as a calibration curve. The system was shown, therefore, to be adapted to photometric analysis. The calibration curve is shown in Figure 1.

Relative light extinction of the acetone-insoluble sols was found likewise to be directly proportional to the break content of the oils. The calibration curve relating per cent break to relative light extinction is shown in Figure 2. Average deviation of the points from the calibration curve was ±0.006 break per cent, while the maximum deviation of any point from the curve was 0.016 break per cent. Average precision

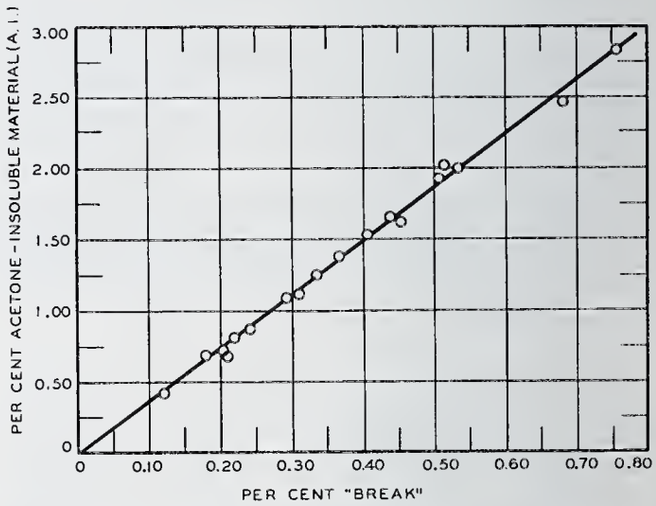

FIGURE 3. RELATION BETWEEN ACETONE-INSOLUBLE MATERIAL AND BREAK IN SOLVENT-EXTRACTED SOYBEAN OIL

of the photometric method was found to be approximately 2 scale divisions, corresponding to 0.005 break per cent. This compares favorably with the precision obtained with the modified Gardner break test. Although it is possible to obtain a high degree of precision with this latter test when duplicate analyses are run by the same analyst in parallel and with careful technique, different analysts will generally find larger differences in results. The photometric procedure is not influenced to so great an extent by differences in technique. Approximately 5 minutes are required for the photometric determination as compared to between 1 and 1.5 hours for the modified Gardner break determination.

Examination of the data in columns 5 and 6 of Table I revealed that a direct empirical relation existed between acetone-insoluble and break. When per cent acetone-insoluble material is plotted as ordinates and per cent break as abscissas, a straight-line curve results which passes through the origin and has a slope of 3.8 (Figure 3). This would indicate that break amounts to approximately one quarter of the acetone-insoluble material. Although the exact chemical composition of neither the acetone-insoluble material nor the break is known, this empirical relation would seem to indicate that the break is formed from the acetone-insoluble material and that this occurs through some stoichiometric reaction. The immediate value of the relation lies in the fact that upon the basis of the relation break can be calculated directly from acetone-insoluble material content.

Summary

A photometric procedure has been described for the determination of acetone-insoluble material and break in solvent-extracted soybean oil. The photometric method is rapid, possesses an average precision of approximately 0.005 break per cent, and is relatively free of errors introduced by differences in technique. A direct empirical relation has been found between acetone-insoluble material and break in solvent-extracted soybean oils.

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Determination of Oxide Copper

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IN RECENT editions of widely used reference books on the chemical analysis of metallurgical products (*1*, *2*), it is stated that cuprite, tenorite, malachite, chrysocolla, and other oxidized forms of copper can be determined by leaching with 3 per cent sulfur dioxide. This method depends on the fact that oxidized copper is soluble in dilute sulfurous acid, whereas copper sulfides are unattacked under these conditions. It is recommended that 1 to 2 grams of 100- to 150-mesh ore be agitated with approximately 100 ml. of 3 per cent sulfur dioxide for 0.5 to 2 hours, filtered, and washed, and copper determined on the filtrate or oxide portion.

In many parts of the world oxide copper is determined by leaching with 5 per cent sulfuric acid saturated with sulfur dioxide, instead of the 3 per cent sulfur dioxide recommended in the literature. During a study of local oxidized copper ores a large number of comparative tests were carried out on mine and concentrator products, using these two solutions. Slightly higher and more consistent results were secured when using 5 per cent sulfuric acid saturated with sulfur dioxide, but in these complex products it is difficult to obtain an absolute or calculated value for the oxide copper present. The authors then turned to pure copper minerals, whose composition they could determine by careful chemical and mineralogical analysis, in their study of the determination of oxide copper.

The material was ground to <100-mesh, and 1 gram was weighed into a copper flask, which was corked after the addition of 100 ml. of leaching solution, and agitated for 1 hour. Prolonging the leaching period did not result in increasing the recovery of oxide copper. The solution in the flask was filtered, and washed thoroughly with hot water, the filtrate boiled to remove sulfur dioxide, nitric acid added, and copper determined electrolytically. Typical results from a series of determinations are given in Table I.

It has long been known locally that, contrary to published information, cuprite cannot be determined by leaching either with dilute sulfurous acid or with dilute sulfuric acid saturated with sulfur dioxide. The fact that under most favorable conditions only 50 to 60 per cent of the copper is recovered, would indicate that the reaction $Cu_2O + H_2SO_4 \rightarrow CuSO_4 + H_2O + Cu$ occurs.

Table I. Recovery of Oxide Copper in Oxidized Ores by Leaching

	Cuprite %	Chrysocolla %	Malachite %	Azurite %
Total Cu	76.50	21.22	54.17	51.68
Oxide Cu, using 3% SO_2	18.48	19.36	43.49	44.27
	17.80	19.69	44.89	42.25
	18.37	19.58	43.91	41.65
Oxide Cu, using 5% H_2SO_4 saturated with SO_2	40.00	21.14	54.16	51.64
	40.05	21.18	54.09	51.65
	39.92	21.12	54.10	51.68

It is also apparent from the above results that 3 per cent sulfur dioxide is unsatisfactory for the determination of oxide copper. Not only are the results low, when carrying out the recommended procedure, but they are also erratic. The 5 per cent sulfuric acid saturated with sulfur dioxide, on the other hand, dissolves oxidized copper completely, with the exception of cuprite, and replicates attest to the satisfactory precision of the method for plant laboratories.

Copper sulfides are scarcely attacked by 5 per cent sulfuric acid saturated with sulfur dioxide. Pure samples of bornite and chalcocite, and a mixture of bornite and azurite, were subjected to this procedure for oxide copper with the results shown in Table II.

Table II. Effect of 5 Per Cent Sulfuric Acid Saturated with Sulfur Dioxide on Copper Sulfides

	Bornite %	Chalcocite %	1 Azurite (51.68% Cu): 1 Bornite (63.61% Cu) %
Total Cu	63.61	78.46	57.65
Oxide Cu, using 5% H_2SO_4 saturated with SO_2			
Calculated	Nil	Nil	25.84
Determined	0.07	0.15	25.83
	0.07	0.14	25.86
	0.06	0.15	25.84

Even excluding the strong possibility that a very small quantity of oxide copper was actually present in the bornite and chalcocite, and assuming that the figures for oxide copper in Table II represent wholly an attack of the sulfides, it is evident that the error is only 0.1 to 0.2 per cent, or below inherent errors in most analytical operations.

In view of the nearly universal presence of varying quantities of iron in mine and concentrator products, and the well-known solvent action of ferric salts on copper and copper sulfides, it was considered advisable to determine the possible solvent action of iron on sulfides during the determination of oxide copper with the authors' procedure. Since the oxide leaching solution is saturated with sulfur dioxide, and ferrous sulfate is not a solvent for copper sulfides, it appeared improbable that marked solvent action by iron would be found, but quantitative data were desirable.

Iron was added to chalcocite in four forms prior to leaching with 5 per cent sulfuric acid saturated with sulfur dioxide: as sulfide in bornite, as oxide in ferric oxide, as a mixture of sulfide and oxide in a mill feed, and finally as sulfate in ferric sulfate. The results are given in Table III. In all this work, of course, any metallic iron introduced through pulverizing

Table III. Effect of Iron in Determination of Oxide Copper

(Using 5% H_2SO_4 saturated with SO_2)

	Oxide Copper Calculated	Oxide Copper Found
	%	%
Chalcocite (78.46% Cu)	Nil	0.15
1 chalcocite : 1 bornite	Nil	0.07
Chalcocite + 4% Fe as Fe_2O_3	Nil	0.07
Chalcocite + 4% Fe as $Fe_2(SO_4)_3$	Nil	2.11
Chalcocite + 15% Fe as $Fe_2(SO_4)_3$	Nil	7.02
Mill feed (3.12% Fe)		3.59
1 chalcocite : 1 mill feed	1.80	1.86

equipment was removed with a magnet prior to the determination.

It is evident from Table III that iron added to chalcocite in the form of sulfide or oxide has no solvent action on the copper sulfide mineral. In fact, the quantity of copper dissolved in these cases is always slightly less when iron is present than where pure chalcocite is leached with the oxide copper solution.

Where ferric sulfate is initially present, however, the addition of the leaching solution leads to immediate attack of the copper sulfide minerals by the ferric salt. It is probable under these conditions that ferric sulfate dissolves appreciable copper sulfide before the sulfur dioxide of the leaching solution reduces the iron to the ferrous state. A similar attack of copper sulfides by ferric sulfate was observed when 3 per cent sulfur dioxide was used as a solvent for oxide copper.

Summary

The use of 3 per cent sulfur dioxide as a solvent for oxide copper, recommended by standard analytical textbooks, has been found unsatisfactory, but the substitution of 5 per cent sulfuric acid saturated with sulfur dioxide has resulted under the same conditions in complete recovery of oxidized copper, except cuprite. In the absence of ferric sulfate, solution of copper sulfides is negligible.

Contrary to the references cited, cuprite cannot be determined by the sulfurous acid method for oxide copper, nor even by employing 5 per cent sulfuric acid saturated with sulfur dioxide.

The presence of iron as sulfide or oxide does not lead to attack of copper sulfide, using 5 per cent sulfuric acid saturated with sulfur dioxide. If ferric sulfate is initially present, appreciable solution of copper sulfide occurs during the leaching period in spite of the presence of sulfur dioxide, and results for oxide copper are not reliable.

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Determination of Thiamine in Vegetables

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The thiamine content of eight frozen vegetables has been determined by the bioassay, thiochrome, and fermentation procedures. When the sulfite cleavage modification of the fermentation method was used, the results were in good agreement with those by the thiochrome procedure. In the case of three vegetables the potency obtained by bioassay agreed with that determined by the thiochrome and fermentation methods, but the other vegetables gave somewhat lower values by the latter procedures. The suggestion is made that the animal diet may not have been complete in other factors.

RELATIVELY rapid methods for the accurate estimation of thiamine are needed both for the determination of the vitamin B_1 content of fresh vegetables and for the study of changes which may occur during their storage, processing, and preservation. Bioassay methods cannot be used, since most fresh vegetables will not keep long enough to permit completion of the assays, and furthermore, even if they do not spoil, their composition is subject to change due to respiration.

Hennessy and Cerecedo (*4*) have proposed a modification of the thiochrome method of determining thiamine which appears to be applicable to vegetables. Schultz, Atkin, Frey, and Williams (*12*) have modified the original fermentation method of determining thiamine proposed by Schultz, Atkin, and Frey (*11*) to include an estimation of the fermentation-stimulating action of substances other than thiamine in the material under test and its subtraction from the total volume of carbon dioxide obtained.

The work reported in this paper is a comparison of analyses by the thiochrome and fermentation methods of determining thiamine in frozen vegetables with those obtained by a growth bioassay procedure. The results should be useful in determining the relative merits of these rapid procedures and standard bioassays.

Since it has been shown that in the case of carotene and ascorbic acid there is little or no loss of vitamin potency in storage at −40° C. (*5*, *6*, *13*, *14*), this mode of storage seemed most feasible in the interim between harvesting and examination of the thiamine content by the three procedures. This supposition in the case of thiamine has been demonstrated at this station (*7*) to be correct.

The vegetables used in this study—peas, broccoli, cauliflower, and spinach—were obtained from the station gardens, cleaned, sorted, blanched in boiling water, and cooled. The drained product was then packed in a cellophane-lined Peters type of carton and frozen in a Birds-eye multiplate freezer. The frozen asparagus, cut corn, string beans, and lima beans were obtained from commercial sources. In Table I the varieties and blanching times of these vegetables are given.

Although the blanching before freezing does not affect the comparison of the three methods of analyses, it does lower the total thiamine figure, since the blanching water extracts some of the thiamine from the vegetable. A study of the losses of thiamine from asparagus and peas during processing prior to freezing has been reported in another paper (*7*).

Table I. Varieties and Blanching Times of Vegetables Studied

Vegetable	Variety	Blanching Time at 212° F., *Min.*
Peas	Thomas Laxton	50 seconds
Asparagus	Mary Washington	3
Cut corn	Golden Cross	7.5
Snap beans	Lowe's Champion	2
Spinach	King of Denmark	1.5
Cauliflower	Snowball	2.5
Lima beans	Henderson Bush	3
Broccoli	Calabrese	2.5

Samples for assay by the three methods were finely ground in a food chopper at −23.3° C. (−10° F.). The samples for bioassay were shipped under dry ice refrigeration to the nutrition laboratory of the State College at Amherst, Mass.

Bioassay Procedure

The two most common methods for the bioassay of thiamine are the growth and curative tests with rats (*8*). Since, with the possible exception of peas, the materials to be assayed were bulky, fibrous, and of low potency, it was necessary to use the growth technique.

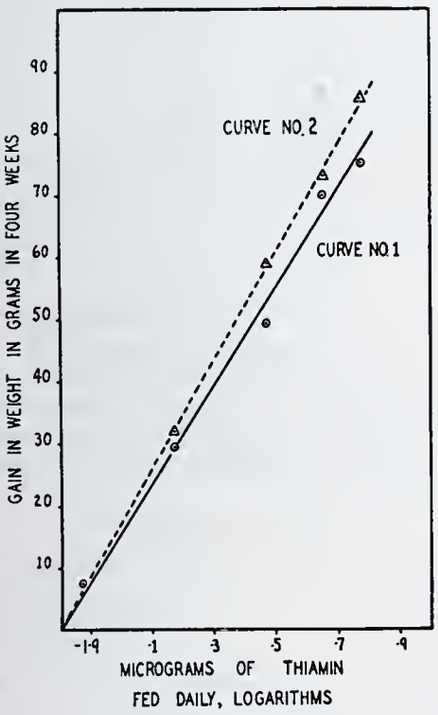

Figure 1. Thiamine in Vegetables

All the animals used in this work were raised in the rat colony of the Massachusetts State College and weighed from 35 to 50 grams at 21 to 28 days of age when placed on the diet recommended by Chase and Sherman (*1*). Within 28 days after the B_1-free diet was started, the rats had definitely stopped growing or had begun to lose weight. This maintenance of or loss in weight was observed from the weights of the individual animals taken every other day over a period of a week and a half towards the end of the depletion period. The animals from the various litters were then selectively randomized into groups of eight, so that each group contained the same number of males and females with no more than one member from each litter in any one group. One of these groups received no thiamine at all—i. e., they were set aside as a negative control—while five groups were given daily 0.75, 1.5, 3.0, 4.5, and 6.0 micrograms of thiamine, respectively. The remaining groups were fed two levels of vegetables. The addenda were administered daily for a period of 28 days. All animals were weighed once a week.

It was not possible to obtain enough animals to complete assays on all vegetables at one time and, therefore, asparagus, snap beans, cauliflower, peas, cut corn, and spinach were assayed during the period from February to April, while broccoli and lima beans were assayed during the months of May and June. Because some time elapsed between the two sets of assays, a second curve of response was determined when the broccoli and lima beans were assayed. These curves are shown in Figure 1, curves 1 and 2 being for the first and second response curves, respectively.

The curves of response were drawn by plotting the average gains in weight, as the ordinate, against the logarithms of micrograms of thiamine, as the abscissa. By dividing the corresponding thiamine units obtained by the grams of vegetable fed, the equivalent amount of thiamine in each gram of food was determined.

Two feeding levels of each vegetable were started, but it soon became apparent that the supply of cauliflower was being rapidly depleted, so that the higher level was discontinued. All eight rats constituting the negative control group died before the assay was completed, except two which remained alive though they showed polyneuritic symptoms. One animal of the group receiving 0.75 microgram of thiamine daily also died.

In Table II the results of the bioassay on the various vegetables are presented. The greatest variation in the thiamine content of a single vegetable, when fed at two levels, was in the case of peas, where the average value differed by 16 per cent from either assay. This divergence may be considered as being within the range of error encountered in bioassays.

Thiochrome Determination

The chemical methods for determining thiamine have been reviewed by Hennessy (*3*). A modification of the Hennessy and Cerecedo (*4*) procedure has been used by Conner and Straub (*2*) for the determinaton of thiamine in frozen spinach, peas, and broccoli, fresh lima beans, string beans, and squash. They found that there was an agreement between the bioassays and chemical analyses which was better than 12 per cent for the two cereal products and frozen peas tested, the results from the chemical method being in all three cases higher.

Recently the Research Corporation Committee in conjunction with the Committee on Vitamin Fortification of the American Association of Cereal Chemists (*9*) suggested a procedure for the determination of thiamine which is simpler than the Conner and Straub method. The details of the modified method which was used in the authors' studies are given below:

Twenty-five grams of vegetable are placed in the cup of a Waring Blendor and the tissues macerated in 150 ml. of 0.1 *N* sulfuric acid for 2 or 3 minutes. Then the Blendor cup is rinsed out with 250 ml. of 0.1 *N* sulfuric acid into a 500-ml. Erlenmeyer flask. The flasks are set in boiling water or on a steam bath. During the first 5 minutes the flasks are frequently agitated and then once every 5 minutes for one-half hour. The flasks are then cooled below 50° C., and 25 ml. of 2.5 *M* sodium acetate solution containing 0.5 gram of Clarase are added. (The Clarase is added to the sodium acetate solution just previous to addition to the extract.) Incubation at 50° C. for 2 hours permits a complete hydrolysis of the thiamine phosphates. Through the addition of distilled water the volume of the extract is made up to 500 ml. and then, after thorough mixing, a portion is filtered. The first 10 ml. of filtrate are discarded and the rest is collected.

The remainder of the procedure—that is, the base exchange purification and the production of thiochrome—was followed exactly as outlined by the committee. The percentage recovery of thiamine added to test samples of vegetables before extraction varied from 93 to 105. The thiamine contents of the eight vegetables as determined by the thiochrome method are presented in column 3 of Table III.

Table II. Thiamine Content of Vegetables as Indicated by Bioassay

Supplement	Amount Fed Daily	Average Gain per Week	Thiamine per Gram of Supplement	Average Thiamine Value
	Micrograms	*Grams*	*Micrograms*	*Micrograms*
Group I				
Thiamine	0.00	...	...	....
	0.75	7	...	....
	1.50	30	...	....
	3.00	49	...	....
	4.50	70	...	....
	6.00	73	...	....
	Grams			
Asparagus	1.00	40	2.00	2.11
	2.00	67	2.23	
Snap beans	2.00	33	0.83	0.76
	4.00	47	0.69	
Cauliflower	4.00	41	0.53	0.53
Peas	0.50	41	4.22	3.64
	1.00	54	3.06	
Spinach	3.00	31	0.52	0.52
	5.00	49	0.53	
Cut corn	2.00	43	1.15	1.20
	3.00	60	1.24	
	Micrograms			
Group II				
Thiamine	0.00	...	...	....
	1.50	33	...	....
	3.00	62	...	....
	4.50	74	...	....
	6.00	86	...	....
	Grams			
Lima beans	1.00	23	1.16	1.17
	3.00	66	1.18	
Broccoli	2.50	59	1.15	1.06
	5.00	78	0.98	

Fermentation Method of Assay

All the experimental work reported in this paper was completed before the paper of Schultz, Atkin, and Frey (*10*) appeared in the press. The fermentometer used was of the older type and consequently in all analyses double the quantity of reagents recommended by the above authors was used for each determination. However, the results obtained do not vary materially from those obtained when the newer type of equipment is used. The amounts of fermentation stimulation expressed as micrograms of thiamine before and after sulfite cleavage are given in columns 4 and 5 of Table III.

From these data it will be seen that the total stimulation was always greater than that considered to be due to thiamine alone. This would seem to indicate that there are one or more substances in vegetables other than thiamine which have a stimulatory effect on the rate of fermentation. The presence of this effect makes necessary the use of sulfite cleavage when determining the thiamine content of vegetables by the fermentation method.

Comparison of Results Obtained by Bioassay, Fermentation, and Thiochrome Methods

The thiamine contents of the eight frozen vegetables as determined by the thiochrome and fermentation methods are compared in Table III with those obtained by bioassay.

Taken as a whole, the thiamine contents of eight vegetables as determined by bioassay, thiochrome, and fermentation "after sulfite cleavage" methods are in good agreement. There is, however, a slight divergence in the case of cut corn, the after sulfite cleavage fermentation results being about 12 per cent higher.

The thiamine content as derived from the total stimulation of gas production in the fermentation method gave results which in four cases out of eight were considerably higher than the bioassay. However, three vegetables, lima beans, cauliflower, and broccoli, gave thiamine values by the total stimulation method which were in agreement with the results of the bioassays.

In the case of frozen peas there is considerable difference between the results obtained by the three methods. The bioassay is the average of two rather widely different values and since there is no reason to discard the higher potency, it must be retained. Nevertheless, were the lower of the two bioassay values to be taken, the thiochrome, total stimulation and after stimulation and after sulfite cleavage fermentation results would only be 15, 5, and 12 per cent, respectively, too low.

There is the possibility that the animal diet was deficient in known or unknown members of the B complex other than thiamine and if these factors were present in the vegetable being tested, a higher response would be obtained than would normally be due to thiamine. In general, the thiochrome and after sulfite cleavage fermentation values agree well. It is assumed that there are substances other than thiamine in vegetables which stimulate the rate of fermentation and therefore the sulfite cleavage procedure should be used.

Table III. Comparative Vitamin B_1 Values Obtained by Bioassay, Fermentation, and Thiochrome Methods

Vegetable	Bioassay	Thiochrome	Fermentation	
			Before sulfite cleavage	After sulfite cleavage
	Micrograms of thiamine per gram			
Peas	3.64	2.60	2.83	2.69
Asparagus	2.11	2.04	2.52	2.12
Cut corn	1.20	1.17	1.50	1.31
Lima beans	1.17	0.89	1.10	0.90
Broccoli	1.06	0.88	1.09	0.81
Snap beans	0.76	0.64	0.87	0.63
Cauliflower	0.53	0.43	0.51	0.39
Spinach	0.52	0.55	0.72	0.55

Acknowledgment

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A summary of part of the thesis submitted in February, 1942, by James C. Moyer to the Graduate School of Cornell University in partial fulfillment of requirements for the degree of doctor of philosophy. Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 516, July 1, 1942. This work was supported largely by grants made by the General Foods Corporation and the Frosted Foods Sales Corporation of New York.

Routine Colorimetric Determination of Titanium in Chromium Steels

Separation of Titanium and Chromium by Perchloric Acid

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THIS paper maps a simple routine for the determination of titanium in certain steels, in which the sample is dissolved in mixed hydrochloric-nitric acids and fumed in perchloric acid, and the titanium is filtered off cold. The inadequacies of the method for certain steels are pointed out.

Chromium trioxide is quantitatively insoluble in cold 69 to 72 per cent perchloric acid (*4*), and it can be shown that small amounts of titanium are soluble in the reagent.

It is common practice to determine titanium (1 to 6 mg.) colorimetrically, using the peroxide method. In steels, titanium can be separated from chromium, nickel, and ferrous iron by cupferron (*2*), then determined colorimetrically. Titanium may also be separated from chromium by the lead perchlorate method (*6*).

The method proposed in this paper can be applied to steels of the 18–8 chromium-nickel type (Bureau of Standards No. 121) and to the 5 per cent chromium–0.5 per cent molybdenum type. The procedure is relatively simple. The steel is dissolved in aqua regia and evaporated to fumes in excess perchloric acid. When cold, the insoluble chromium trioxide is filtered off, and titanium is determined in the filtrate.

Vanadium can be separated in a similar manner (*4*), and the problem is now being further investigated.

Experimental

TITANIUM-CHROMIUM STEELS. One-gram samples of the steels were dissolved in mixed nitric-hydrochloric acids, and 30 cc. of 72 per cent (technical) perchloric acid were added to each beaker. The beakers were heated until chromium was oxidized to chromium trioxide, then several minutes more, and were then removed to a cool place in the hood. At the same time a wash solution of perchloric acid was made by heating 72 per cent perchloric acid to fumes and cooling to room temperature.

For filters, Gooch crucibles with asbestos pads or fritted-glass crucibles (Pyrex M style) were used in conjunction with a bell-jar type of suction apparatus.

The cold steel solutions were filtered through dry crucibles. When using fritted-glass crucibles small glass stirring rods were used to agitate the insoluble red residues. Each crucible was washed five or more times with 5-cc. portions of the cold wash solution. The apparatus was disassembled, and the residues in the crucibles were washed into the original reaction beakers with water.

An additional 15-cc. portion of 72 per cent perchloric acid was added to each beaker of chromium trioxide, and the contents were heated to fumes of perchloric acid. After cooling, the solutions were filtered through dry crucibles, and the precipitates were washed with wash solution.

Titanium was determined in each filtrate in the customary manner, by diluting with water, adding 5 cc. of 3 per cent hydrogen peroxide, and comparing with a standard titanium solution. For these comparisons, the cupferron method was used with Bureau of Standards Sample No. 121 (0.394 per cent titanium).

TITANIUM, COLUMBIUM, TUNGSTEN, MOLYBDENUM, AND CHROMIUM STEELS. Three procedures were investigated.

METHOD A. The steel (2.00 grams) was decomposed by hydrochloric acid, and the titanium precipitated by cupferron. After filtering, the residue is expected to contain titanium, columbium, and tungsten. The paper and contents were then treated with nitric (*5*) and perchloric acids, and the beaker was heated to fumes of perchloric acid. After cooling and diluting with water, the insoluble matter was filtered off. Titanium was determined in the filtrate by the peroxide method. The residue was ignited in a platinum crucible and cooled. Then the contents of the crucible were treated with sulfuric and hydrofluoric acids, the former in great excess. The crucible was heated till heavy fumes of fulfuric acid appeared. After being cooled and diluted with water, the crucible contents were transferred to a glass cylinder, and titanium was determined by the peroxide method, without filtering off the insoluble columbium and tungsten.

METHOD B. The steel (2.00 grams) was decomposed by mixed hydrochloric and nitric acids, then heated to fumes with perchloric acid. After cooling, 100 cc. of water were added, chlorine was boiled out, and the insoluble matter was filtered off on paper. Titanium was determined in the residue, as in Method A; and in the filtrate, the solution was evaporated to perchloric acid fumes, the beaker cooled, and the titanium filtered from the chromium and washed with "cold perchloric acid" through a fritted-glass crucible, and then determined by the peroxide method.

METHOD C. In the third method, used merely for comparison purposes, the steel (2.00 grams) was decomposed by mixed hydrochloric and nitric acids, fumed with perchloric acid, and cooled, and the titanium was filtered and washed, in the manner proposed for steel not containing columbium, tungsten, or molybdenum.

Results

Table I shows results with steels of the titanium-chromium-molybdenum type and the (18–8) titanium-chromium-nickel type, using Bureau of Standards No. 121 as the ultimate standard; the titanium was separated by the cupferron method. Comparison of the cupferron method with the proposed method shows that all the titanium may not be recovered with five perchloric acid washings, even though this was accomplished with steel sample 3.

Table II concerns two steels of the titanium-columbium-tungsten-molybdenum-chromium type, and the ultimate standard is Bureau of Standard Steel No. 121.

The figures given in these tables were obtained by a routine analysis of the group. No "corrections" were made by using

TABLE I. DETERMINATION OF TITANIUM

Type of Steel	Titanium, Cupferron Method	Titanium, Proposed Method 1st filtrate	Titanium, Proposed Method 2nd filtrate
	%	%	%
No. 1, 5% Cr, 0.6% Mo, 0.1% C	0.50	0.49	0.02
No. 2, same type	0.48	0.50	0.02
No. 3, 18% Cr, 10% Ni	0.34[a]	0.34	0.0
No. 4, B. of S. No. 121 (0.394% Ti)	0.39[b]	0.37	0.02

[a] Manufacturer, 0.33%.
[b] Used as color standard for cupferron method and for proposed method.

TABLE II. COMPARATIVE RESULTS WITH TITANIUM-COLUMBIUM-TUNGSTEN-MOLYBDENUM-CHROMIUM STEELS

	Titanium Sample 1	Titanium Sample 2
	%	%
Method A		
Ti in cupferron precipitate		
Soluble in dilute perchloric acid	0.38	0.34
Recovered from residue	0.08	0.05
Method B		
Ti soluble in dilute perchloric acid	0.40	0.30
Ti recovered from residue	0.03	0.03
Method C		
Ti, first filtrate	0.38	0.35
Ti, second filtrate	0.02	0.02
Manufacturer's results	0.45	0.35

results of analyses with more exhaustive washings and attentions other than routine.

Discussion

The proposed method for the determination of titanium in steel is of special advantage for analysis of steels of the 5 per cent chromium–0.6 per cent molybdenum type. This alloy dissolves slowly in strong hydrochloric acid, but rapidly in mixed hydrochloric-nitric acids. The test solution is free of color, while with the cupferron method chromium, as carbide, remains with the titanium cupferron precipitate. A disadvantage is the fact that all the titanium may not be contained in the filtrate from the chromium trioxide (0 to 0.02 per cent).

In the case of the (18–8) steels containing titanium, the advantage of speed in solution of the steel is no longer marked. Some nickel will be found in the titanium filtrate, but this error is easily overcome by adding nickel sulfate to the test solutions, or standard, previous to peroxidation.

When the alloying elements columbium, tungsten, molybdenum, chromium, and nickel are also present, difficulties are encountered. When the cupferron separation was used (Method A, Table II) considerable titanium was left in the residues. Method A does not use an oxidizing acid attack. Method B involves an oxidizing acid attack, a perchloric acid attack, and a water extraction. Method C is proposed for titanium-chromium-molybdenum steels, but with a double extraction. Of the three procedures used, the best seems to be Method A, unless the sample will not respond to hydrochloric acid attack. Method B is unusually long, while Method C may require three or more filtrations.

In a columbium steel, the columbium carbide is resistant to attack of aqua regia and fuming perchloric acid (*3*); and if a titanium-columbium steel is similarly treated most of the titanium is to be found in the filtrate (Method B); but if titanium and columbium salts are fumed with perchloric acid (*1*) a separation cannot be made. The apparent deduction is that columbium carbide is an insoluble entity and has no solvent action on titanium (whatever the latter's chemical state may be).

In contrast, tungstic and molybdic oxides can be expected to retain small amounts of titanium. For this reason, one must expect to determine titanium in these alloyed steels by recovering the titanium in a soluble and in an insoluble portion of the sample, as indicated in Table II.

Proposed Procedure for Titanium in Chromium-Molybdenum-Nickel Steels

Transfer 1 gram of steel to a 150-cc. beaker, add 15 cc. of mixed acids (300 cc. of hydrochloric acid, 100 cc. of nitric acid, 400 cc. of water), and warm to complete solution. Add 30 cc. of 70 to 72 per cent (technical) perchloric acid, heat till red chromium trioxide appears, and several minutes more, and cool.

Prepare a wash solution by heating perchloric acid in a small beaker until heavy fumes appear. Cool.

Use a bell-jar type suction or a filtering desiccator apparatus. Filter through a Gooch crucible with an asbestos pad, or better, a Pyrex M filtering crucible. Wash with five or six 5-cc. portions of wash solution, using a small glass rod to agitate the precipitate. Catch the filtrate and washings in a 150-cc. beaker.

Evaporate the filtrate to 10 to 15 cc., cool, add 25 cc. of water, and boil out chlorine. Dilute with 5 per cent sulfuric acid solution. Add 5 cc. of 3 per cent peroxide, and compare with a standard titanium solution.

Standard. Bureau of Standards No. 121 (0.394 per cent titanium) may be prepared as above, except that the insoluble chromium trioxide residue is redissolved in water, fumed in perchloric acid, and cooled, and the washings are added to the first filtrate. Dilute to 197 cc. with 5 per cent sulfuric acid.

Vanadium, if present, accompanies titanium. Decolorize the yellow titanium solution with hydrofluoric acid. A residual brown color indicates vanadium.

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Removal of Adsorbents from Chromatographic Tubes

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THE contents of a developed chromatographic tube are usually removed by laying the tube horizontally on a piece of paper and extruding the contents from the top of the tube by pressing the bottom of the tube with a wooden stick or the like (*1*, *2*). The method, however, involves considerable skill, since the material in the tube must be neither too dry nor too wet to slip out easily.

The following method has been found particularly effective and has reduced the breakage of chromatographic tubes to a negligible point in undergraduate courses.

After development of the chromatogram the solvent is allowed just to disappear from above the surface of the tube and suction is discontinued. The tube is placed horizontally on a table with paper to receive the contents of the tube. A rubber stopper of a size to fit the bottom of the chromatographic tube is attached to a compressed gas line and by means of this stopper gas pressure is applied gently and periodically to the bottom of the tube. The flow of gas through the tube not only presses against the solid contents of the tube but also dries the adsorbent to such a state that the contents slip out easily.

The pressure must be regulated either by a valve in the gas line or by placing the rubber stopper sideways in the bottom of the tube thus permitting part of the gas to leak through the space between the stopper and the tube. There is first a period of drying and packing the adsorbent, which normally requires greater pressure than the second period of extrusion. Consequently the pressure is applied intermittently for short periods, noting the results of each successive application. The column will finally start to move, often in segments. The pressure and the time of application must be decreased and the process is continued until the contents are pressed out of the tube. If the pressure is not controlled either by a valve or by periodic application of the rubber stopper for short times to the bottom of the tube, the adsorbent will shoot out of the tube across the laboratory. If the adsorbed material is sensitive to oxygen, the gas may be oxygen-free nitrogen or carbon dioxide.

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A Rapid X-Ray Diffraction Method

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SINCE the inception of the powder method of x-ray photography in 1916 (*6*, *7*), numerous techniques have been devised to improve the photographic pattern, shorten the exposure time, and simplify the method of preparing the specimen.

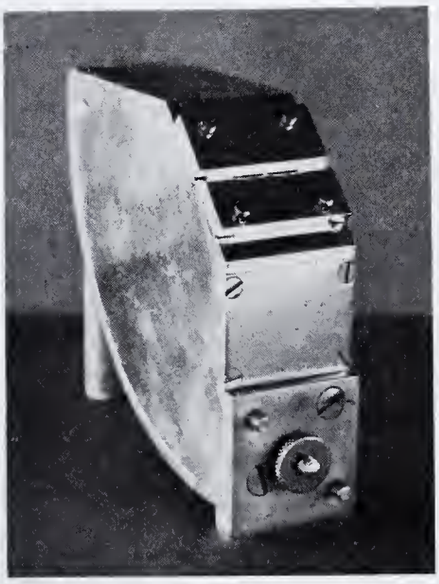


FIGURE 1. CAMERA

The photographic pattern is a compromise between the intensities of the general radiation which produces only background and the particular radiation which produces the diffraction pattern. A technique which will reduce the background intensity without materially reducing the diffraction pattern intensity is desirable, in that faint diffraction lines may then be more easily distinguished.

Only a small percentage of the incident x-ray beam is diffracted to produce the photographic pattern, so that long exposures are frequently necessary to obtain sufficient blackening of the film to overcome the general background and develop sufficient contrast to define readable lines.

The earlier methods involve packing the powder in a capillary of very thin-walled, lead-free glass, with an inside diameter of 0.6 mm., the sample being packed into a length of about 1 cm. This filling of capillaries is tedious and difficult and the glass always produces some background. The time of exposure is usually many hours, the time increasing with the coefficient of absorption of the sample. Davey (*5*) overcame the last difficulty by mixing the sample with an inert material such as starch, gum tragacanth, or flour, the proportions of sample and inert depending on the coefficient of absorption of the sample, but dilution also reduces the intensity of the diffraction pattern. Morse (*8*) built a mold to produce rods 0.6 mm. in diameter. The specimens are mounted vertically in the camera, and are fragile. These rods are completely bathed in the x-ray beam and produce very wide lines.

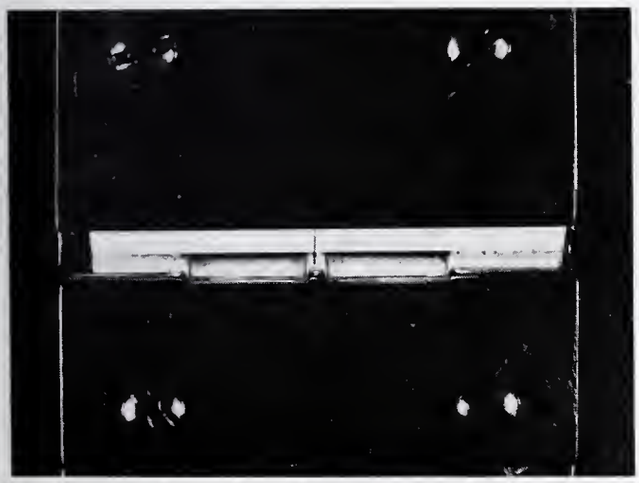


FIGURE 2. SLIT ARRANGEMENT

In another method a thread or filament is moistened with adhesive and dipped into the sample. The quantity of sample used is small and the exposure time necessarily long.

To obtain a greater amount of reflection such capillary specimens, rods, and threads are rotated or oscillated.

St. John (*9*) applied Seemann's slit principle to powder specimens and greatly increased the exposure time. In this method the coefficient of absorption of the sample is a major factor in exposure time considerations and St. John mixed his samples with inerts to correct for variations in absorption in different samples. The patterns are never very clear and the background is heavy.

Seemann and several other workers (*1–3*, *10*) dusted or cemented powder on plane surfaces and by fitting these surfaces to the curve of a camera evolved the focusing type of camera. It is very difficult to obtain a uniform distribution of powder on such a surface, and very fine entrance slits are required in the camera, thus increasing the exposure time. The definition, line width, and dispersion vary along the length of the film. More recent methods involve the use of a solidly packed specimen, usually in the form of a wedge or block, with or without diluent.

New Technique

In the new technique, specimens are produced in the form of pressed rods 15 to 20 mm. long, 3 mm. in diameter. The powder sample is mixed on a glass plate with a small amount of binder, glyceryl phthalate dissolved in mineral naphtha, until a mass is obtained that appears dry but will pack when pressed with a spatula. About 0.2 to 1.0 gram of sample and 2 to 4 drops of binder are generally used. By tapping a glass tube of 3-mm. inside diameter into the mixture of sample and binder, about 30 mm. of mixture may be forced into the tube. The tube is held vertically on a glass plate and an iron rod, which fits the tube snugly, is pressed down on the sample to compress it to about 15 to 20 mm. The rod is then used as a plunger to extrude the specimen. Glyceryl phthalate was chosen as the binding agent because it produces no appreciable fogging from x-rays, it does not wet glass when used in the manner described, and may be baked at 100° C. to a hard resin. When possible without detriment to the material, the rods are baked for 1 hour at 100° C. Otherwise, they may be air-dried, or if carefully handled, used immediately. It has been demonstrated that the closer the rods approach perfect cylinders, the clearer will be the x-ray pattern. With a little practice specimen rods can be produced at the rate of about 16 per hour.

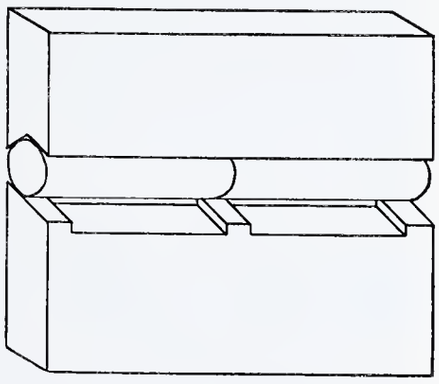
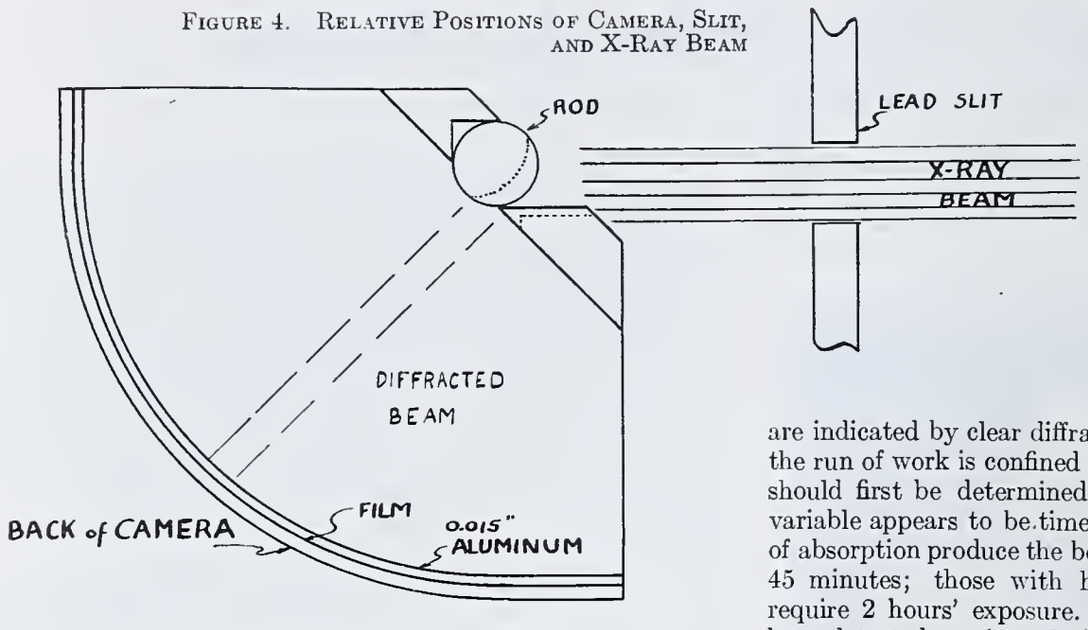


FIGURE 3. SLIT

The rods are mounted on the camera to form one side of an 0.375-mm. (0.015-inch) slit, the other side being brass. The camera is shown in Figure 1. A close-up of the slit arrangement is shown in Figure 2, and a drawing of the slit in Figure 3. The relative positions of camera, slit, and x-ray beam are shown in Figure 4. The x-ray beam is limited by two slits; the first is of lead, and is 0.375 mm. (0.015-inch) wide, and the second is formed by the specimen rod and a piece of brass. Adjustment is made so that the beam impinges on the rod, and a very small amount passes, free, through the slit. The brass block serves a multiple purpose. It is an essential support for the specimen, it is used to limit the width of the primary beam and as a consequence the width of the primary beam imprints on the film, and it effectively reduces the scattered radiation which would normally fall on the film. When used in the manner described, no brass diffraction lines have been recorded during the photographing of several hundred samples. For samples which are opaque to

FIGURE 4. RELATIVE POSITIONS OF CAMERA, SLIT, AND X-RAY BEAM



x-rays only a thin surface of the rod produces the diffraction pattern, but where the coefficient of absorption is small virtually all of the specimen exposed to the rays takes part in producing the pattern.

Brill and Pelzer (*4*) suggested the use of hollow cylindrical rods of sample in their work on particle size determination. Such a specimen represented a rod with a minimum of absorption in the specimen. They also used a rod of lead glass coated with the powder sample to obtain a rod possessing complete absorption within the specimen. However, they did not use such rods as part of the defining slit in their camera but placed them so that they were completely bathed in the x-ray beam. In their technique, double lines are formed from the two sides of the specimen rod.

By the present technique a very considerable portion of the material inside the rod may take part in producing the diffracted pattern, and only when the absorption of x-rays is considerable does the immediate surface of the specimen become the only source of diffraction. Because of the method of mounting the specimen rod, only one side of the rod is used to diffract the beam, so that single lines are always obtained except where resolution of the α_1 and α_2 wave lengths occurs.

Patterns are obtained with a 45-minute exposure at 32-kv. peak or in 10 minutes at 42-kv. peak, using 20-milliampere tube current. A General Electric Ca-2 tube is used. The molybdenum K α radiation is filtered through zirconium dioxide and the photographic film is covered with a strip of pure aluminum 0.375 mm. (0.015 inch) thick.

The cameras are built to hold two rod samples, so that two separate diffraction patterns may be obtained on the same film. With this arrangement comparisons with standards are easily made. With molybdenum K α radiation, this method is superior to that of Wyckoff (*13*).

Because a large amount of sample (0.1 to 0.6 gram) is used to produce the diffraction pattern, a more nearly representative picture is obtained without rotation or oscillation of the specimen, and satisfactory for quantitative determination. When widely diversified materials are being examined, it is only necessary to obtain one exposure, since samples possessing such widely varying coefficients of absorption as metallic lead, iron, zinc oxide, magnesium oxide, titanium oxides, and metallic aluminum all produce very readable patterns in 1 hour.

While a standard 45-minute exposure at 20 milliamperes and 32-kv. peak is suitable for identification, for any particular material there is an optimum set of conditions for securing the clearest photographs, wherein small percentages of one or more components are indicated by clear diffraction lines. Consequently, when the run of work is confined to similar materials, the optimum should first be determined by trial. The most important variable appears to be time. Materials with low coefficients of absorption produce the best patterns in something less than 45 minutes; those with high absorption coefficients may require 2 hours' exposure. One per cent of zinc oxide has been detected consistently in titanium dioxide and 2 per cent of anatase titanium dioxide has been distinguished in rutile.

Certain hydrous metallic oxides which have been termed amorphous from previous x-ray study produce definite patterns when examined by this technique and are truly crystalline. For example, hydrous titanium oxide precipitated from sulfuric acid solution at 25° C. with ammonia has previously been considered amorphous to x-rays (*11*) while electron diffraction patterns of a similar hydrate show distinct crystallinity (*12*), not corresponding to rutile or anatase. By the x-ray method described herein, however, such precipitates exhibit a clearly defined band pattern which apparently corresponds fairly well with the electron diffraction pattern of Weiser and Milligan (*12*).

Using the Seemann slit method it was observed that with a particular instrument using 32-kv. peak, K α molybdenum radiation, zirconium dioxide filter, and 20-milliampere filament current, plates of most pigmentary materials 2 to 3 mm. thick were able to stop enough radiation that no diffraction pattern was obtained, although the main beam penetrated the sample.

Lambert's absorption law is described in the equation

$$I = I_0 e^{-kx} \qquad (1)$$

where I_0 = initial intensity of x-ray beam
I = intensity after traversing a thickness x of a material
k = absorption coefficient of the material

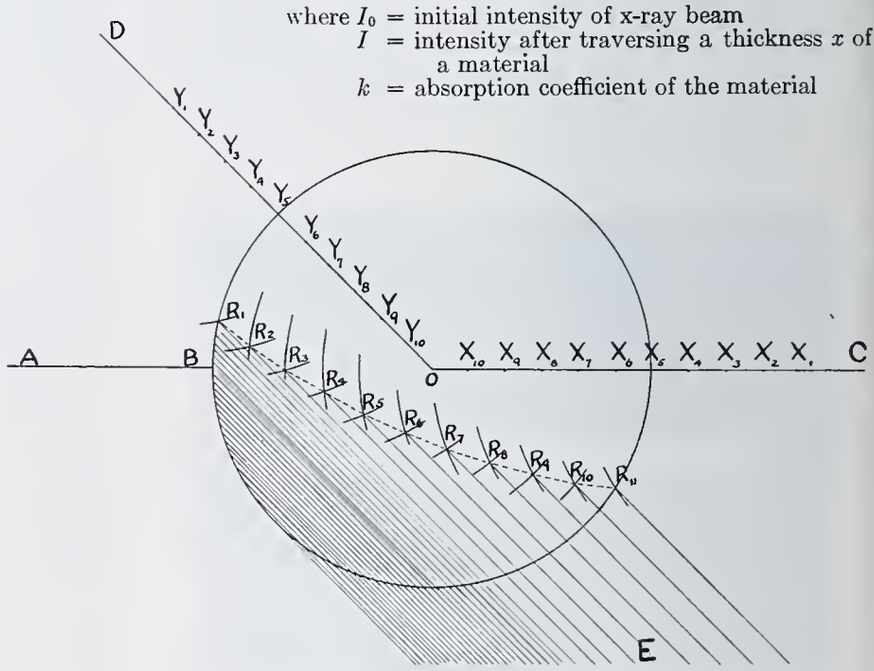


FIGURE 5. SECTION OF ROD

If a beam penetrates a sample to a depth x, then

$$I = I_0 e^{-kx} \quad (2)$$

At point x part of the beam may be diffracted, if the ratio of the intensity of the diffracted to incident beam is p, then the intensity of the diffracted beam $I_d = (pI_0e^{-kx})\ e^{-ky}$

$$I_d = (pI_0e)^{-k(x+y)} \quad (3)$$

where y is the distance from point of diffraction to surface of solid in the direction of diffracted ray.

Equation 3 may be interpreted to mean that the intensity of a diffracted beam at any point in a substance is a function of the sum of the distances traversed by the incident and diffracted beam but independent of the point at which diffraction occurs in the total path.

Figure 5 represents a section of a rod of sample, 3 mm. in diameter. By supposing that the material of which the rod is composed can completely absorb a diffracted beam when its total path ($x + y$ in Equation 3) is 2.5 cm., following the geometric construction delineates the region of the rod which acts to diffract the x-ray.

Let the incident beam be in the direction AB and the diffracted beam in the direction OE. At O draw a line OC in the direction of the impinging x-ray beam, AB. Mark off a distance $OX_1 = 2.5$ cm. and divide it into a number of equal lengths (10 in this case) at X_1, X_2, X_3, X_4, etc.

Draw a line OD parallel to the direction of the diffracted beam OE, mark off a distance $OY_1 = 2.5$ cm., and divide it into the same number of equal lengths as OX_1. With a radius equal to that of the rod, draw arcs using X_1 and O, X_2 and Y_{10}, X_3 and Y_9, etc., as centers. These pairs of arcs intercept at points R_{11}, R_{10}, R_9, R_8, etc., which may be joined to produce a smooth curve. All the material in the rod below this curve can act to diffract x-rays and produce a resulting beam in direction OE, because, if from any point on line R_1, R_{11}, lines are drawn parallel to the impinging and diffracted x-ray beam and touching the circumference of the circle, the sum of the lengths of the two lines is 2.5 cm., the optimum distance a beam may travel and suffer diffraction in the sample before being completely absorbed.

Figure 6 shows a set of x-ray diffraction patterns taken in the manner described for diverse materials, with 45-minute exposure, using molybdenum K α radiation, filtered through zirconium dioxide at 32-kv. peak and 20-milliampere filament current. The film was shielded by 0.375 mm. (0.015 inch) of aluminum sheet. This set of patterns is included to show that materials such as p-aminobenzoic acid and zinc sulfide, possessing extremes in x-ray absorption and diffraction characteristics, produce good working patterns with equivalent exposure times. Patterns 3 and 4 were obtained on the same film in the same camera at a single exposure. It has been observed that patterns of equivalent sharpness but with increased background are obtained with 10 minutes' exposure at 42-kv. peak and 20-milliampere current, and more intensely contrasting patterns, possibly not so desirable for accurate measurement, are obtained by 90 minutes' exposure to 32-kv. peak and 20-milliampere radiation. The lines of the diffraction pattern produced by this method possess one very clear edge; the other edge is somewhat diffused. This characteristic is typical of the technique and results in faint lines standing out clearly. When photographed against sodium chloride the pattern of an unknown may be measured with great accuracy.

Conclusion

A technique has been described whereby powder samples for x-ray diffraction work may be rapidly prepared. The sample is formed into a rigid rod 3 mm. in diameter and is inserted in the camera to form one side of the defining slit. Under average conditions patterns are obtained which are sufficiently clear to identify crystalline powders, irrespective of their coefficients of absorption. An optimum set of condi-

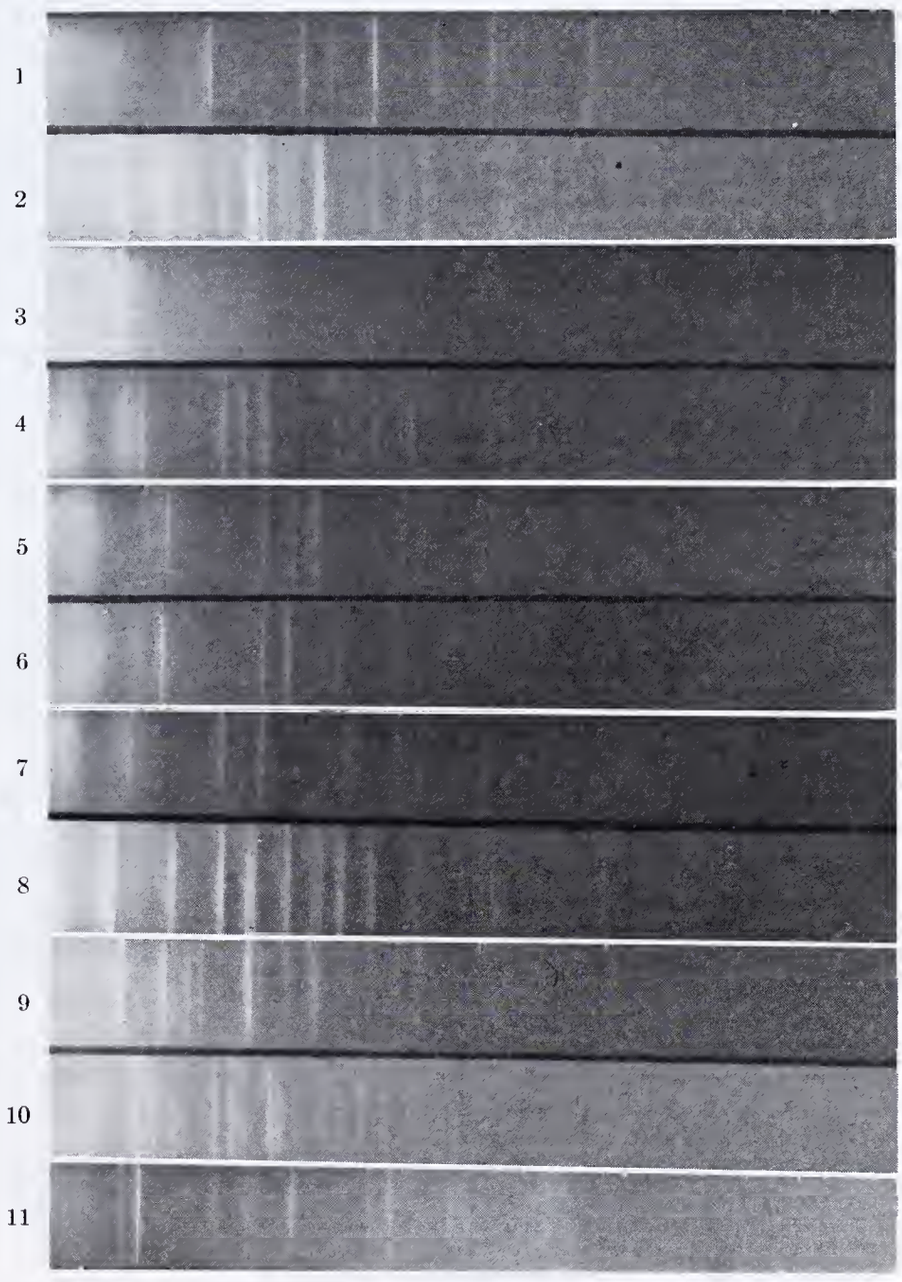


FIGURE 6. X-RAY DIFFRACTION PATTERNS

1. Iron
2. TiO_2-rutile
3. p-Aminobenzoic acid
4. ZnS-wurtzite
5. Zinc oxide
6. Zinc titanate
7. ZnS-sphalerite
8. TiO_2-anatase
9. TiO_2-rutile
10. Cadmium titanate
11. Lithium plumbite

tions may be defined for any mixture of two or more substances, so that analyses may be made with improved accuracy.

Acknowledgment

The author wishes to acknowledge the help and work of P. F. Kerr, professor of geology, Columbia University, in the development of this technique.

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Electrical Ignition of the Spectrographic Arc

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IN APPLYING the Hasler and Harvey (*1*) method of a high-streaming-velocity arc to the analysis of brasses the authors experienced considerable difficulty in obtaining reproducible exposures free of objectionable background. In this method the sample is completely consumed in a direct current arc in less than 10 seconds, so that exposures of 8 seconds were used. With such short periods, the time for manually striking the arc represented an appreciable fraction of the exposure time and the incandescent electrode ends produced background that varied with the rapidity with which the operator succeeded in separating the electrodes. Below is described a circuit which enables one to strike an arc instantaneously and with the electrodes separated at the working distance. The system operates so satisfactorily that in this laboratory it is used as the sole means of striking the direct current arc. It also serves to maintain the arc with difficultly burning materials in the electrodes.

In this scheme a high-potential, high-frequency voltage of low energy is superimposed upon the direct current supply, so that the gap is readily bridged. Once bridged, the arc is maintained by the direct current supply. Pfeilsticker (*2*) has described an interrupted arc in which this type of ignition is applied.

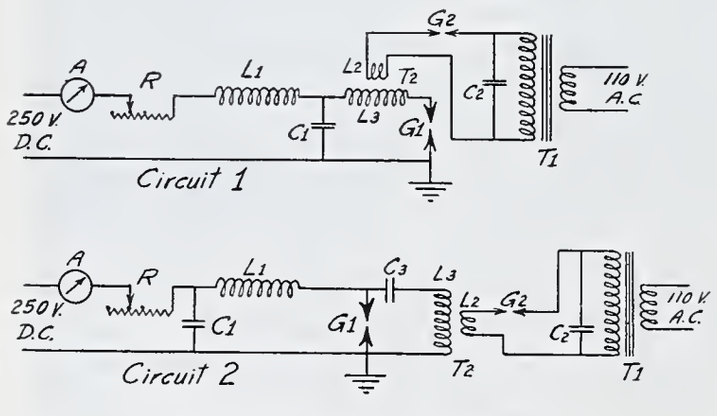

FIGURE 1. CIRCUIT DIAGRAMS

A. Direct current ammeter, 0 to 15 amperes
R. Resistor, 17.5, 35, and 70 ohms
L_1. Filter choke. 116 turns, 12.0-cm. (4.75-inch) diameter, 12 turns per inch, 16-gage bare copper wire on threaded, insulated form
C_1. 0.03-mfd., made from window glass and copper foil, immersed in oil; 39 electrodes, each 15 × 18.75 cm. (6 × 7.5 inches)
C_2. 0.002 mfd. Cornell-Dublier mica capacitor, 12,500 volts direct current maximum
C_3. Same as C_2
G_1. Analytical gap
G_2. Auxiliary gap, 0.63-cm. (0.25-inch) diameter brass rod, 1.56-mm. (0.0625-inch) gap
T_1. 110/8000 volts, 250-volt, 60-cycle Thordarson transformer
T_2. Air core transformer, Barker and Williamson type 80-T1 (L_2 2 turns, L_3 26 turns)

Either circuit 1 or 2 in Figure 1 performs satisfactorily. Circuit 1 is used here because it requires one less circuit element.

CIRCUITS. In circuit 1, C_2, L_2, G_2 is the conventional spark high-frequency generator. C_2 is charged by T_1 to the breakdown potential of gap G_2 and then discharges through L_2, followed by an oscillatory discharge, the frequency of which is determined by C_2 and L_2. Depending upon the separation of G_2, the breakdown of this gap may occur one or more times in each alternation of the 60-cycle supply. During the oscillatory discharge a voltage is induced in L_3 which is equal to that across L_2 multiplied by the step-up ratio of T_2. This voltage appears across the analytical gap, G_1, because the impedance of C_1 to the high-frequency current is very small: G_1 is thus broken down. Inductor L_1 serves to isolate the high-frequency energy from the direct current line since the impedance of L_1 is large at the frequencies used.

The operation of circuit 2 is similar in the essential details.

In the authors' arrangement, the natural frequency of the C_2L_2 circuit is about 4 megacycles. At this frequency the reactance of C_1 is 1.3 ohm while that of L_1 is about 16,000 ohms. The step-up ratio of T_2 is 13.

Resistor R is the customary series resistor to limit the arc current.

The filter choke, L_1, is located at some distance from T_2 and is positioned so that L_1 and T_2 are not in inductive relation.

Since the frequency of the ignitor circuit is high, it is important to keep all leads, including those to the arc stand, as short as possible.

The energy in the spark produced across G_1 is small and is negligible when compared with the energy dissipated in the arc from the direct current line. All spectra produced have been arc spectra.

This ignitor will strike the 250-volt direct current arc with pointed or flat-ended carbon electrodes up to 9.38-mm. (0.375-inch) diameter (the largest available here) at 5-mm. separation with any of the three values of series resistor R. Larger gaps can be ignited if the limiting resistor is set to lower values (a 10-mm. gap will be ignited if R is reduced to 17.5 ohms).

Iron arcs are ignited with more difficulty. However, the authors have found it convenient to produce a reference iron spectrum by using an iron and a carbon electrode (upper electrode carbon, negative; lower electrode iron, positive). This combination is ignited as readily as a carbon arc.

Copper arcs are intermediate in ease of ignition—e. g., a 5-mm. gap will be ignited if R is either 17.5 or 35 ohms.

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Gravimetric Determination of Aluminum in Magnesium Alloys

Benzoate-Oxine Method

V. A. STENGER, W. R. KRAMER, AND A. W. BESHGETOOR
The Dow Chemical Company, Midland, Mich.

RAPID routine analyses of magnesium alloys for aluminum are generally carried out by potentiometric (*1*) or spectrographic (*5*) methods. There is a need, however, for a more accurate procedure suited to referee work and other special purposes. The difficulties encountered in separating aluminum from much magnesium and also from zinc are well known (*3*). In the usual hydroxide precipitation method several precipitations must be made, and care is required to avoid errors in weighing the hygroscopic aluminum oxide. In addition, removal of or correction for silica is necessary in many cases.

The 8-hydroxyquinoline (oxine) reagent (*2*) is useful for determining aluminum in magnesium alloys low in manganese, zinc, and other interfering elements, but since most commercial alloys contain one or more of these, preliminary separations are required. To precipitate the aluminum as oxyquinolate from ammoniacal tartrate solution, which in the authors' experience yields somewhat more precise results than precipitating from an acetic acid–acetate buffer, involves also the prior removal of magnesium. Addition of ammonium benzoate to a weak acid solution of the metals has been shown (*4*) to give a virtually complete separation of aluminum from most of the divalent elements, in one step. The benzoate precipitate is readily soluble in warm ammoniacal tartrate solution, from which aluminum may be precipitated with oxine. This combination of methods has been in use in laboratories of The Dow Chemical Company for nearly 5 years. In view of the present great practical importance of the magnesium alloys, and since the procedure is currently being considered for adoption as a tentative standard method of the American Society for Testing Materials, it seems advisable that a description, together with results on known mixtures, should be published at this time.

Reagents

Ammonium benzoate solution. Dissolve 100 grams of pure ammonium benzoate (preferably recrystallized) in 1 liter of warm water and add 1 mg. of thymol as a preservative.

Benzoate wash solution. To 100 ml. of the ammonium benzoate solution add 900 ml. of warm water and 20 ml. of glacial acetic acid.

Oxine solution. Dissolve 50 grams of 8-hydroxyquinoline in 1 liter of water containing 120 ml. of glacial acetic acid. Filter if necessary and keep in a dark bottle.

Ammoniacal tartrate solution. Dissolve 30 grams of ammonium tartrate in 1 liter of water containing 120 ml. of concentrated ammonium hydroxide.

Ammonium hydroxide, 1 to 1.

Hydrochloric acid, C. P., concentrated.

Acetic acid, glacial.

The magnesium and aluminum chloride solutions used in testing the method were prepared from weighed amounts of the pure metals dissolved in hydrochloric acid and made to known volume. Other elements were added as solutions of the C. P. chlorides.

Procedure

Weigh out a sample calculated to contain 0.2 to 0.5 gram of aluminum, place in a 250-ml. beaker with 25 ml. of water, and dissolve by adding in small portions a total of 10 ml. of hydrochloric acid for each gram of sample. When dissolved, cool to room temperature and dilute to 500 ml. in a volumetric flask. Any residue of copper or carbon should be allowed to settle out, but undissolved silica, which might contain some occluded aluminum, should be kept in suspension. Pipet a 50-ml. aliquot into a 400-ml. beaker and dilute with 50 ml. of water.

Add 1 to 1 ammonium hydroxide drop by drop with stirring until the precipitate which forms as each drop strikes finally redissolves only very slowly—that is, until nearly all the free hydrochloric acid is neutralized without permanent precipitation of aluminum hydroxide. Add 1 ml. of glacial acetic acid, about 1 gram of ammonium chloride, and 20 ml. of ammonium benzoate solution. Heat to boiling while stirring well, keep at gentle boiling for 5 minutes, then filter with the aid of suction on a previously weighed glass crucible, Gooch type, with fritted disk of fine porosity. Wash eight to ten times with hot benzoate wash solution, making no effort to transfer all precipitate to the crucible. Return the precipitate to the beaker, transferring most of it with the aid of the stirring rod and washing the remainder back with water. Dissolve a small part remaining in the crucible by washing with five 10-ml. portions of hot ammoniacal tartrate solution and combine with the precipitate in the beaker. Heat to 70° to 90° C., add 25 ml. of oxine solution, and digest for 30 minutes without boiling. Filter on the same crucible and wash eight times with water, transferring all precipitate. Dry for 1.5 to 2 hours at 120° to 130° C., cool, and weigh as $Al(C_9H_6ON)_3$.

$$\% \text{ Al} = \frac{Al(C_9H_6ON)_3 \times 0.05873 \times 100}{\text{grams of sample in aliquot}}$$

Tests on Known Solutions

The magnesium alloys to which this method applies ordinarily contain more than 88 per cent magnesium, up to 10 per cent aluminum, up to 3 per cent zinc, and 0.1 to 0.3 per cent manganese. There are also individual alloys which contain 1.5 per cent manganese in one case, 3.5 per cent cadmium in another, and 0.5 per cent silicon in a third. Generally copper and iron will be below 0.04 per cent, as will silicon also except in the case just noted. Special treatment would be necessary for certain less common alloys high in copper, tin, or silver. The effects of some of these elements

TABLE I. ANALYSIS OF KNOWN MIXTURES

Aluminum Taken *Mg.*	Additions *Mg.*	Aluminum Found *Mg.*	Error %
25.00	None	24.92	−0.32
25.00	None	24.98	−0.08
50.00	None	50.08	+0.16
10.00	500 Mg	9.99	−0.10
25.00	500 Mg	25.05	+0.20
50.00	500 Mg	49.86	−0.28
25.00	25 Zn	25.09	+0.36
10.00	500 Mg, 10 Zn	9.96	−0.40
10.00	500 Mg, 10 Zn	10.01	+0.10
25.00	500 Mg, 10 Zn	24.96	−0.16
25.00	500 Mg, 10 Zn	25.04	+0.16
10.00	500 Mg, 25 Zn	10.02	+0.20
10.00	500 Mg, 25 Zn	10.06	+0.60
25.00	500 Mg, 25 Zn	24.93	−0.28
25.00	500 Mg, 25 Zn	25.01	+0.04
25.00	500 Mg, 25 Mn	24.93	−0.28
25.00	500 Mg, 25 Mn, 25 Zn	24.96	−0.16
25.00	500 Mg, 2 Mn, 15 Zn	24.92	−0.32
50.00	500 Mg, 1 Mn, 10 Zn	49.92	−0.16
25.00	500 Mg, 25 Cd	24.88	−0.48
50.00	500 Mg, 10 Cu	50.36	+0.72
50.00	25 Cu	50.60	+1.2
25.00	500 Mg, 25 Sn	25.25	+1.0
25.00	500 Mg, 25 Sn	25.30	+1.2
25.00	500 Mg, 1 Fe	25.19	+0.76
25.00	500 Mg, 1 Fe	25.24	+0.96

on the determination of aluminum have been tested, with results as shown in Table I.

In general the deviations fall within acceptable limits except in the presence of considerable copper, tin, or iron, which is not likely to be encountered. Copper would largely remain insoluble in hydrochloric acid, the amount going into solution being too small to cause serious interference. Tin is preferably precipitated with hydrogen sulfide, the filtrate then being boiled and cooled before neutralization and precipitation with benzoate. The small amounts of iron usually present as an impurity have negligible influence on the aluminum determination. Larger quantities present as the result of some special condition may be precipitated by passing hydrogen sulfide through the ammoniacal tartrate solution of the benzoate precipitate. The filtrate is then warmed and treated at once with oxine reagent, without removal of excess sulfide. Enough ammonia should be present to keep any free sulfur in solution as polysulfide, yet an excess should be avoided as it may cause precipitation of the oxine.

Behavior of Silicon

Since one of the advantages of the method is that silicon does not have to be removed, it is of interest to consider what happens to silicon in the analysis. The alloy having the highest content of silicon, 0.5 per cent, contains 10 per cent aluminum. Even if all of the silicon stayed with the aluminum and were weighed as silicon dioxide, the ratio of silicon dioxide to the total weight of the precipitate would amount to only 0.0063 because of the large molecular weight of aluminum oxyquinolate. This would correspond to an error percentage of 0.63 based on the aluminum; in other words, 10.063 per cent aluminum might be reported instead of 10.00 per cent.

In practice, up to about 0.15 per cent silicon dissolves in the dilute hydrochloric acid. This soluble silica does not interfere. Known solutions of aluminum and magnesium chlorides have been analyzed with and without the addition of small amounts of sodium silicate, no significant differences being observed in the results. The remainder of the silicon evidently dissolves but reprecipitates as a flocculent hydrous oxide. This again can partly dissolve when the benzoate precipitate is taken up in ammoniacal tartrate. The final percentage of error on the aluminum can thus drop to about 0.3, which is not too serious even in this unfavorable case. Since the majority of the alloys contain much less silicon, the error generally introduced in the aluminum figure is negligible even for referee purposes.

Summary

A fairly rapid gravimetric procedure, suitable for referee analyses, is presented for the determination of aluminum in magnesium-base alloys. Aluminum is precipitated with ammonium benzoate, dissolved in ammoniacal tartrate solution, and reprecipitated with 8-hydroxyquinoline. None of the common alloy constituents interferes, and no separation of silica is necessary.

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Studies on the Carotenoids

Spectrophotometric Determination of the Carotenoids of Yellow Corn Grain

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In 1937, Clark and Gring (*4*) presented a method for determining the carotenoids of corn [*Zea Mays L.*] but did not separate the carotene from cryptoxanthol. Consideration of cryptoxanthol in analytical methods for corn pigments is important because of its provitamin A activity. These workers reported the concentration of "xanthophyll" in several corn varieties, but the standard absorption coefficients which they used for this determination were much lower than those reported by others, leading to erroneous results for "xanthophyll" concentration. Later, Buxton (*2*) described a method for the determination of "carotene and/or cryptoxanthin" in yellow corn. He used adsorption on calcium carbonate for separation of carotene from cryptoxanthol in his final solution. Both workers used 90 per cent methanol for the separation of zeaxanthol from the provitamin pigments. Fraps and Kemmerer (*5*) recently reported an adsorption method for the determination of the pigments of corn. They separated the pigments epiphasic to 90 per cent methanol into five compounds: beta-carotene, alpha-carotene, K carotene, cryptoxanthol, and neocryptoxanthol. An abridged method for routine determination was developed in which the pigments were divided into two groups, carotenes and cryptoxanthol plus neocryptoxanthol, by adsorption on magnesium carbonate. Calculation of the provitamin A potency of these two groups was based on their average composition as found in the preliminary study. This method is probably the best of the three, though losses from incomplete recovery of adsorbed pigments must not be overlooked. It is also necessary to test and standardize each new lot of adsorbent.

White, Zscheile, and Brunson (*11*) demonstrated the presence of luteol and gamma-carotene in corn, and noted the occurrence of unnamed carotene 1 which may be identical with the K carotene reported by Fraps and Kemmerer (*5*). In this paper it is shown that large amounts of neo isomers are present in corn extracts and must be considered in analysis. The solvents suggested in a previous paper (*10*) for the separation of the pigments into three groups—carotene, cryptoxanthol, and carotenol—have been applied to the separation of corn-grain pigments. Where possible, account is taken of the presence of pigments other than beta-carotene, cryptoxanthol, and zeaxanthol.

Experimental

For immature corn grain, the sample (20 to 40 grams, depending on water content) was extracted 5 minutes in a Waring

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Blendor with 150 to 200 ml. of acetone. Mature corn grain was ground in a hammer mill and 15 to 20 grams of the meal were tempered with about 6 ml. of water for 5 minutes and extracted in a Soxhlet extractor with acetone until all visible color was removed. One volume of ether was added to the acetone solution from either extraction and the pigment was transferred to ether by cautious addition of 3 volumes of water. The ether solution was washed cautiously five times with water and saponified by refluxing for 0.5 hour with 10 ml. of concentrated ethanolic potassium hydroxide. The alkali and soaps were washed from the ether solution and it was evaporated on a steam bath with a boiling stick in the beaker. The beaker was removed from the steam bath just before it became dry.

The residue was dissolved in a mixture of hexane and 78.5 per cent diacetone alcohol (100 parts of diacetone alcohol to 28 parts of water) and transferred to a separatory funnel. The volumes of the two phases were adjusted to approximately 25 ml. and after vigorous shaking the diacetone alcohol phase was removed. The hexane was extracted twice with fresh diacetone alcohol solution. The diacetone alcohol solutions were combined and extracted with about 15 ml. of hexane, which was in turn extracted with an equal volume of diacetone alcohol solution. An equal volume of ether was added to the combined diacetone alcohol extracts, the solution was shaken, and 2.5 to 3 volumes of water were added to force the pigments into the ether phase. The combined hexane extracts and the ether solution were each washed five times with water. The ether was evaporated on the steam bath with precautions noted above and the residue (carotenols except cryptoxanthol) dissolved in ethanol for spectrophotometric measurement.

The hexane solution was added to 25 ml. of 91.8 per cent (100 to 9) 2-methyl-2,4-pentanediol in a dry separatory funnel and extracted three times with that solvent. About 15 ml. of hexane were added to the combined extracts and the cryptoxanthol was transferred to hexane by the addition of 300 ml. of water. Occasionally a second extraction was necessary. The original hexane solution and the cryptoxanthol solution were each washed five times with water and made to volume for spectrophotometric measurement. It was not necessary to dry the solutions for clarification.

Absorption spectra of these three solutions showed the type of "degradation" from the curves of the pure pigments that is caused by the presence of neo-type pigments (*1*). [Absorption spectra were determined on an improved photoelectric spectrophotometer, previously employed in pigment studies (*1*, *12*, *13*), of the type described by Hogness *et al.* (*6*).] Chromatography on magnesium oxide–Supercel did not separate the neo pigments from their isomers. Absorption spectra of the pigments from single zones indicated the presence of neo isomers.

Carotene Fraction. In addition to the presence of neo-beta-carotene, the spectra of the carotene fractions showed the presence of unnamed carotene 1, as noted previously (*11*). Attempts were made to analyze the carotene fraction as a beta-carotene–neo-beta-carotene system as outlined by Beadle and Zscheile (*1*). Agreement between wave lengths 4780 and 4850 Å. was poor, owing to the presence of other colored compounds (compare values of R_2 in Table I with those of Table III).

Table I. Ratio Values for Certain Carotenoids

Pigment	$R_1 = \frac{\text{log at 4350 Å.}}{\text{log at 4250 Å.}}$	$R_2 = \frac{\text{log at 4950 Å.}}{\text{log at 5000 Å.}}$
β-Carotene	1.08	1.73
Neo-β-carotene	1.16	1.77
Unnamed carotene 1	0.35	..
γ-Carotene	0.98	0.83
33% neo–67% β-carotene	1.10	1.74

Table II. Analytical Constants for Carotenols

Wave Length Å.	Specific Absorption Coefficients in Ethanol Solution: Zeaxanthol	Luteol	Neozeaxanthol II
	Liters per gram cm.		
4275	*ca.* 172	*ca.* 172	*ca.* 172
4500	247	247	174
4783	219	219	103
4850	203	152	59.5
4900	166	98.3	35.5
4950	119	53.7	23.0

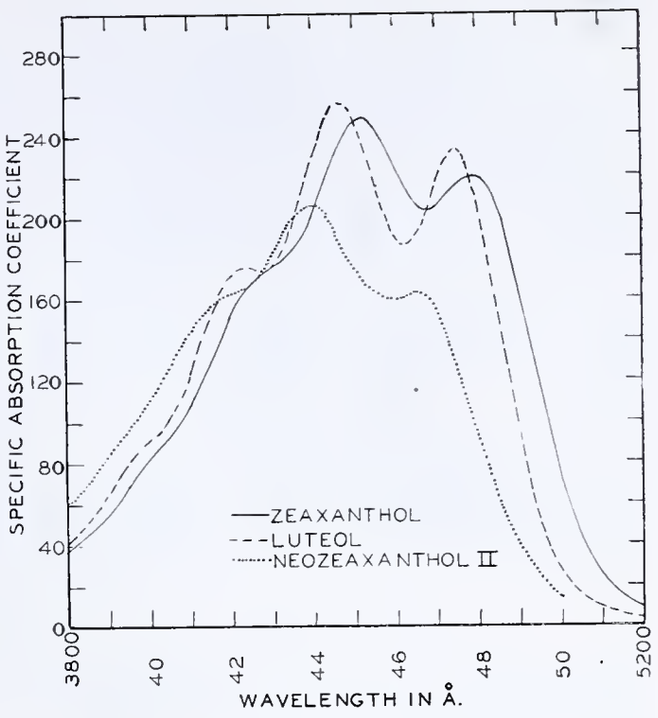

Figure 1. Absorption Spectra of Carotenols in Ethanol

Accurate analyses for unnamed carotene 1 and for gamma-carotene are not possible because quantitative absorption spectra for these components are not yet available. As an indication of the presence of these compounds in corn-grain extracts, the ratios of $\log \frac{I_0}{I}$ values at 4350 Å. to those at 4250 Å. were determined for the carotene fractions from corn, as well as the ratios of $\log \frac{I_0}{I}$ values at 4950 Å. to those at 5000 Å. Table I shows these ratios for certain pure pigments for comparison with the values for corn extracts reported in Table III.

Cryptoxanthol Fraction. The cryptoxanthol fraction was treated as a mixture of cryptoxanthol and neocryptoxanthol for analysis, using the absorption spectra previously presented (*11*, *13*).

The composition of these extracts in terms of percentage neocryptoxanthol varied from 28 to 90 per cent. The average values are considerably higher than those present in equilibrium mixtures of pure cryptoxanthol and neocryptoxanthol. Since agreement between wave lengths 4500 to 4850 Å. and 4500 to 4900 Å. was only fair, other compounds probably interfered with this analysis (*11*).

Carotenol Fraction. The carotenol fraction from a corn-grain extract was chromatographed on magnesium oxide–Supercel. The zeaxanthol zone was eluted and the pigment adsorbed on calcium hydroxide–Supercel, resulting in 3 zones, similar in appearance to those resulting from the isomerization of zeaxanthol (*11*). Analysis of the carotenol fraction as a mixture of zeaxanthol, luteol, and neozeaxanthols I and II (*11*), ignoring the presence of neoluteols, is impractical because the curves for luteol and neozeaxanthol I are so close together.

Therefore, the carotenol fraction was analyzed as a mixture of zeaxanthol, luteol, and neozeaxanthol II by the procedure given below. Figure 1 shows the spectra (*11*, *13*) used as standards for the analysis and Table II presents the analytical constants at selected wave lengths.

Step 1. At wave lengths 4500 and 4783 Å. the curves for zeaxanthol and luteol intersect and are considerably higher than the curve for neozeaxanthol II. The method of simultaneous

Table III. Carotenoids of Corn Grain

Age	Water Content	Total[a] Carotenes	Total[b] Cryptoxanthols	Zeaxanthol	Neozeaxanthol	Luteol	Ratio R_1	Ratio R_2
Days after pollination	%	*Micrograms per gram dry weight*						
Inbred K-156								
32	55.2	2.48	2.25	0.53	8.40	12.8	1.00	1.73
Mature	9.9	2.30	1.67	1.03	7.83	9.30	0.97	1.66
Mature	..	2.01	1.74	0.83	6.71	11.1	0.98	1.38
Inbred 38-11								
40	35	5.17	6.54	15.6	5.68	5.78	0.98	1.28
44	38	5.70	5.58	17.4	6.76	6.79	0.99	1.35
44	..	..	6.30	17.4	5.48	7.45	..	..
Mature	9.0	5.06	2.98	20.7	7.78	4.45	0.93	1.37
Mature	9.6	3.42	3.52	18.9	8.48	4.36	..	..
Mature	..	4.40	3.43	16.8	6.17	3.69	..	..
Inbred M4B								
24	71.5	5.87	10.3	9.71	9.41	18.0	1.06	1.47
28	65.2	6.71	7.97	7.72	8.76	16.4	1.04	1.58
36	48.8	6.96	7.95	16.0	5.62	13.7	0.92	1.18
40	48.0	7.35	12.6	16.2	3.92	18.3	0.96	1.09
Mature	11.6	7.41	10.4	14.0	7.32	12.8	0.87	1.30
Mature	..	8.95	10.7	14.5	7.98	11.9	0.90	1.31
Inbred Kys								
32	54.4	8.51	14.7	33.1	8.87	10.7	0.90	1.57
36	47.5	8.75	15.7	17.7	7.65	11.3	0.86	1.71
40	..	9.45	12.1	21.8	3.88	6.53	0.86	1.62
Mature	9.1	5.64	6.78	21.3	11.3	3.33	0.73	1.54
Mature	..	5.53	7.36	20.7	10.3	4.54	0.73	1.60

[a] Sum of beta-carotene and neo-beta-carotene.
[b] Sum of cryptoxanthol and neocryptoxantbol.

equations which has been previously applied in such cases (*5*) was used here to obtain the concentrations of luteol plus zeaxanthol and of neozeaxanthol II.

Step 2. At wave lengths 4850, 4900, and 4950 Å. in the region of greatest spread between the spectra of luteol and zeaxanthol, the absorptions $\left(\text{absorption} = \frac{\log_{10}\frac{I_0}{I}}{l}\right)$ due to neozeaxanthol were calculated from the concentration of neozeaxanthol obtained from step 1 and from its absorption coefficients. These absorptions were then subtracted from the observed absorptions of the mixture at these wave lengths to give the absorption due to the luteol and zeaxanthol.

Step 3. Then with the value for concentration of luteol plus zeaxanthol from step 1 and the absorption of luteol plus zeaxanthol from step 2, the percentage of luteol was calculated by the method used by Beadle and Zscheile (*1*) for beta- and neo-beta-carotenes.

Step 4. Since all three absorption spectra are close together at 4275 Å. an approximate value for the total concentration of all three components can be obtained by use of an approximate absorption coefficient at this wave length. This value should check the sum of the two concentrations obtained in step 1.

Results of the application of the above methods to several inbred corn lines are shown in Table III. The inbreds were self-pollinated and each sample is a composite from at least 3 ears.

Chromatographic Confirmation of Spectrophotometric Results. The approximate amounts of cryptoxanthol in the carotene fraction and vice versa were determined on the solutions from the mature samples with the aid of chromatographic technique. This separation was approximately 80 per cent efficient (*10*).

The carotenol fractions from the same samples were transferred to ether and chromatographed to obtain values representing the relative amounts of zeaxanthol and luteol present. Pigments were eluted from zones and made to volume and the concentration was determined spectrophotometrically. Results are shown in Table IV.

These data indicate that the determination of all the neotype pigments in the solution as neozeaxanthol II did not introduce serious error as far as the relative amounts of luteol and zeaxanthol were concerned

The carotenol fraction of a corn sample was analyzed spectroscopically as described above and also as a mixture of luteol, zeaxanthol, and neozeaxanthol I. Results are given in Table V.

The agreement between determinations of total pigment content of the carotenol solution by step 1 and step 4 is shown in Table VI.

Discussion

It is evident that accurate spectroscopic determination of the carotenoids is complicated by the presence of neo-type isomers as well as by the occurrence of pigments not heretofore recognized in corn extracts. In some applications it may be desirable to employ a single wave length to determine pigment groups. In analysis for total pigment content of the carotene, cryptoxanthol, and carotenol fractions, a careful choice of wave lengths is necessary to minimize errors due to varying composition. This is accomplished by selection of wave lengths where the absorption spectra of the component pigments are coincident or nearly so.

For the carotene fraction a specific absorption coefficient of 185 at 4325 Å. is obtained by assuming a specific absorption coefficient for unnamed carotene 1 of 250 at 4250 Å., and using the spectrum for gamma-carotene reported by Kuhn and Brockmann (*7*). The absorption coefficients at 4325 Å. are then: beta-carotene, 187; neo-beta-carotene, 182; gamma-carotene, 170; and unnamed carotene 1, 150. Since most of the pigment present is beta- or neo-beta-carotene, 185 is taken as a weighted mean.

In a solution in which the only significant components are cryptoxanthol and its neo isomer, the choice of the average coefficient 186 of the two components at 4375 Å. as a standard absorption coefficient should give results with small error, since the curves are only 12 units apart.

As previously pointed out, determination of total carotenols at 4275 Å. gives good agreement with spectrophotometric analysis for the components. The specific absorption coefficient used was 172.

Table IV. Comparison of Chromatographic and Spectrophotometric Determinations of Luteol

Inbred	Luteol Content of Carotenol Fraction: By adsorption	By spectroscopic analysis
	%	%
Kys	27	18
M4B	46	45
38-11	22	18
K-156	83	90

Table V. Analysis of Carotenol Fraction by Two Systems

System	Zeaxanthol	Luteol	Neozeaxanthol	Total at 4275 Å. (Step 4)	Total by Addition (Step 1)	Luteol
	Mg./l.	*Mg./l.*	*Mg./l.*	*Mg./l.*	*Mg./l.*	%
Neozeaxantbol II system	1.21	1.26	0.75	3.21	3.22	49
Neozeaxanthol I system	1.39	1.51	0.33	3.25	3.23	48

Table VI. Reliability of Carotenol Determination

Inbred	No. of Determinations	Average Difference between Results by Steps 1 and 4	Maximum Difference
		%	%
Kys	17	3.3	7.7
M4B	11	1.9	4.0
38-11	19	2.0	11.4
K-156	4	2.4	3.0

TABLE VII. COMPARISON OF RESULTS

	Carotene	Cryptoxanthol / Carotene Ratio	Carotene plus Cryptoxanthol	Total Carotenols
	Micrograms/g.		*Micrograms/g.*	*Micrograms/g.*
Buxton (*2*)	0.34–0.85	5.9–13.5	4.2–9.3	.....
Clark and Gring (*4*)	.....	.....	0.10–1.11	2.2–33.0
Kuhn and Grundmann (*9*)	0.5–0.7	6.5–14.0	5.1–7.5	12.7–14.5
Peterson, Hughes, and Payne (*10*)	.....	.....	0.8–9.4	0.1–26.0
Fraps and Kemmerer (*6*)	0.4–3.7	0.73–2.14	0.9–7.7	.....
This paper (mature samples)	2.0–8.9	0.7–1.2	3.7–19.6	18.2–35.9

A comparison of the results of several investigators is shown in Table VII.

Wide variation is found among corn varieties with respect to pigment content. All varieties studied here were inbreds; this was not true of the other work reported in Table VII. The grain of inbred lines usually has a higher proportion of pigmented hard starch than the grain of hybrids, which may account for the relatively high values obtained in this study. Clark and Gring (*3*) found an inverse relationship between grain size and carotenoid content. The outstanding differences between the results presented here and those of other workers are the higher values for carotene and the consequently lower cryptoxanthol-carotene ratio.

The method of separation of carotene and cryptoxanthol used here tends to give slightly high results for carotene because of the inclusion of about 10 per cent of the cryptoxanthol in the carotene fraction. Since a corresponding percentage of the carotene is found in the cryptoxanthol fraction, error is introduced by incomplete separation only when the cryptoxanthol-carotene ratio deviates appreciably from unity. The results of Fraps and Kemmerer show ratios of the same order of magnitude as those reported here.

Approximation of the amount of unnamed carotene 1 in the inbreds from R_1 values in Table III gives results varying from 5 to 35 per cent of the total carotene fraction. Fraps and Kemmerer (*5*) reported K carotene in 22 corn varieties to average 15 per cent of the total carotene fraction.

Summary

A method is presented for the determination of the carotenoids of corn grain which involves a separation of the pigments into three fractions by partition between immiscible solvents and spectrophotometric estimation of the pigment concentration.

The analysis of the carotene and cryptoxanthol fractions of corn-grain carotenoids in terms of normal and neo-type pigments was not highly successful, but it is probable that analyses of the fractions for total pigment content are reliable at 4325 Å. for the carotene fraction and at 4375 Å. for the cryptoxanthol fraction.

Analysis of the carotenol fraction for luteol, zeaxanthol, and total neocarotenols was made by spectrophotometric methods. Analysis for total pigment in this fraction was made at 4275 Å.

Four inbred corn lines were studied. Variations in content of mature grain were as follows: total carotenols twofold, total carotenes fourfold, total cryptoxanthols sixfold, luteol fourfold, and zeaxanthol twenty-five fold.

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JOINT contribution from the Departments of Agricultural Chemistry and Botany of the Purdue University Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. Journal Paper No. 35 of the Purdue University Agricultural Experiment Station. Studies on the Carotenoids No. 5.

Apparatus for Iodometric Determination of Tin

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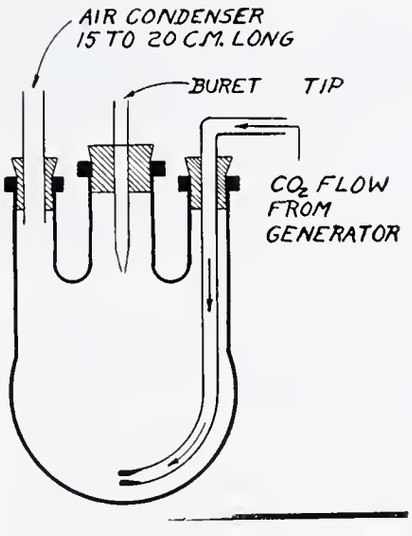
IN THE volumetric determination of tin it is necessary to keep the solution of stannous chloride under an atmosphere of a neutral gas, such as carbon dioxide, while the solution is being reduced and then titrated with a standard oxidizing solution such as potassium iodate.



A 500-ml. 3-necked distilling flask has been found to be a very suitable container for the solution during reduction and titration. The middle neck accommodates the buret, and carbon dioxide is supplied through one of the side necks. The other side neck serves not only as an outlet for the gas but also as a convenient avenue for the introduction of potassium iodide and starch indicator. If the side neck acting as the entrance for the carbon dioxide is supplied with a glass tube projecting below the surface of the solution as shown in the accompanying sketch, carbon dioxide will bubble through the solution, indicating the rate of flow of gas, and solution will be thoroughly agitated at all times.

The use of a 90-cm. (36-inch) length of rubber tubing (longer if necessary) to connect the distilling flask and the carbon dioxide generator will greatly facilitate moving the flask from the hot plate to the sink for cooling purposes. After the entire unit has been assembled, the complete procedure may be carried out without taking the apparatus apart and without interrupting the flow of carbon dioxide.

Standardization and Stability of 0.1 *N* Sodium Thiosulfate Solutions in Hot Weather

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SEVERAL investigators have shown that, by properly choosing experimental conditions, various standards for 0.1 *N* sodium thiosulfate solutions may give results agreeing within 0.1 per cent (*13*). Of these standards, potassium dichromate is one of the best because of its ease of purification and the stability of its solutions. The usual standardization methods even for this reagent, however, become unreliable at the high summer temperatures prevalent in many localities (*11, 18*). The problem is further complicated by the possible instability of the thiosulfate solutions themselves (*7*).

Various methods of standardizing and stabilizing 0.1 *N* sodium thiosulfate solutions have been systematically studied under hot weather conditions. In the course of the investigation a dichromate procedure was developed which has given highly accurate results over a temperature range of 23° to 40° C.

Table I. Effect of Temperature on the Vosburgh Method

Reaction Temperature ° C.	Titration Temperature ° C.	$Na_2S_2O_3$[a] *N*
23	23	0.10165
40	25	0.10169
25	40	0.10182
40	40	0.10192

[a] Each value represents average of at least 3 determinations; normalities are corrected to 23° C.

Standardization Methods

The effect of temperature on the Vosburgh dichromate method (*17*) was first studied.

Vosburgh Method. Accurately weigh 0.2 to 0.22 gram of purified potassium dichromate into a 750-ml. Erlenmeyer flask. Add 100 ml. of distilled water and when the dichromate is dissolved add 5 ml. of 6 *N* hydrochloric acid. Add 2.5 grams of analytical grade potassium iodide crystals, stopper the flask with a clean stopper, and then rotate the flask to mix the solutions thoroughly until the iodide has dissolved. Set the flask in the dark for 5 minutes. Then add 300 ml. of distilled water, and titrate with 0.1 *N* sodium thiosulfate solution of known temperature until within 3 to 5 ml. of the end point, being careful not to rotate the flask too vigorously. Add starch and complete the titration.

Reaction and titration temperatures were varied separately by adding distilled water of the proper temperature at convenient points of the procedure. If a high reaction temperature was desired, the flask was placed in an oven set at that temperature during the dichromate-iodide reaction.

The results summarized in Table I show that the reaction temperature had little effect on the final result, although an increase in temperature doubtless accelerated the reaction. On the other hand, an increase in temperature of the solutions during the titration always resulted in higher normalities, indicating a loss of iodine by volatilization. It was found that this loss could be largely avoided, even at 40° C., by increasing the potassium iodide concentration at the beginning of the titration from 0.5 to 1.4 per cent. Likewise, reversing the order of addition of acid and the iodide should minimize a possible initial loss of iodine which otherwise might occur before the reagents are completely mixed.

The possibility of the iodide ion undergoing atmospheric oxidation was considered. Results obtained by carrying out the reaction at 40° C. in an atmosphere of nitrogen did not differ significantly from those obtained in air. The use of bicarbonate, as in the Willard and Furman method (*19*), is therefore believed to be unnecessary.

Incidental experiments were carried out to determine whether a shift in the starch-iodine end point occurred as the temperature was increased to 40° C. The effect proved to be negligible, certainly not more than equivalent to 0.02 ml. of thiosulfate solution.

The proposed procedure for thiosulfate standardization followed directly from the above experiments.

Proposed Dichromate Method. Accurately weigh 0.2 to 0.22 gram of purified potassium dichromate into a 500-ml. Erlenmeyer flask. Carefully wash down any dichromate adhering to the funnel or sides of the flask. Dilute to 125 ml. with distilled water, and add 5 grams of potassium iodide, low in iodates. Rotate the flask until the iodide has dissolved. Add 5 ml. of 6 *N* hydrochloric acid with constant swirling. Carefully wash down the sides of the flask, so that a layer of water is formed on top of the solution. Stopper the flask and set the sample in the dark for about 6 minutes. Add 165 ml. of distilled water. Titrate with 0.1 *N* sodium thiosulfate solution until the solution becomes greenish-yellow, rotating the flask so that no excess of sodium thiosulfate is in contact with the acidic solution at any time. Add 0.6 per cent starch solution, using from 2 ml. at 20° to 3 ml. at 40° C. Wash down the sides of the flask, and continue the titration to the end point. For a corresponding volumetric procedure: add about 45 ml. of 0.1 *N* potassium dichromate solution, accurately measured, to the Erlenmeyer flask, and proceed as described above.

To determine the accuracy of the dichromate procedures, it appeared desirable to compare them with an exact method involving the use of pure iodine.

The iodine was weighed in a mixture of potassium iodide and water, as suggested by Treadwell and Hall (*16*). It was found that weighing bottles containing mixtures of 2.5 grams of the iodide with 0.5 to 0.8 ml. of water lost weight at the same rate when the stopper was removed, whether iodine had been added to the solution or not. Hence, losses resulting from the vaporization of iodine are believed to be negligible. In order to determine the quantity of water vapor lost during the transfer of the crystalline iodine, weighed samples of cadmium iodide, clay plate, sodium nitrate, and iodine itself were poured into the potassium iodide–water mixtures contained in 50 × 15 mm. weighing bottles with male ground stoppers. The results showed that a positive correction of 0.20 ± 0.05 mg. must be applied. The uncertainty in the correction for a 0.5-gram sample of iodine is about 0.01 per cent of the total weight. These experiments were made in a room thermostatically controlled to 23° to 25° C. and having a low relative humidity, and no claim is made for the reliability of the method under other atmospheric conditions.

The procedure given below was adapted from that of Treadwell and Hall in the light of the above experiments and of the results obtained by Popoff and Whitman (*13*). Under these conditions very closely reproducible results were obtained. The method was of great value in this research, but it cannot be recommended for routine work.

Baker's resublimed iodine was further purified by sublimation, first from a potassium iodide mixture and then alone. A third sublimation in a current of dry nitrogen (*2*) did not perceptibly change the results.

Iodine Method. Add 0.7 ml. of distilled water directly to 2.5 grams of potassium iodide in a 50 × 15 mm. weighing bottle (male ground stopper) by means of a 1-ml. pipet. Let the bottle remain open for a few minutes to permit the evaporation of any

Table II. Standardization of Thiosulfate Solution at 23° C.

	Vosburgh Method	Iodine Method	Willard and Furman Method[a]	Iodine Method	Proposed Method	Iodine Method
	0.10080	0.10092	0.10091	0.10117	0.10085	0.10078
	0.10093	0.10087	0.10096	0.10113	0.10082	0.10079
	0.10086	0.10086	0.10101	0.10113	0.10080	0.10087
	0.10088	0.10093	0.10097	0.10113	0.10078	0.10077
	0.10088	0.10074	0.10118	0.10117	0.10068	0.10082
	0.10084	0.10085	0.10111	0.10117	0.10076	0.10086
	0.10085	.....	0.10120	.....	0.10074	.....
	0.10090	.....	.....	.....	0.10068	.....
	0.10080	.....	.....	.....	0.10084	.....
	0.10098	.....	.....	.....	0.10081	.....
Average	0.10087	0.10086	0.10105	0.10115	0.10078	0.10082
Range	0.00018	0.00019	0.00029	0.00004	0.00017	0.00010
Standard deviation × 10^5	5.6	6.8	11.5	2.2	6.1	4.2
Difference from iodine value		+0.00001		−0.00010		−0.00004

[a] Each value represents average of two determinations made with same dichromate solution. Standard deviation of the two values in each pair, a measure of the titration errors, was found to be 4.8×10^{-5}.

Table III. Standardization of Thiosulfate Solution at 40° C.

(Normalities corrected to 23° C.)

	Vosburgh Method	Willard and Furman Method[a]	Proposed Method	Iodine Method[b], 23° C.
	0.10164	0.10145	0.10120	.....
	0.10160	0.10129	0.10117	.....
	0.10172	0.10126	0.10122	.....
	0.10138	0.10138	0.10120	.....
	0.10174	0.10135	0.10124	.....
	0.10155	.....	0.10122	.....
	0.10156	.....	0.10126	.....
Average	0.10160	0.10135	0.10122	0.10115
Range	0.00036	0.00019	0.00009	0.00004
Standard deviation × 10^5	12.1	7.7	2.9	2.2
Difference from iodine value	+0.00045	+0.00020	+0.00007	.....

[a] Each value represents average of two determinations made with same dichromate solution. Standard deviation within pairs, due to titration errors, was found to be 6.0×10^{-5}.

[b] These data, from Table II, are included for comparison with dichromate method values obtained in hot room.

water from the inner wall of the bottle; then stopper and weigh accurately. Add 0.4 to 0.5 gram of purified iodine directly to the moist salt with a small porcelain spoon. Stopper the bottle, tap it gently for a few seconds until all the iodine dissolves, and weigh. The difference in weights plus 0.20 mg. equals the weight of the iodine added. Loosen the stopper, and drop the weighing bottle followed by the stopper into a 500-ml. Erlenmeyer flask containing a solution of 2 grams of potassium iodide in 150 ml. of distilled water. Rotate the flask only enough to submerge the weighing bottle. Titrate at once with 0.1 *N* sodium thiosulfate solution with no swirling at all to within 3 ml. of the end point (calculated approximately). Then slowly rotate the flask so that most of the iodine is reduced before reaching the surface. Continue the titration to a pale yellow, slowly rotating the flask. Add 2 ml. of 0.6 per cent starch solution, and complete the titration.

Final Tests. The different dichromate procedures were tested in rooms thermostatically controlled to 23° to 25° C. and 38° to 40° C. The determinations in Table II for a given dichromate method, together with corresponding iodine tests, were carried out on the same day. All the determinations given in Table III were completed during a single day, as were those of Table IV, the dichromate methods being alternated to minimize the relative effect of any change in temperature, lighting, or alertness of the operator. Separately weighed samples were used for the determinations by the Vosburgh and proposed methods in Tables II and III.

The Willard and Furman method is not well adapted to the use of individually weighed samples; consequently, dichromate solutions were made up from accurately weighed portions of the salt, and aliquot portions were taken for the trials by this method. These solutions were also used for all the determinations in Table IV. The normality of the thiosulfate solutions as determined by the iodine method two days before the hot-room tests was taken as the true normality of the solutions during the tests. All normalities were calculated for 23° C., taking account of the expansion of both water and glass. Baker's analytical grade potassium dichromate was further purified for these determinations by thrice recrystallizing and fusing.

It is evident from the data in Table II that satisfactory results may be obtained at moderate temperatures (20° to 25° C.) by any of the dichromate procedures under consideration. Results obtained at 40° C. by the proposed method (Table III) do not differ significantly from the true value, but those obtained by the Vosburgh and the Willard and Furman methods are high by 0.45 per cent and 0.20 per cent, respectively, and are not very consistent. Cooling the solution before titrating (Table IV) is unimportant in the proposed dichromate method even at 30° to 40° C., provided that the starch solution is reasonably sensitive. The proposed method is simpler than the Willard and Furman method and is adapted to the use of individually weighed samples as well as the more rapid volumetric procedure. The method is therefore recommended for the standardization of 0.1 *N* thiosulfate solutions, particularly at temperatures of 30° C. and higher.

Table IV. Standardization of Thiosulfate Solution at 38° to 40° C. with Cooling before Titration[a]

(Normalities corrected to 23° C.)

	Vosburgh Method	Willard and Furman Method[a]	Proposed Method	Iodine Method[b], 23° C.
	0.10120	0.10113	0.10116	.....
	0.10117	0.10129	0.10115	.....
	0.10134	0.10127	0.10111	.....
	0.10107	0.10123	0.10113	.....
	0.10126	0.10128	0.10119	.....
Average	0.10121	0.10124	0.10115	0.10115
Range	0.00027	0.00016	0.00008	0.00004
Standard deviation × 10^5	10.1	6.6	3.0	2.2
Difference from iodine value	+0.00006	+0.00009	0.00000	.....

[a] Determinations were carried out in hot room, but cold distilled water was added to solutions before titration. Average titration temperatures were 24°, 30°, and 28° C. for Vosburgh, Willard and Furman, and proposed methods, respectively.

[b] These data, from Table II, are included for comparison with dichromate method values obtained in hot room.

Stability Tests

The decomposition of thiosulfate is generally attributed to the action of certain bacteria, light, oxygen of the air catalyzed by traces of copper ions, and possibly carbon dioxide (*7*). A large number of stabilizers have been advocated (*4–7, 10, 15, 20*), but apparently little is known as to their effectiveness in hot weather. The present investigation involves the periodic testing of variously treated thiosulfate solutions kept in a hot-air bath at 40 ± 1° C., and of control solutions kept at 20° to 25° C.

The solutions were made with freshly distilled water and stored in clean bottles, sterilized by steam. The bottles were of amber glass except two of colorless glass which were used for solutions to be exposed to the light. An individual Normax pipet was used for each solution, chiefly to avoid the possible spread of bacterial infection. The pipets to be used for the 40° solutions were kept in the hot-air bath.

Storage Tests. The normalities of the various thiosulfate solutions were determined by titrating 25-ml. portions against 0.1 *N* iodine solutions, which were made up as suggested by Hillebrand and Lundell (*3*), and were standardized by the arsenious acid method described by Popoff (*12*). Alkali-treated solutions were first neutralized with 0.01 *N* hydrochloric acid. Suitable volume corrections were applied to compensate for differences in temperature of the solutions.

Table V. Storage Tests on Thiosulfate Solutions[a]

(Normalities corrected to 20° C.)

		Nov. 7, 8	Nov. 17, 18	Dec. 8, 9, 10	Jan. 5, 6	Feb. 3, 6, 7	Mar. 9, 10, 13
A. No special treatment							
1[b]. No preservative	N	0.10086	0.10100	0.10107	0.10118	0.10100	0.10104
	pH	6.5	6.8	6.7	5.5[c]	5.0	4.9
2. No preservative	N	0.10073	0.10116	0.10089	0.10102	0.10105	0.10083
	pH	6.7	6.9	7.1	7.3	6.8[c]	7.4
3. No preservative, light	N	0.10062	0.10093	0.10057	0.10067	0.10060	0.10061
	pH	6.5	6.7	7.1	7.2	7.1[c]	7.5
B. Alkali treatment							
4[b]. Sodium hydroxide, 0.15 g./l.	N	0.10058	0.10042	0.10008	0.09977	0.09932	0.09941
	pH	11.1	11.1	11.2	10.8	10.5	10.2
5. Sodium hydroxide, 0.15 g./l.	N	0.10042	0.10100	0.10082	0.10086	0.10076	0.10072
	pH	11.1	11.1	11.4	11.2	11.2	11.2
6[b]. Sodium carbonate, 0.2 g./l.	N	0.10062	0.10081	0.10080	0.10087	0.10049	0.10061
	pH	10.3	10.3	10.2	10.1	10.0	9.9
7. Sodium carbonate, 0.2 g./l.	N	0.10062	0.10100	0.10105	0.10117	0.10093	0.10083
	pH	10.4	10.3	10.4	10.4	10.4	10.4
8. Sodium carbonate, 0.2 g./l., light	N	0.10066	0.10093	0.10095	0.10099	0.10083	0.10088
	pH	10.3	10.3	10.4	10.4	10.3	10.2
9[b]. Sodium carbonate, 0.2 g./l., chloroform, 1 ml./l.	N	0.10073	0.10062	0.10054	0.10059	0.10045	0.10050
	pH	10.3	9.1	8.3	7.6	7.7	7.6
10. Sodium carbonate, 0.2 g./l., chloroform, 1 ml./l.	N	0.10062	0.10062	0.10074	0.10086	0.10093	0.10076
	pH	10.3	10.3	10.2	10.1	9.9	9.8
C. Germicidal treatment							
11[b]. Chloroform, 1 ml./l.	N	0.10093	0.10104	0.10103	0.10094	0.10073	0.10057
	pH	7.5	6.1	6.6	4.7[c]	4.6	4.7
12. Chloroform, 1 ml./l.	N	0.10101	0.10104	0.10090	0.10102	0.10105	0.10083
	pH	7.8	6.9	7.4	7.2	7.1[c]	7.4
13[b]. Mercuric iodide, 10 mg./l.	N	0.10104	0.10104	0.10107	0.10126	0.10096	0.10092
	pH	6.9	6.5	6.1	5.2	4.9[c]	4.8
14. Mercuric iodide, 10 mg./l.	N	0.10101	0.10096	0.10080	0.10086	0.10083	0.10076
	pH	7.2	6.7	6.8	6.7	6.9[c]	7.4
15[b]. Mercuric cyanide, 0.1 g./l.	N	0.10116	0.10119	0.10050	0.09973	0.09932	0.09910
	pH	9.0	8.5	8.4	4.6[c]	4.5	4.6
16. Mercuric cyanide, 0.1 g./l.	N	0.10101	0.10112	0.10116	0.10133	0.10120	0.10118
	pH	8.9	8.7	8.4	8.1	8.2	8.1
17[b]. Toluene, 1 ml./l.	N	0.10101	0.10112	0.10126	0.10130	0.10123	0.10127
	pH	7.9	7.3	7.8	5.7	5.1	4.9[c]
18. Toluene, 1 ml./l.	N	0.10108	0.10104	0.10101	0.10113	0.10108	0.10106
	pH	7.7	7.7	7.8	6.8	7.0	7.4

[a] Approximate sulfate plus sulfite concentrations after storage-test period were: solutions 4 and 15, 0.005 *M*; other 40° solutions, 0.0005–0.0015 *M*; control solutions 2, 7, and 12, 0.0001 *M* or less.
[b] Hot-air bath; temperature of solutions, 39.0–40.5° C.
[c] Finely divided sulfur first observed on surface of solution.

Tests indicated that a change as small as 1 part in 1000 in normality of a solution during storage can be detected with practical certainty, while changes of less than 1 part in 2000 are not necessarily real.

The pH values of the solutions were determined with a Beckman pH-meter, model G. A sodium-ion correction was applied to solutions having pH values higher than 9.5.

The combined sulfite and sulfate content of some of the solutions was roughly determined by treatment with iodine followed by barium chloride (*9*). The resulting turbidity was compared with that produced in standard solutions of barium chloride treated with sulfate.

Results. A summary of the storage tests is given in Table V. Each solution in the hot-air bath is followed by a control solution kept at 20° to 25° C. The difference in time intervals should be noted.

The untreated solutions (Section A) proved to be surprisingly stable. Thus, after a 10-day aging period, the maximum observed variation in normality during 4 months was 0.18 per cent for solution 1 kept at 40° C. The effect of light (solution 3) appears to be almost negligible.

Despite widespread belief in their efficacy as stabilizers (*6*, *7*, *10*, *15*) sodium hydroxide and sodium carbonate (Section B) were found to be actually detrimental to stability, especially at 38° to 40° C. For instance, the normality of solution 4 containing 0.015 per cent sodium hydroxide decreased 1.2 per cent in 3 months. A similar change occurred in solution 15 containing mercuric cyanide (Section C).

Of the various other stabilizers tried, chloroform and mercuric iodide proved to be the best; they were effective even at 40° C. for about 2 months. During this time the chloroform apparently hydrolyzed, forming an acid which later accelerated the decomposition of the thiosulfate.

Fluctuations in normality of thiosulfate solutions are commonly attributed to the formation of sulfite and sulfide ions followed by their atmospheric oxidation to sulfate and free sulfur, respectively. Reactions like these tend to produce an occasional rise in pH, and also a significant rise in normality if an antioxidant is present; toluene seems to act as such. But regardless of the type of treatment, all the thiosulfate solutions stored at 40° C. became definitely more acidic, their normalities fell noticeably, and sulfate was formed. These facts are best explained by the following reaction, which obviously would be favored by the presence of alkalies (*8*):

$$S_2O_3^{=} + 2O_2 + H_2O = 2SO_4^{=} + 2H^+$$

The control solutions kept at 20° to 25° C., in contrast, formed very little sulfate or sulfite. Significant changes in their effective normalities must therefore be due to the initial formation of such products as sulfide, tetrathionate (*14*), and perhaps trithionate (*1*).

Summary

An improved dichromate procedure for the standardization of 0.1 *N* sodium thiosulfate solutions has been developed, particularly for determinations to be carried out at 30° to 40° C. The accuracy of the method has proved to be within 0.07 per cent over a temperature range of 23° to 40° C. The Vosburgh method (*17*) and the Willard and Furman method (*19*), although satisfactory at moderate temperatures, gave high and somewhat erratic results at 40° C. The accuracy of the different dichromate procedures was determined by comparison with the iodine method of Treadwell and Hall (*16*), modified as described in this paper.

A number of 0.1 *N* sodium thiosulfate solutions were treated in various ways and stored at 20° and 40° C. for approximately 4 months. The maximum variation in the effective normalities of untreated solutions during this time was about 0.3 to 0.4 per cent. Chloroform and mercuric iodide, which proved to be the most satisfactory of the preservatives tried, were effective for only about 2 months at 40° C. At that temperature, sodium hydroxide and sodium carbonate, instead of acting as stabilizers, actually accelerated the decomposition of the thiosulfate.

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Carbon Dioxide Generator

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A SOURCE of carbon dioxide gas is frequently desirable in connection with laboratory or research work. Where the gas is used under low pressure, and especially where considerable quantities are desired on short notice, it is usually cheaper and more convenient to generate the gas from dry ice than to purchase it in cylinders.

The generator shown in Figure 1 has been in successful use for some time in the Chemical Engineering Laboratories at Yale University. This generator is simple in principle, is inexpensive to make, and is free from operational difficulties encountered in previous designs.

The body of the generator, *A*, is made of a 24-gallon Hackney steel drum with a removable bolt-on cover. The cover is provided with a 2-inch discharge line, *B*, a 6-inch handhole, *C*, and a stuffing box, *E*, for the rod supporting the wire basket, *D*. The discharge line has a pop safety valve set at 10 pounds per square inch, and a pressure gage for indicating the pressure in the drum. The handhole cover is grooved for a rubber gasket and is also provided with a small vent line which serves to vent the air trapped in the handhole during filling. This cover is held in place by hinged bolts which fit into slots in a bar running across the top of the cover. This arrangement permits removal or replacement of the cover in a few seconds.

An important feature of the generator is the wire basket, *D*. It is a circular basket made of strap iron covered with 0.25-inch mesh wire cloth. The basket is supported by a steel rod which passes through a stuffing box in the cover. This arrangement permits the basket to be lifted out of the water which fills the lower part of the generator. By adjusting the height of the basket the rate of generation of carbon dioxide can be controlled.

The generator is also provided with a steam coil, *J*, and a valve at the bottom for draining the drum, *G*.

To operate the generator valves *F* and *G* are closed, the handhole cover is removed, and the generator is filled about half full of water. Next about 5 pounds of dry ice, broken up into 1 to 1.5-inch lumps, are placed on the basket which has been lifted out of the water. The handhole cover is replaced and the basket is lowered into the water. Generation of carbon dioxide starts immediately. After venting the air from the system through the safety valve and the vent line, valve *F* is opened to allow the carbon dioxide to go into the holder.

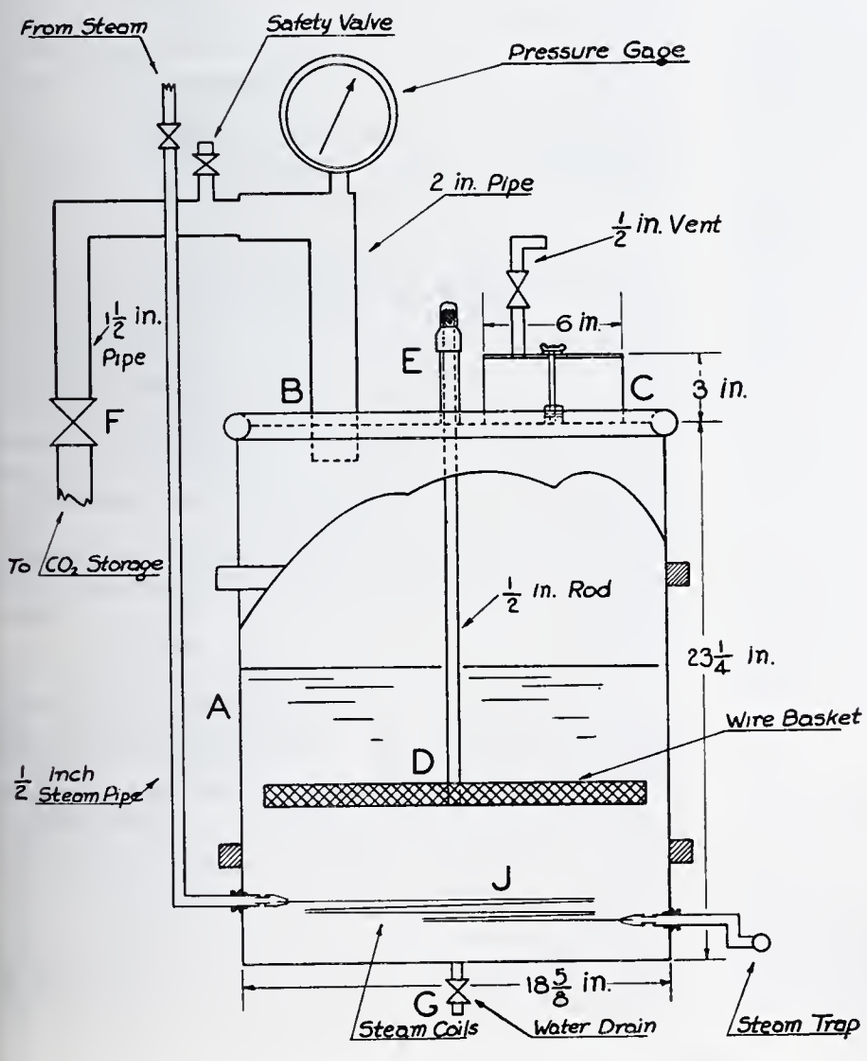

FIGURE 1. GENERATOR FOR CARBON DIOXIDE GAS

With this generator it is possible to generate about 50 cubic feet of gas in 15 to 20 minutes by the method of operation described. If a higher rate is desired or if the generator is used frequently, it is necessary to use the steam coils to keep the water lukewarm.

The purity of the gas sent to the holder can be controlled by limiting the amount of air in the generator at the time of filling and by purging before allowing any gas to go to the holder.

Extraction of Metallic Constituents from Oils

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THE determination of the metallic constituents of organic compounds generally involves conversion to an inorganic form, with removal of the organic material.

The simplest method of accomplishing this is ashing, and standard methods have been published for petroleum oils in general (*1*) and drying oils (*3*); however, some oxides are lost by volatilization and by reaction with the crucible. Gottsch and Grodman (*8*) have shown that the ash method gives low results on lead and zinc driers. Sulfating the ash may minimize losses, but even ferric sulfate is appreciably volatile (*12*).

To avoid the inaccuracies inherent in the ash method, the organic matter may be destroyed by wet oxidation, as with sulfuric and nitric acids. This is a standard method for the analysis of lead driers (*4*). However, the size of sample which can be handled conveniently in this way is limited.

The metallic soaps may be decomposed, the metal converted to a water-soluble salt, and the organic matter separated by extraction. Many published methods are based on this principle—for instance, manganese driers may be decomposed with hot hydrochloric acid, and the organic matter dissolved in beeswax, and so removed (*4*). Zinc driers may be decomposed with cold hydrochloric acid, and the organic matter separated by the use of petroleum ether (*4*). Lead soaps in oils and greases are decomposed in a homogeneous phase containing glacial acetic acid and benzene, followed by dilution and washing with water in a separatory funnel (*9*). Tetraethyllead may be converted to the inorganic form by shaking with bromine (*6*) or cold nitric acid (*5*), or by refluxing with hot hydrochloric acid (*2*). The metal content of organomercurials may be determined by dissolving the sample in petroleum ether and shaking with hydrochloric acid (*10*).

This article describes one extraction method by which all the above determinations, and many others, may be made. This method has been applied successfully to used oils in which the metallic constituents were suspended as well as dissolved, paint driers, greases, and compounded lubricating oils containing a wide variety of additives.

Refluxing with hot acid gives more rapid conversion to the inorganic form than does shaking with cold acid. Consequently, the ideal apparatus for the extraction process would be a separatory funnel in which the organic and aqueous layers could be boiled and refluxed. These unusual requirements are met in a piece of equipment, known as the tetraethyllead extraction apparatus (*2*), which is now obtainable from many laboratory supply houses. This equipment was designed to extract the lead from gasoline by refluxing for 30 minutes with 12 *N* hydrochloric acid. Without any modifications, the same equipment may be used to extract the calcium from a lime-base grease by refluxing for 10 minutes with 0.5 *N* hydrochloric acid. For certain special cases, even water is satisfactory. Between these extremes of time and normality, all the other extractions have been found to be complete. Fifteen minutes of refluxing with 6 *N* hydrochloric acid, have been found to be most widely applicable. Gottsch and Grodman (*8*) have suggested the use of this equipment for paint driers.

Extraction Method

Fifty milliliters of the appropriate extraction medium (hydrochloric acid or other agent) are introduced into the extraction apparatus.

The sample is weighed in a beaker, the accuracy of weighing depending upon the desired precision of results. Only sufficient sample is taken to give a convenient amount of the metal sought. No lower limit to the size of the sample need be set. Not over 200 ml. of a light product, such as gasoline, can be handled conveniently. With heavier products, such as lubricating oils, the use of more than 20 grams of sample may cause bumping.

The sample is transferred to the extraction flask by the use of sufficient petroleum naphtha to give a total organic layer of approximately 100 ml. A convenient distillation range for this naphtha is from approximately 200° to 250° F. If the sample is difficultly soluble in naphtha, toluene may be used. Even greases may be handled in this way, the sample being dispersed with some of the toluene by creaming with a spatula.

Five milliliters of butyl alcohol are added to prevent emulsions.

The current is turned on, and boiling is continued until the reaction is complete. The length of time required depends upon the nature of the sample, but not over 30 minutes have been found necessary so far. The current is then turned off, and the layers are allowed to separate.

If the separation of the layers is difficult or slow, more butyl alcohol may be added. This, with a judicious application of heat, serves to break the emulsion and give a clear separation.

The aqueous layer is drawn off, and additional extractions, or washes with distilled water, may be made in the same fashion. By titration of the acidity of the water wash, following a hydrochloric acid extraction, the holdup of the aqueous layer in the extractor was found to average 2.5 per cent. One water wash, therefore, gives about 99.94 per cent recovery.

TABLE I. PRECISION OF RESULTS

	Ash	Extraction
	%	%
Used motor oil		
Lead	0.66	0.68
Iron	0.062	0.059
Cobalt drier dissolved in naphtha		
Cobalt	0.035	0.035
	1.20	1.22
	3.46	3.45
Diesel lubricating oil		
Calcium	0.046	0.048
Semifluid grease		
Aluminum	0.033	0.033
Extreme-pressure lubricant		
Ash, operator 1	3.02	...
Ash, operator 2	3.61	...
Lead, operator 1	2.4	3.05
Lead, operator 2	2.8	3.03

The aqueous extract may be analyzed for its metallic content by any desired method. Generally, as in the determination of tetraethyllead, the hydrochloric acid is evaporated. If small amounts of organic matter which are extracted by the acid are likely to interfere with the subsequent analysis, they may be destroyed with sulfuric, nitric, or perchloric acid, with hydrogen peroxide, or by other appropriate means.

The organic layer also may be used for further tests, since the fatty acids from the soaps may be washed, saponified, extracted, etc., in the same apparatus.

The extraction method has been found to be as rapid as the ashing method, and to require less attention on the part of the operator. The relative precision of results is illustrated by Table I.

In the analyses shown, the ashes were dissolved in hydrochloric acid, and the extractions were effected with the same acid. The acid solutions from ashing and extraction were treated identically in subsequent analysis.

The petroleum naphtha used in the extractions was Sovasol No. 3, the metallic content of which was shown to be zero by extraction. All reagents used were of the C. P. grade. The various metals were determined by methods described by Treadwell and Hall (*11*), except cobalt, when anthranilic acid was used (*7*).

In testing the extreme-pressure lubricant, operator 2 was careful to leave an appreciable amount of carbon in the ash, in order to avoid loss of lead due to strong heating, yet actually lost 8 per cent of the lead. Operator 1 burned out all the carbon, obtaining a large proportion of metallic lead, and lost 21 per cent of the lead. Obviously the ash figure itself is meaningless.

Used lubricating oils containing tin, copper, cadmium, and aluminum, in addition to lead and iron, have been analyzed successfully by this method. No work has been done on paints containing titanium oxide or lithopone, but the proper choice of extraction acid and time of refluxing should give a complete extraction, though not solution.

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An Efficient Column Suitable for Vacuum Fractionation[1]

Concentric Tube Type

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THE design of laboratory fractionating columns especially suitable for operation at reduced pressure has received relatively little attention. Where a column is intended for vacuum operation, pressure drop constitutes a particularly critical factor because the high vapor velocities prevailing result in high pressure drop and tendency to flood. Most columns, including those of high efficiency, when operated under vacuum may suffer so seriously in some of their operating characteristics (reduction in throughput, increase in pressure drop, and increase in holdup) as to be impracticable for some purposes.

Among the recently reported high-efficiency packed columns (glass construction), the simple concentric tube type (2-tube system) of Craig (*7*) and the more complex one of Selker, Burk, and Lankelma (*18*) have been shown to possess particularly low pressure drop and low holdup characteristics. The very low H. E. T. P. (height equivalent to a theoretical plate) obtainable with this concentric tube type (2-tube system) was first demonstrated by Craig (*7*), and recently even more strikingly by Naragon, Burk, and Lankelma (*11*). Both of these columns, however, were of small diameter (5 to 7 mm. in inside diameter of outer tube) and of very short length (25 to 31 cm.). [Performance of straight open tube (including concentric tube type) columns from a theoretical standpoint have received critical analysis by Westhaver (*20*).] No such low H. E. T. P., however, has been demonstrated for concentric tube columns of any appreciable length (8 feet or more). This circumstance may be due in part to an inherent lack of accord in the H. E. T. P. values of short and long columns, as indicated by some authors (*1*, *4*, *5*, *9*), but is perhaps, in a larger measure, due to difficulties of construction, as experienced in this laboratory. Although Selker *et al.* (*18*) succeeded in constructing a column 5 feet (142.5 cm.) long, ordinarily the difficulties encountered are so serious as to render the use of such columns impracticable, except for short lengths. As found in this laboratory, this is primarily due to "bowing" and lack of uniformity in diameter (inner and outer diameter for the entire length of the tubing). Glass tubes (in sufficient length) of such precision measurements as to permit the narrow clearance necessary (1 to 2-mm. annular space), without having the concentric tubes touch each other when the column is in operation, are virtually unobtainable. This difficulty is obviously multiplied in the case of the multiple-tube system of concentric tubes such as that of Selker *et al.*, a circumstance which, together with the difficulties incident to the proportionation of condensate to the respective tube surfaces, may account for the inability of Bailey (*1*) to duplicate the low H. E. T. P. findings of these authors.

As observed in this laboratory, contact of the inner and outer tubes at various points (due to bowing and variations in tube diameter) interrupts the flow of condensate over the cylindrical surfaces, giving rise to the formation (at the points of contact) of "rivulets" of reflux liquid. Thus the reflux, in a large measure, is made to course down the column in the form of thickened streams or rivulets instead of a thin even film, a condition obviously unfavorable for bringing about the desired intimacy of contact between vapor and liquid.

For a 2-tube concentric tube system the construction problem has been satisfactorily solved, as described in this paper, by departing from the use of the single central tube and using instead a series of tube segments, fitting them end to end (interlocking) in quasi chain form. Further, to ensure even distribution of condensate, each segment is closely wound with fine glass thread, a device which serves only as a mechanism to distribute the liquid in a fine film and to give turbulence, but not as a spiral path for the liquid as in the case of the spiral-type columns (*15*). In the form described in this paper the flow of condensate is restricted to the surface (convex) of the chain segment packing. [It has been found advantageous to restrict the reflux to the convex surface. Studies are in progress of the relative efficiencies obtainable by the wetting of convex and concave surfaces, respectively, the simultaneous distribution of reflux over both surfaces (in a 2-tube system, with the column of vapors sandwiched in between), as also the effect of other variables (annular space and curvature). These will be the subject of a future paper.]

Actual centering of the segments is obtained by the use of simple pronged centering collars (at the tops of segments). These, together with the interlocking system between tops and bottoms of the entire series of segments, ensure centering

[1] Application made for U. S. Service Patent by S. Palkin and S. A. Hall, Serial No. 424,476.

and the maintenance of a uniform annular space. Moreover, the centering prongs which make contact with the concave surface of the outer column tube are upward pointing. This effectively prevents the liquid from being diverted to, and the formation of rivulets on, the concave surface of the enclosing or outer tube, a requirement found vital for the efficient performance of this type of column.

Experimental

Construction of Column and Housing. The front and side views of the column assembly are shown in Figure 1. The column (13 mm. in inside diameter) is enclosed in an air jacket tube (30 mm. in inside diameter). To minimize radiation losses, the jacket tube is covered with tinfoil, except for a 2.5-cm. (1-inch) width in the front to permit full visibility of the column packing. The air-jacketed column and stillhead are enclosed in a box housing (about 8 × 6 inches × 9 feet), similar to that of Bruun (*5*) but made from 85 per cent magnesia slab, supported in a simple frame of galvanized sheet iron angles. The full-length window in front is made of two panes of double-thick window glass (about 0.3 cm., 0.25 inch, apart) set in galvanized iron channels and cushioned with asbestos. Four space heaters (Aminco; 750 watts each, except for the top heater which is 500 watts) are fastened on a galvanized sheet iron channel (10 cm., 4 inches wide) which fits in back of the magnesia box housing as shown in Figure 1 (side view). Each heater is individually controlled by rheostat and thermostat (Fenwal) placed as shown.

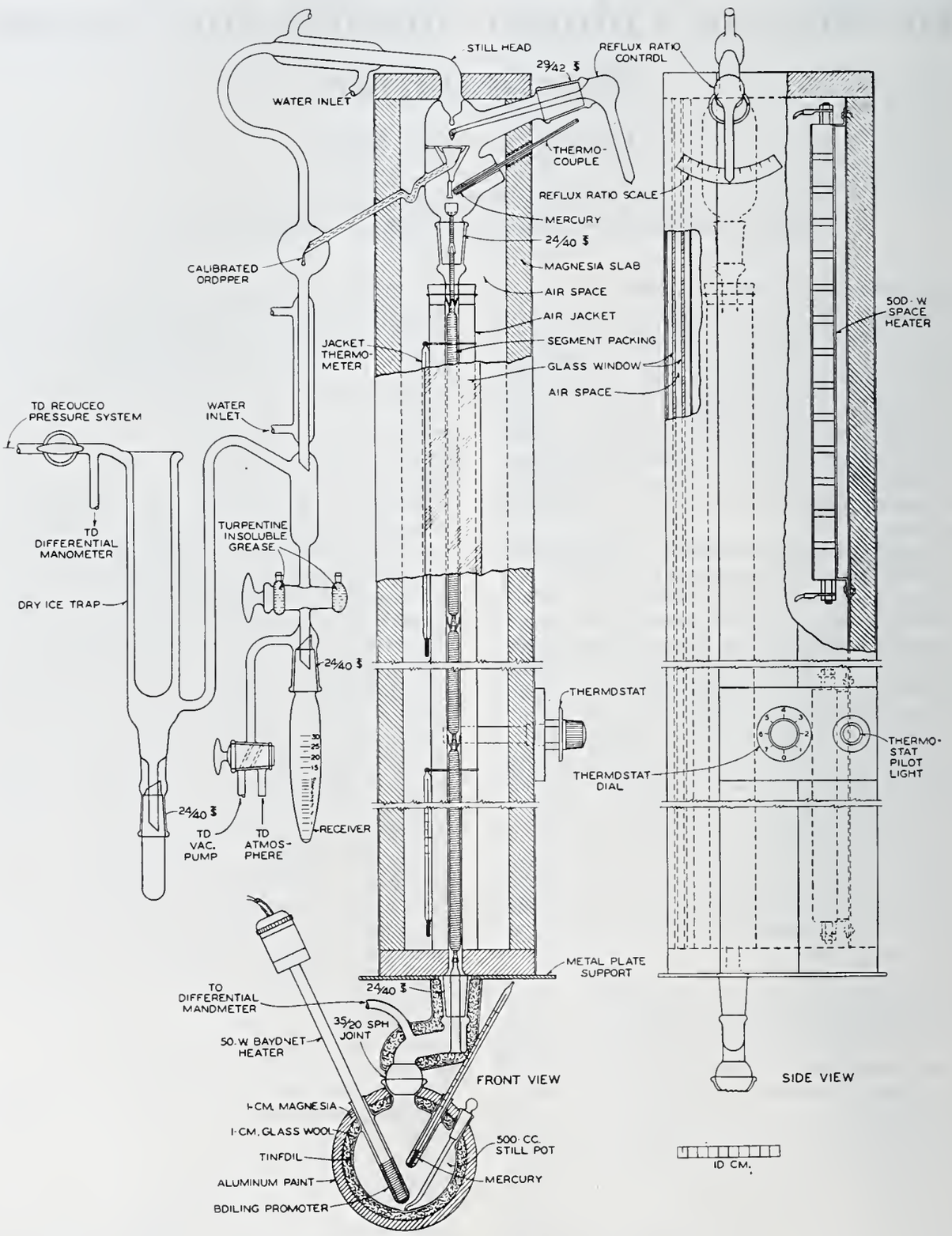

Figure 1. Front and Side Views of Column Assembly

The jacket temperature gradient, when once set by adjusting the thermostats, is thus maintained automatically, and changes in settings are made manually from time to time as the progress of a distillation demands.

Column Packing. The packing consists of 8 segments of tubing (10 mm. in outside diameter and sealed at both ends) each approximately 30 cm. long, which gives a total packed length of 238 cm. A short piece of glass rod (4 mm. in diameter and shaped as shown in Figure 2) is sealed to the top. To the bottom of each segment is sealed a short piece of glass tubing (6 mm. in outside diameter), which serves as a receptor for the glass-rod top of the next lower segment. This simple mechanism constitutes what might be considered a loosely fitting and interlocking male-female joint system which, with a centering mechanism, ensures

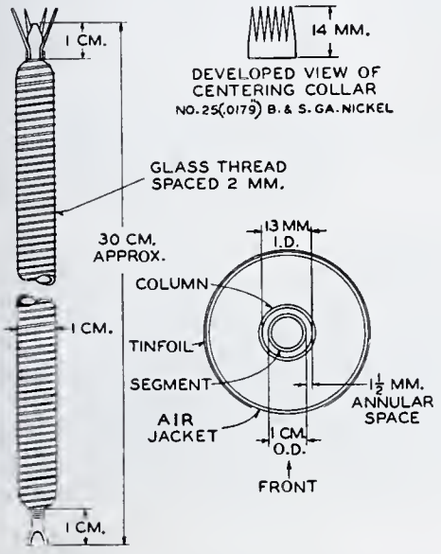

Figure 2. Column Packing

proper alignment of the segments within the tube. Small multi-pronged metal collars fitting snugly around the glass rod top of each segment provide the centering mechanism for a pair of segments. This centering collar is cut from sheet metal. Nickel (No. 25, B. & S. gage) was used, but other noncorroding metal of convenient gage might serve equally well. The prongs are bent outward at about a 45° angle and the collar portion is curved and closed (using long-nosed pliers) snugly around the glass-rod top of the segment.

Accurate centering of the metal collar on each segment was accomplished as follows: Two pieces of glass tubing about 40 cm. long were selected, one (11 mm. in inside diameter) to serve as a "sleeve tube" for the segment, and the other (about 14 mm. in inside diameter or approximately the same inside diameter as the column tubing) to slide over this sleeve tube. After the segment was placed in the sleeve tube with collar projecting, the prongs were adjusted by slight bending to permit sliding the outer tube over the assembly, allowing just enough springiness to remain so that, after removal of the sleeve tube, when the segment was pushed through the outer tube it not only remained properly centered but slid through with some but not excessive resistance.

The uppermost segment in the column differs from the others in its extended upper portion by having a small glass cup, perforated at the bottom, sealed to a short piece of 6-mm. tubing as shown in Figure 1. This serves to collect and convey the reflux liquid to the chain of segments below. The segments are each wound spirally with fine glass thread (about the thickness of No. 35 B. & S. gage wire) with spacing approximately 2 mm. between turns. [Glass thread sometimes tends to fray (perhaps owing to use of short staple in manufacture). Care should be taken to prevent formation of fine filaments, as these by touching the column tube cause rivulets. Use of a little water glass to cement filaments at frayed portions may be necessary. Experiments on the use of fine metallic wire (in place of glass thread) are in progress.] The segments thus prepared are then ready for insertion in the column tube. The lowest segment is kept centered at the bottom by a vertical glass rod having a conical top like the male end of a segment tube sealed into the L-shaped removable bottom closure at the base of the column as shown in Figure 1.

The still pot is made from a 500-cc. round-bottomed flask with a slight depression blown in the bottom. For heat insulation it is first covered with tinfoil, then with about 1 cm. of glass wool and 1 cm. of 85 per cent magnesia as paste, and after drying in an oven is finally coated with aluminum paint. Two opposite holes (2.5-cm., 1-inch diameter) are provided in the still-pot insulation to permit observation. To minimize heat losses these holes are normally kept stoppered. For withdrawing samples of liquid (in H. E. T. P. tests) a small siphon is used; it is made to fit the 10/30 female opening of the still-pot withdrawal tube. During column operation this opening is closed with a 10/30 male plug.

Heat for the distillation is applied through an internal glass "boiling tube" wound with glass thread and sealed into the still pot to promote smooth boiling (*13*). Arochlor to a depth of about 3 cm. in the boiling tube is heated by a 50-watt bayonet type heater and serves as the heat transfer medium. Distillation rate is kept relatively constant through automatic control of the heat input to the still pot by means of an oil manometer-manostat assembly (*10*) actuated by column pressure drop. One arm of the manometer-manostat is connected to the still pot and the other arm to the still head by way of the dry ice trap. The manometer-manostat serves both as a manometer for precise reading of the pressure drop, and for the control of distillation rate (*10*). A precision oil manometer (*6*) is used for reading actual distillation pressure which is automatically and precisely controlled (*12*). Reflux ratio is controlled by means of the low-holdup arc flow divider previously described (*14*).

Column Operation. The jacket heaters were adjusted to maintain a temperature gradient at least 1° C. below the gradient in the column. Upward adjustment of jacket temperatures during the progress of a fractionation was made by simply turning up the thermostat settings, each of the four jacket heaters being controlled by its own thermostat. The setting of the reflux ratio (to start "taking off") was made in the morning after equilibrium conditions had been established by an overnight run of the column at "total reflux". For a separation requiring the highest efficiency of the column, the throughput was set at about 80 cc. per hour and the reflux ratio at about 100, which is approximately equal (*17*) to the number of theoretical plates at this low throughput. Reflux rate was determined by counting drops at the still head.

Column Holdup and Pressure Drop. The holdup of the column when in operation was determined directly by boiling

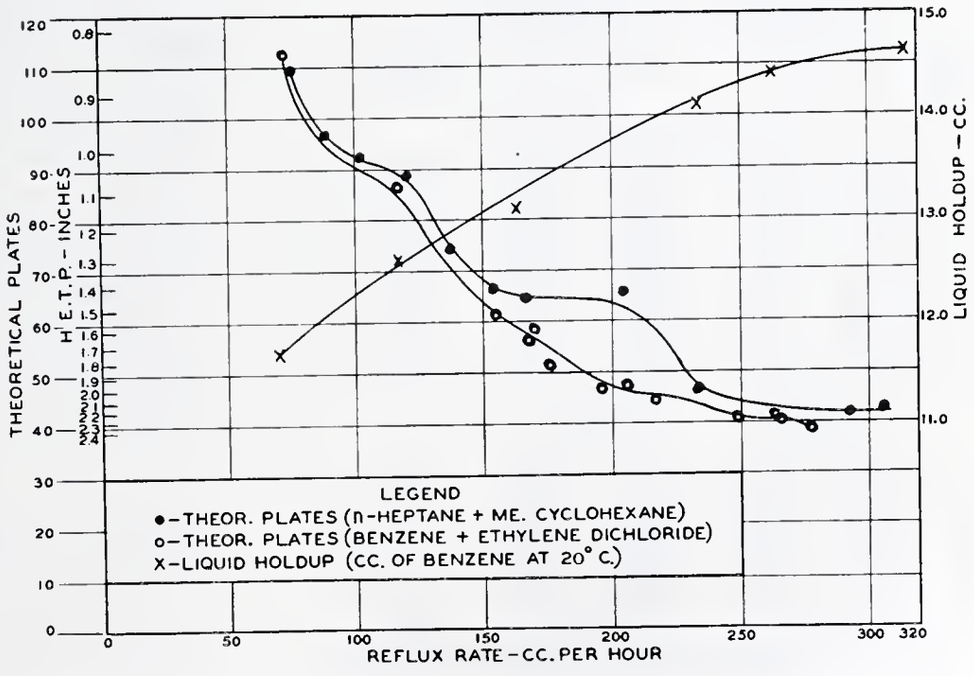
THEORETICAL PLATES
H.E.T.P. - INCHES
LIQUID HOLDUP - CC.
REFLUX RATE - CC. PER HOUR
LEGEND
●-THEOR. PLATES (n-HEPTANE + ME. CYCLOHEXANE)
o-THEOR. PLATES (BENZENE + ETHYLENE DICHLORIDE)
X-LIQUID HOLDUP (CC. OF BENZENE AT 20° C.)

Figure 3. Holdup Data

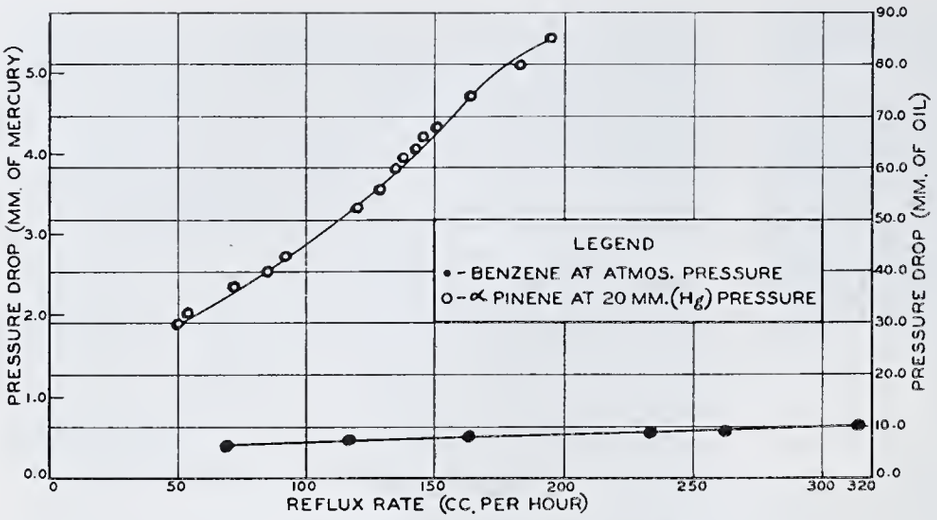
PRESSURE DROP (MM. OF MERCURY)
PRESSURE DROP (MM. OF OIL)
REFLUX RATE (CC. PER HOUR)
LEGEND
• - BENZENE AT ATMOS. PRESSURE
o - α PINENE AT 20 MM.(Hg) PRESSURE

FIGURE 4. PRESSURE DROP CURVES

benzene in the still pot at a given steady throughput, with the jacket temperature maintained at 79° C. This was accomplished as follows: When smooth boiling was attained and distillation rate stabilized the still pot was removed and quickly replaced with a special receiver fitting the bottom spherical joint which normally connects the column to the still pot. The heat was then shut off. During the change-over only a few drops at most of the benzene were lost. The column was then allowed to drain into the receiver for one hour and the volume of benzene thus collected was measured at 20° C. To this was added the nondrainable "static" holdup (0.6 cc.) determined as the difference between a known volume of benzene introduced at the top of the dry column and the volume collected in the receiver at the bottom after one hour of drainage. This direct method of obtaining dynamic holdup values was used in preference to the stearic acid method (*9*) because the former was found more reliable in giving check results.

The holdup data obtained are given in Table I and shown as a curve in Figure 3.

The pressure drop curves for distillation of benzene at 760 mm. and of α-pinene at 20 mm. (pressures at the still head) are shown in Figure 4.

EFFICIENCY TESTS. H. E. T. P. determinations were made at total reflux with two binary mixtures: (1) benzene and ethylene dichloride, and (2) *n*-heptane and methyl cyclohexane.

The components of these mixtures had been purified by fractionation until their respective physical constants checked with those given in the literature. Sufficient volume (200 cc.) of each binary mixture was used in the still pot to make the effect of the column holdup negligible in the H. E. T. P. calculations. Initially the column was run at total reflux and at fixed throughput for at least 16 hours. Still-head samples of a few drops were taken at intervals of one hour or more between consecutive samples until check results for refractive index were obtained. A small sample of liquid was then siphoned from the still pot.

The refractive indices were taken at room temperature with an

TABLE I. HOLDUP DATA

Reflux Rate[a]	Holdup of Benzene at 20° C.	No. of Theoretical Plates	Holdup per Theoretical Plate
Cc./hr.	*Cc.*		
70	11.7	110	0.11
117	12.6	89	0.14
163	13.1	66	0.20
234	14.1	46	0.31
263	14.4	42	0.34
315	14.6	40	0.37

[a] Measured at still head by counting drops.

TABLE II. EFFICIENCY TESTS

[Total packed length 238 cm. (95.3 inches)]

Reflux Rate	Refractive Index at 25.2° Head	Refractive Index at 25.2° Still	Mole Fraction Benzene Head	Mole Fraction Benzene Still	No. of Theoretical Plates	H. E. T. P.
Cc./hr.						*Inches*
		Benzene–ethylene dichloride				
73	1.4976	1.4432	0.9966	0.0189	112.5	0.83
117	1.4976	1.4437	0.9966	0.0283	86.6	1.08
155	1.4948	1.4436	0.9492	0.0264	61.1	1.53
168	1.4927	1.4435	0.9136	0.0245	56.4	1.66
170	1.4935	1.4435	0.9271	0.0245	58.3	1.61
176	1.4909	1.4438	0.8830	0.0302	51.2	1.83
196	1.4890	1.4441	0.8508	0.0359	46.8	2.00
206	1.4895	1.4441	0.8593	0.0359	47.5	1.97
217	1.4860	1.4439	0.8000	0.0321	44.6	2.10
249	1.4827	1.4440	0.7431	0.0340	40.9	2.29
264	1.4820	1.4438	0.7310	0.0302	41.5	2.26
266	1.4815	1.4439	0.7224	0.0321	40.5	2.31
278	1.4803	1.4440	0.7017	0.0340	39.0	2.40
	Refractive Index at 20.0° C.		Mole Fraction *n*-Heptane			
		n-Heptane–methyl cyclohexane				
75	1.3880	1.4210	0.9903	0.0550	109.4	0.86
88	1.3884	1.4210	0.9774	0.0550	96.8	0.97
102	1.3887	1.4210	0.9697	0.0550	92.3	1.01
120	1.3889	1.4210	0.9613	0.0550	88.6	1.06
138	1.3907	1.4210	0.9032	0.0550	74.1	1.26
154	1.3921	1.4208	0.8594	0.0600	66.4	1.41
167	1.3930	1.4210	0.8313	0.0550	64.6	1.45
205	1.3928	1.4210	0.8375	0.0550	65.3	1.43
234	1.3992	1.4206	0.6412	0.0650	47.0	1.99
293	1.4026	1.4207	0.5429	0.0525	41.6	2.25
306	1.4023	1.4208	0.5514	0.0600	42.7	2.19

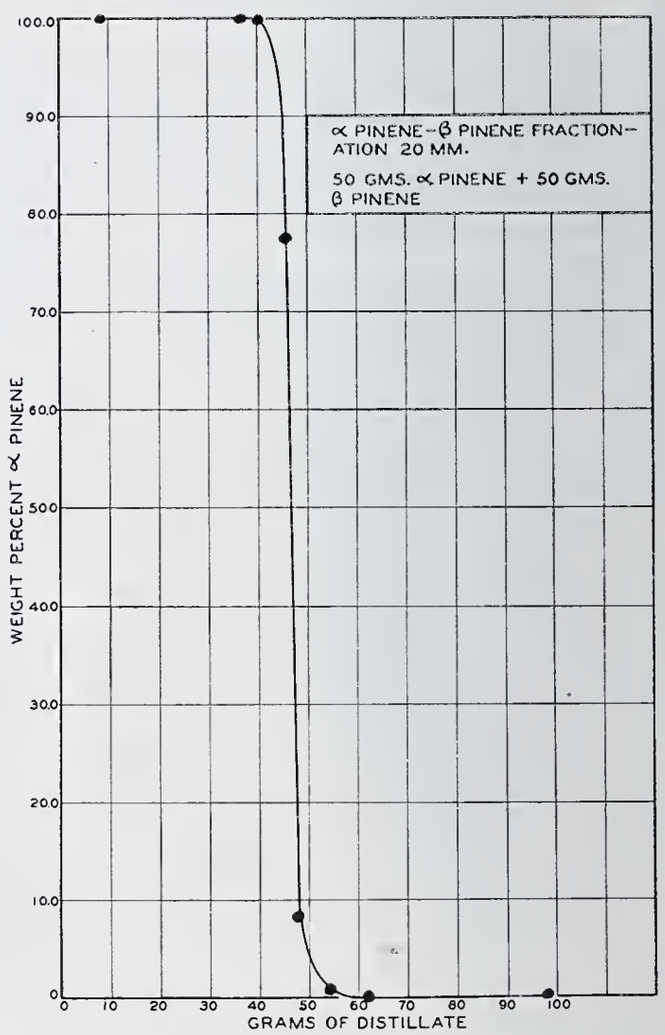

FIGURE 5

Abbe refractometer and then corrected. In order to obtain data for correcting observed refractive indices of the samples to the temperatures for which the published analytical data used are given, refractive indices of the respective pure components of the binary mixtures were determined at room temperature immediately after the readings on samples. This was found more reliable than using a temperature coefficient for correcting refractive index to the temperature desired. For the benzene–ethylene dichloride mixture the analytical data at 25.2° C. of Rosanoff and Easley (*16*) were used. The data at 20.0° C. of Bromiley and Quiggle (*3*) were used for the *n*-heptane–methyl cyclohexane mixture. The number of theoretical plates (minus one for the still pot) was calculated by the equation of Beatty and Calingaert (*2*). Relative volatility (*2*, *19*) values of 1.11 and 1.07 were used, respectively, in the calculations for benzene–ethylene dichloride and *n*-heptane–methyl cyclohexane mixtures.

These data are given in Table II and the efficiency curves in Figure 3.

Fractionation of a 50–50 α- and β-Pinene Mixture. A binary mixture composed of 50.0 grams of pure α-pinene and 50.0 grams of β-pinene was charged in the still pot. The pure components had the following properties:

	B. P. (20 Mm.) ° C.	$[\alpha]_D^{25°}$ (10-Cm. Tube)
α-Pinene	52.3	+29.81°
β-Pinene	59.5	−18.95°

The column was run at total reflux at a rate of 90 cc. per hour for 22 hours, after which take-off was begun with the reflux ratio set at approximately 100 to 1. The optical rotations of the fractions (at 20° C. in a 10-cm. tube) were determined, from which their respective compositions were obtained by applying the Biot equation (*8*).

The curve obtained (Figure 5) shows an intermediate fraction between pure α-pinene and pure β-pinene of about 17 grams, which is somewhat greater than the holdup of the column at this throughput (90 cc. per hour).

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Constant-Level Device for Hot Water Baths

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A METHOD was developed in this laboratory for maintaining a constant level in a water bath using distilled water, with a 10-gallon reserve supply of distilled water available through an automatic series–parallel siphoning feed system. The apparatus requires a modicum of care, the only attention necessary being the addition of distilled water at table height about once every 2 weeks and positive pumping to the level of the reservoir.

The advantages of the system are fourfold: (1) the water in the bath is always kept to a predetermined optimum level; (2) distilled water may be used at all times, thus eliminating scale formation commonly seen where tap water is employed; (3) additions to the supply in the reservoir may be made at table level rather than at reservoir level; and (4) the system requires attention only once in 2 weeks of constant use.

Figure 1 discloses the operation of the system. Bottles *A*, *B*, and *C* are 5-gallon distilled water bottles, common equipment in many laboratories. *B* and *C* constitute the reservoir; *A*, which is easily detachable, is for adding water to the reservoir. The reservoir is supported by a shelf at a convenient height above the water bath, *D*. This height is not limited by atmospheric pressure, which controls the height to which siphoning action is effective, but is governed by the hydrostatic pressure exerted upon the stopper in *A*. If the distance between shelves is greater than about 4.5 feet (a possible effective hydrostatic head of about 6.5 feet, after making allowance for the height of tube *J* above the upper shelf), it is difficult to avoid having the stopper of *A* blow out while water is being pumped into *B*.

When it is desired to pump water into the reservoir, pinchclamp *F* is opened and pinchclamp *G* is closed. As pressure is exerted inside *A* through rubber bulb *E* and water is forced into the bottle through *J*, the water in tube *H* descends until *H* is empty. After sufficient water has been pumped into *B*, *F* is closed and *G* is opened. Water immediately flows into the bath through *N* until its level is coincident with that of the lower open end of *H*, whereupon it enters *H* and rises to the level of the water in the reservoir. The water level in *B* and *C* is equilibrated by siphoning action through tube *K*.

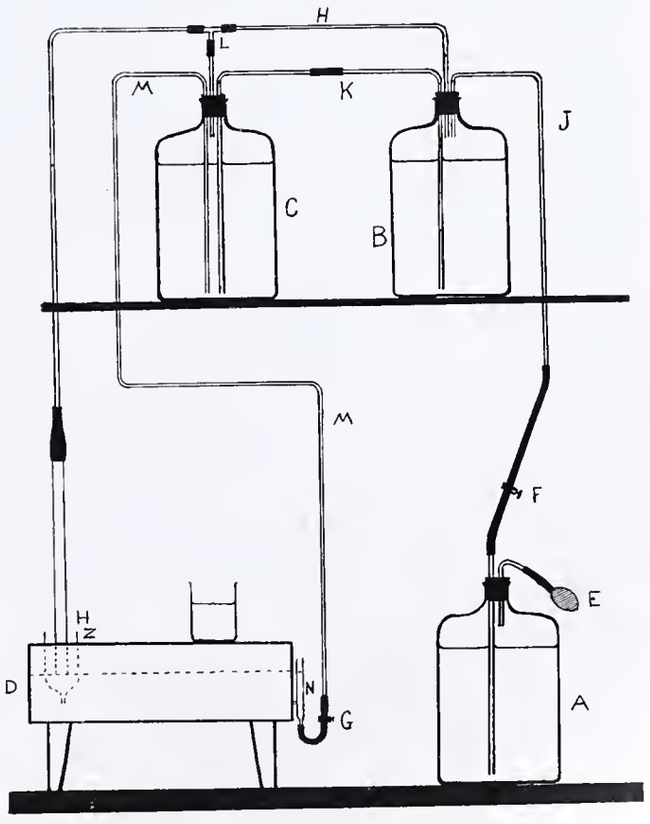

Figure 1. Constant-Level Device

The various possible effects of atmospheric pressure, while all are not pertinent to the efficiency of the system, are nevertheless interesting. The water in *H* rises until its level is the same as that obtaining inside the reservoir. If the reservoir were 40 feet above the water bath and the barometric pressure were 32 feet of water, the water in *H* would rise to a level 32 feet above the water in the bath. At this point a balance would be reached between atmospheric pressure and the weight of the column of water in *H*, and the two would offset each other. Inasmuch as the siphoning action in tubes *M* and *K* is dependent upon the ability of atmospheric pressure to force water to a height equal to the height to which these tubes extend above the level of the water in *C* and/or *B*, any nullification of the effects of atmospheric pressure stops the flow of water through the system. Such a nullification may be complete, as in the case just cited, or it may constitute a nullification of the "atmospheric head", as is the case during normal operation at heights less than about 30 feet. By atmospheric head is meant that portion of atmospheric pressure which is required to raise the level of the water in *H* to the same height as the level in the reservoir.

The lower portion of *H* should be of fairly large diameter to eliminate surface tension interferences. When sufficient water has been lost from the bath by evaporation to lower the level below the opening of *H*, air enters *H* and water runs down *H* into the bath. This results in a lowering of the water level in *H*, which usually empties, and the siphoning action in tube *M* begins. Since the level in *C* is thereby lowered, the siphoning action in *K* sets in. The complete cycle takes place about every half hour in actual practice. Inasmuch as the automatic emptying of *H* is accompanied by considerable agitation, this tube should be fastened securely in such a position that its open end is kept at the optimum level for the water in the bath. A convenient location for the entrance of *H* into the bath is through the smallest of the usual four sets of concentric rings. Tube *L* is a glass T held in place by rubber tubing.

As is shown in Figure 1, *H* opens into vessel *Z*, which is a modification of the Berzelius beaker suggested by Holmes (*6*). This vessel consists of a 1-liter conical glass percolator, the bottom opening of which is connected by rubber tubing to a glass capillary of 1.5-mm. inside diameter. The percolator is fastened in place so that approximately its upper two thirds extends above the opening of *H*.

Z accomplishes two things. First, if, as Holmes (*6*) states, a large flask immersed in the liquid of the bath is abruptly removed, the level of the water in *Z* is lowered so slowly (by virtue of the fact that the water must drain out through the small capillary) that the water outside of *Z* will revert to its original level before all the water in *H* runs out and allows the air pressure within the reservoirs to rise to atmospheric. The entrance of air into *H* lowers the water in *H* and increases the air pressure within the reservoirs slightly—just enough to start the flow of water into the bath and maintain it until the level is brought back to normal—but even if there were a sufficient lowering of the bath level to allow all the water in *H* to run out, the presence of *Z* makes it run out in slowly repeated small increments. The chances of such a large beaker being removed from the bath as to interfere seriously with the stabilizing action of *Z* are very remote.

The disadvantage of allowing the air pressure within the reservoirs to rise to atmospheric rests in the fact that water will not stop flowing into the bath until the water again rises in *H* to the same level as that obtaining in the reservoirs. From the time water begins to flow back into *H* until these equilibrium levels have been reached, water continues to flow into the bath, and when this finally ceases, the water in the bath is likely to be undesirably high. This condition is prevented by *Z*. If the bath is small, or changes of volume of the water are small, or if larger changes in level due to siphoning of water into the bath through *M* are not objectionable, *Z* may be omitted.

Secondly, in pumping water into the reservoirs from *A*, *Z* has sufficient water to stabilize the action of the *H* tube, through which excess air escapes during the filling process. At the conclusion of this pumping, in order to prevent the air pressure within the reservoirs from rising to too great an extent, the stopper in *A* should be removed, allowing all the water in *J* to flow back into *A*, the stopper should be replaced, *F* should be closed, and *G* should be opened, in this order.

It is the use of *L* in place of the Mariotte arrangement of Gerdel (*4*) which permits refilling of the reservoir. If it is desired, only one 5-gallon bottle may be used as a reservoir; on the other hand, more than two may be used, depending on the length of time the operator wishes the bath to be without attention.

This apparatus has been in daily use in this laboratory for over 6 months, and has been satisfactory in every way. The apparatus is of simple construction, requiring no glass blowing, but only the usual laboratory equipment such as standard tubing, bottles, etc. The literature contains a considerable number of articles on constant-level devices for water baths (*1–3, 5, 7–14*). The writer is indebted to the authors of all these papers for their several valuable contributions in tracing the history of constant-level devices up to the present time. That paper which reports a device most closely approximating the writer's is the one by Gerdel (*4*).

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A Simple Thyratron Circuit

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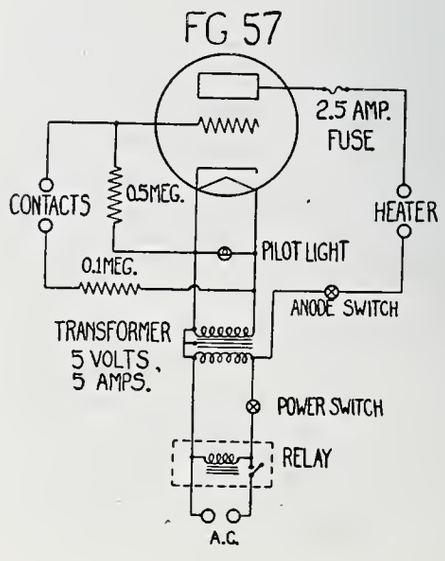
MANY circuits which employ a thyratron in conjunction with thermostat regulation have been described. While no particular claim of originality is made, the circuit shown in the figure is very simple. The values of the resistors employed are not critical and may be varied somewhat. The relay unit, although not essential for the operation of the thyratron, is included in order to prevent damage to the thyratron following periods of line failure, while in operation.

With line failure, the relay opens the circuit and must be closed mechanically to reapply power, after the anode switch is opened. The usual procedure is followed of allowing the cathode to come to operating temperature before closing the anode switch, which takes about 5 minutes.

The low current at the thermoregulator contacts (measured at 14 microamperes with the suggested resistor values) suggests other uses where such an on-off control may be used.



It is important that the secondary and primary of the transformer be connected so that measurement of the alternating current voltage between the two free ends shows a greater voltage than that across the primary.

Qualitative Analysis of Microgram Samples

General Technique and Confirmatory Tests

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The technique of working in the capillary cone has been extended to permit the performance of confirmatory tests after the separation of constituents. The tests are carried out on a circular plateau of 0.03- to 0.2-sq. mm. area obtained by cutting a glass thread of 0.2- to 0.5-mm. diameter. The thread is drawn out from the end of a short glass rod of 5-mm. diameter, which serves at the same time as condenser for concentration of the light in the plateau. The identification of approximately 0.001 microgram of the more common ions of the hydrogen sulfide group is assured in this manner.

The cell for the performance of the operations has been modified to accommodate a greater variety of tools, and better control of the operation of the micropipet has been obtained with the use of a plunger advanced by means of a screw with fine thread.

A PRECEDING paper (*2*) described a general working technique, which can be applied to the qualitative analysis of samples of 1 microgram mass or less. The procedure employed in separating the constituent ions was outlined, but the performance of confirmatory tests was not mentioned. This report may be considered an extension of the first paper, dealing with confirmatory tests and some modifications of the working technique. Various changes in the design of apparatus are in part required to facilitate the performance of confirmatory tests and in part suggested by the accumulation of experience in work of this kind.

Apparatus

CARRIER. To enable the mounting of a larger number of reagent capillaries, capillary cones, etc., the dimensions of the carrier slide have been changed to 35 × 25 × 7 mm. It may be cut from plate glass, or microscope slides of the proper dimensions may be cemented together to give the thickness desired. The thick slide is easier to handle and has the further advantage that it supports the capillaries in approximately the level in which the platform of the condenser rod is located.

MOIST AND DRY CHAMBER. The all-glass chamber, recommended for biological work by Chambers (*5*) and used in the preliminary experiments (*2*), was found unsatisfactory for two reasons: (1) It does not provide ample room to accommodate a condenser rod and a capillary carrier of the above-specified dimensions which would comfortably hold as many as six or seven capillaries without crowding; (2) it causes difficulties in manipulations, especially in removing and introducing capillaries.

The new chamber shown in Figure 1 has been found entirely satisfactory. The sides, *a*, consist of pieces of brass 67 mm. in length, 6 mm. in thickness, and 11 mm. in height. Four grooves run lengthwise, and, in addition, each bar has a vertical groove, *f*, at one end. The two pieces forming the side of the chamber are attached with screws to a brass frame, *b*, which in turn is mounted by means of De Khotinsky cement on a glass plate, *h* (68 mm. × 60 mm.), serving as the bottom of the chamber. The brass frame is 1 mm. thick and made to fit plate *h*, so as to give a border 5 mm. wide all around. This leaves an area approximately 48 × 57 mm. through which light can pass. A piece of glass slide, *c*, which fits the vertical grooves, *f*, forms the back of the cell. The top plate, *d*, is cut from an old projection slide, so that it can be freely moved when inserted into the uppermost of the horizontal grooves of the brass bars.

Thus, the dry chamber resembles a chess box with the cover sliding in and out and one end open. To convert it into a moist chamber, the remaining horizontal grooves of the brass bars are lined with cotton, *e*, which, if slightly moistened with water, produces a damp atmosphere in the cell.

FORCEPS. At least two forceps, one of which should be cork-tipped, are required. They should be made of stainless steel and have smooth (not corrugated or toothed) parallel tips. Cork-

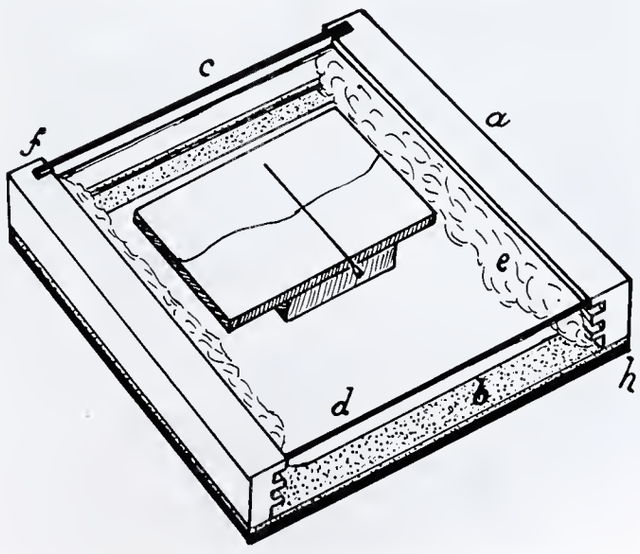

FIGURE 1. MOIST CHAMBER

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tipped forceps are necessary, for they prevent the loss of capillaries which often slip the grasp of plain forceps. The loss of a capillary cone may mean the untimely end of an investigation on which a great deal of work has already been spent.

Illumination of the Microscopic Field. Quick change from observation with transmitted light to observation with reflected light is most conveniently obtained by means of two microscope lamps. Any inexpensive source of light may be used for the illumination of the mirror of the microscope. The second lamp should be equipped with condenser lens and diaphragm, so as to give a narrow pencil of intense light which is sent in a horizontal direction through the rear window (*c*, Figure 1) into the interior of the chamber. To this end, the lamp is placed on one side of the microscope and mounted at the height of the stage of the microscope. Switches for both lamps should be located side by side within easy reach of the experimenter.

Micropipets. Micropipets having a bore of approximately 1μ at the opening were used at the outset and operated by means of a hypodermic syringe according to the directions of Chambers (*5*). It was found more convenient in work with capillary cones to give the micropipet a wider opening and to control the flow of liquid mechanically by means of a plunger, the motion of which is regulated by a screw with fine thread. The micropipets were prepared with the use of Rachele's device (*4*), and a portion of the gradually tapering fine tip was snipped off with a pair of forceps to give an opening 30 to 40μ in diameter.

Plunger Device. For use with micropipets of the above indicated wide opening at the tip, the plunger device designed by Johnson and Shrewsbury (*6*) was adopted. It is visible in the foreground of Figure 2. A flexible copper tubing connects the tubular chamber with the pipet holder in the clamp of the manipulator. The plunger is operated by rotation of the milled head. Tubular chamber, copper tubing, and pipet holder are filled with distilled water from which air has been removed by boiling. The water is allowed to occupy the first third of the shank of the micropipet. The rest of the shank remains filled with air which serves to separate the liquids in the micropipet proper from the hydraulic water.

Reservoir for Cleaning Fluid. A short buret is most convenient for use in cleaning micropipets. A fresh drop of cleaning fluid, usually water, is supplied for every rinse, so that it remains hanging from the tip of the buret. The micropipet is introduced into the hanging drop, and suction is applied with the plunger. When enough liquid has been taken in to fill shaftlet and taper, the pipet is withdrawn from the drop of cleaning liquid and its contents are expelled by touching a piece of filter paper to the opening of the micropipet and reversing the motion of the plunger. The manipulator used permits rotation around its vertical column as axis. The buret with the cleaning fluid is placed so that it is only necessary to withdraw the micropipet from the moist chamber and rotate the manipulator through 90 degrees to get the pipet to the tip of the buret.

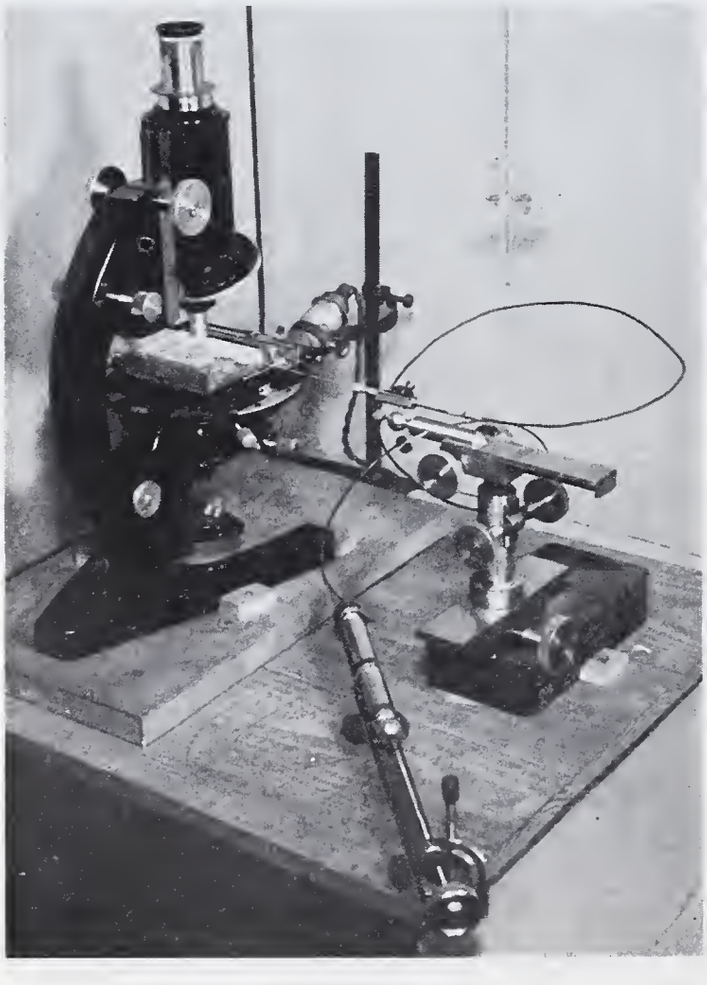

Figure 2. Working in the Capillary Cone

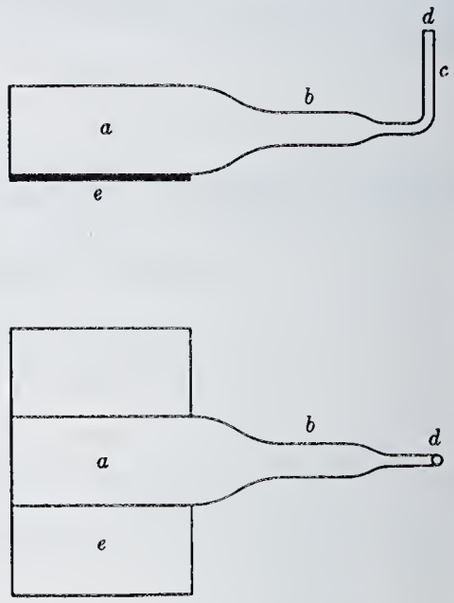

Figure 3. Condenser Rod

Condenser Rod. The condenser rod used in the performance of confirmatory tests is made of glass which shows only a little color when viewing through a layer 3 cm. thick. A rod about 4 to 5 mm. in diameter is drawn out to a finer rod (*b*, Figure 3), which is then cut so as to leave approximately 7 cm. attached to the original rod. Next, *b* is heated at a distance of 10 mm. from the taper and pulled out to a fine thread, *c*, of 0.2- to 0.5-mm. diameter, which is cut 20 mm. from the taper. The bend at *c* is obtained by holding the rod horizontal and approaching the thread with a small flame. If not too much heat is applied, the end of the thread will slowly swing through 90 degrees as it follows the pull of gravity. The original rod, *a*, is next cut to a length of 10 to 12 mm., scratched with a file, and then broken while held at *a* by means of a pair of pliers. Thread *c* is then dipped into molten paraffin and slowly withdrawn again. When the paraffin coating has solidified, the thread is cut approximately 5 mm. above the bend and broken off with forceps. The break should be clean and, as closely as possible, at a right angle to the axis of the thread. By means of some Duco cement, the rod is finally mounted on a small cover slide, *e*, so that the thread is vertical to the cover slip.

Confirmatory Tests

Qualitative analysis of samples of 1-microgram mass must permit the identification of 0.001 to 0.01 microgram of a minor constituent. These quantities are smaller than the limits of identification of most slide tests, and it follows that general use of the customary slide test technique is impossible with samples of 1 microgram mass. The limit of identification of the slide tests can be improved to meet the requirements by reducing the volumes of the test drops to 0.01 to 0.001 cu. mm. Even then, the crystals of the precipitates are so small, for lack of material, that as a rule their shape can no longer be safely discerned under the microscope. Thus, the use of the crystal form as criterion for the identification frequently has to be abandoned, and one is then reduced to the criteria employed with test-tube tests: appearance or disappearance of phases, and color phenomena. As in test-tube experiments, preference will be given to tests which give characteristically

and intensely colored precipitates and thus assure a high degree of specificity and sensitivity.

The tests could have been performed on the underside of the cover glass of the moist chamber by using either of the two techniques employed by Rachele (*4*). To prevent the spreading of the test drops, the cover slide would have to be coated with some material which repels the test solutions. The performance of confirmatory tests in this manner would be rather cumbersome, since a micropipet with a straight tip, which is required for the withdrawal of the test solution from a capillary cone, cannot very well deposit the solution on the cover of the cell without preliminary adjustments. In addition, the efficient illumination of small test drops located on the top plate of a cell 11 mm. high would suggest the use of specially designed condensers. It was therefore decided to try the confirmatory tests on the end of a rod condenser drawn out to a very fine tip.

The condenser rod (Figure 3) is placed in the moist chamber, so that the circular end surface of rod *a* faces the rear window of the chamber and is close to it. The condenser rod as well as the carrier, which is placed next to it, is kept from sliding about by wetting the under sides with a small drop of water. The film of water forming between the base plate of the chamber and the glass plates of the apparatus holds the latter in place. The moist chamber is mounted on the stage of the microscope so that the rear window faces the lamp mounted on the side of the stage, and the pencil of light is concentrated on the end surface of rod *a*. Once in the rod, most of the light is held there because of total reflection, concentrated into the narrow thread, and led around the bend so that it emerges as a narrow, vertical bundle through the small end surface, *d* (*7*). The tests are carried out on this end surface which has been called the "plateau". The paraffin coating of thread *c* prevents the test drops from flowing over the edge of the circular surface, and in this way it is possible to confine the drops within areas of 0.03 to 0.2 sq. mm., depending upon the diameter of *c*. In most of the work reported below, plateaus of 0.3-mm. diameter—i. e., 0.07 sq. mm. area—were employed.

It would be inconvenient to have the plateau less than 2 mm. below the top of the moist chamber. The objective used for the observation of the test will have to possess, therefore, a working distance of 3 mm. or more. Thus, the objective magnification is limited to approximately 20, and the plateau will not fill the field of vision of the microscope.

Test solution and reagent are transferred to the plateau by means of the micropipet. The plateau is focused with the microscope, and then the micropipet is guided with the manipulator, so that its tip appears in sharp focus close to the plateau. The pipet is slightly raised and moved horizontally, so that the opening of the tip appears above the plateau about half way between the center and the near edge of the platform (Figure 4, left). The pipet is next lowered so that it actually touches the platform and the image of the tip again becomes sharply defined. Application of pressure by means of the advancing plunger delivers the solution from the micropipet to the plateau. The pipet is then raised and withdrawn. The same general procedure is followed in adding reagent solutions. Slight pressure, however, is applied with the plunger before the opening of the micropipet is lowered into the test drop on the platform. This starts the outflow of the reagent the moment the opening of the pipet makes contact with the test solution, and clogging of the pipet by formation of precipitates inside the tip is prevented.

The use of solid reagents as in slide tests is not practical. It is obvious that tiny particles of somewhat hygroscopic substances will liquefy immediately when introduced into the moist chamber. More important are the difficulties connected with measuring small quantities of solid reagents. Thousand times smaller amounts of reagents are needed than in the analysis of milligram samples. The use of liquid reagents and the rapid mixing of the tiny drops of test solution and reagent may promote the formation of many small crystals of precipitate.

For determining the sensitivity of the tests to be described, the volumes of the test solutions were determined in measuring capillaries and those of the reagent solutions in the reagent containers. Both procedures have been described in the preceding paper (*2*). As a rule, 0.001 cu. mm. of the test solutions was taken. The limit of identification of the cadmium test was established as 0.01 microgram of cadmium. All the other tests gave positive results with 0.001 cu. mm. of 0.1 per cent solutions of the ions—i. e., 0.001 microgram of the metals concerned. It seems obvious that the sensitivity of most of these tests might be improved by the use of smaller drops on smaller plateaus.

Silver. An acidified silver nitrate solution 4 *M* in nitric acid was treated with an approximately equal volume of a saturated solution of potassium dichromate. The characteristic reddish-brown precipitate separated immediately, and a number of spear-shaped crystals could be discerned (Figure 4, right).

Mercury. Mercuric chloride solution containing 0.2 mole of nitric acid per liter was transferred to the platform of the condenser rod and treated with a slight excess of a freshly prepared solution of 1 part of *s*-diphenylcarbazide in 100 parts of ethyl alcohol. The violet precipitate was observed with a magnification of 150 diameters. The alcoholic solution of the carbazide assumes a light pink coloration on standing. It then becomes rather difficult to differentiate between the color of the reagent and that of the precipitate.

Lead. Triple nitrite reagent was prepared following the directions of Adams, Benedetti-Pichler, and Bryant (*1*). To prevent dilution, the container with this reagent was introduced into the moist chamber just previous to use. Four volumes of a solution containing approximately identical quantities of lead and copper were treated with 1 volume of the reagent solution. The characteristic black squares and rectangles of $K_2PbCu(NO_2)_6$ separated in a short time. With 0.001 microgram of lead it was necessary to cause partial evaporation of the final test drop by opening the top of the chamber somewhat.

Bismuth. A black precipitate of metallic bismuth was obtained immediately on adding a small excess of freshly prepared stannite solution (*3*) to solutions of bismuth nitrate.

Alkaloid iodobismuthites were investigated in the hope of getting strongly colored precipitates, and it was discovered that quinine permits immediate differentiation between bismuth and antimony because of the difference in the color of the precipitates. One gram of quinine bisulfate is dissolved in 50 ml. of warm water to which a drop of concentrated nitric acid has been added. A second solution is prepared by dissolving 2 grams of potassium iodide in 50 ml. of water. Small quantities of the reagent are made just previous to use by mixing equal volumes of the two solutions. Discoloration and separation of a precipitate will occur, if the ready mixed reagent is stored for more than a few days. In the preformance of the test, the bismuth solution is treated with an equal volume of the reagent. A brown precipitate of quinine iodobismuthite separates immediately, and some characteristic spherulites may usually be recognized.

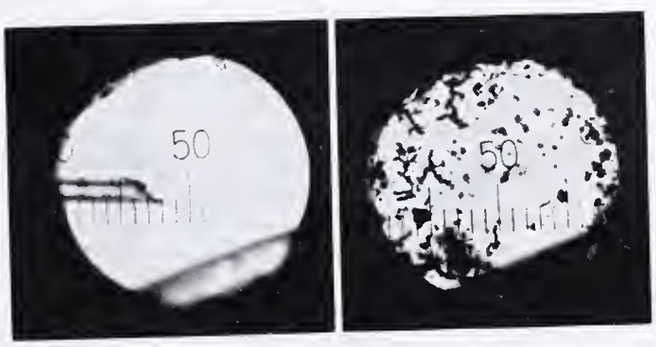

Figure 4. Confirmatory Tests on Platform of Condenser Rod

Diameter of field of vision of microscope is approximately 6 times diameter of plateau.
Left. Tip of micropipet above plateau
Right. Precipitate of silver dichromate

Copper. The copper solution was made to evaporate on the plateau of the condenser rod by partly opening the top of the moist chamber. The residue was then treated with a volume of a solution of salicylaldoxime (1 gram in 5 ml. of ethyl alcohol and 95 ml. of water) equal to the volume of test solution used. A greenish-yellow precipitate was easily discerned with low magnifications.

Cupric mercuric thiocyanate was precipitated by treating the copper test solution with an equal volume of ammonium mercuric thiocyanate reagent (8 grams of mercuric chloride and 9 grams of ammonium thiocyanate dissolved in 100 ml. of water). The

yellow precipitate obtained with 0.001 microgram of copper was distinctly visible when using a magnification of 150 diameters.

CADMIUM. The test solution was treated with an equal volume of a solution of sodium sulfide (10 grams of sodium sulfide novahydrate in 100 ml. of water). The yellow sulfide precipitate obtained with 0.01 microgram of cadmium was distinctly visible with the use of low magnifications.

ARSENIC. The test solution containing arsenate or arsenic acid was evaporated on the plateau of the condenser rod by partly opening the top of the moist chamber. The residue was treated with 0.001 cu. mm. of silver nitrate in acetate buffer solution (1 gram of silver nitrate, 7.7 grams of ammonium acetate, and 6 ml. of glacial acetic acid in 200 ml. of water). The reddish-brown precipitate of silver arsenate was easily discerned under low magnification.

ANTIMONY. The acid test solution was treated with the quinine–potassium iodide reagent as outlined for bismuth. A yellow precipitate was obtained.

TIN. The solution of stannic chloride was treated on the platform of the condenser rod with an equal volume of concentrated hydrochloric acid and then with a like volume of a saturated solution of rubidium chloride. After adding the reagents, the top of the cell was slightly opened to cause partial evaporation of the test drop. Crystals of rubidium chlorostannate separated in a short time. Using a magnification of 150 diameters, there was difficulty in discerning the shape of the crystals when 0.001 microgram of tin was taken for the test.

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Qualitative and Quantitative Analysis of Hydrocarbon Mixtures

By Means of Their Raman Spectra

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IT IS well known that Raman spectra and infrared absorption spectra are as characteristic of organic materials as any of the more common physical constants. Infrared absorption has been used frequently in recent years to solve qualitatively and quantitatively certain analytical problems both in fundamental research and in plant control. If the characteristic frequency of any infrared absorption band of a particular molecule and its extinction coefficient are known, the basis is laid for the quantitative determination of this molecular species in a mixture of compounds, providing no other complicating factors are introduced by the mixture.

There are undoubtedly many times when infrared analysis is too cumbersome, complicated, and expensive for the solution of certain analytical problems. The purpose of this paper is to add to the knowledge and technique of Raman spectra, using a relatively simple procedure, so that it is possible to make quantitative analyses of hydrocarbon mixtures within certain definite limits. It will be shown further that the method of internal standards provides a very satisfactory means of determining scattering coefficients. Scattering coefficient for the present purposes is defined as the ratio of the intensity of the hydrocarbon line in question to the intensity of a particular line of the internal standard. Seven and four-tenths volume per cent of carbon tetrachloride at 25° C. has been used in all cases as the internal standard. The use of internal standards is a commonly used spectroscopic procedure. All scattering coefficients refer to the ratio of the intensity of the hydrocarbon line in question, to the intensity of the 459 cm.$^{-1}$ frequency of this standard, unless stated otherwise. These scattering coefficients for analytical purposes are as truly physical constants of the molecule in question as the Raman frequencies themselves.

Mixtures of hydrocarbons can be analyzed by the above method or by any spectroscopic method, only if the characteristic lines or bands can be distinguished from each other. If too many coincidences occur in the spectrum, analysis is obviously impossible. The present paper deals only with binary mixtures. Three- and four-component mixtures have been analyzed in favorable cases. Thus the method is not a cure-all; there are definite limitations to its applicability.

References to the wave number determination of the Raman frequencies of the hydrocarbons investigated here have not been given because of their multiplicity. Ample information can be obtained from the extensive bibliographies on this subject (*4*, *5*).

Experimental Procedure

PRODUCTION OF THE SPECTRA. The Raman spectra (*7*, *8*, *9*) were in all cases excited by the λ4358 group of lines from the mercury arc. The light of the arc was made practically monochromatic in so far as excitation of Raman spectra in the blue and violet is concerned, by means of the sodium nitrite filter before impinging on the scattering material. This filter effectively removes wave lengths shorter than λ4358. The removal of the shorter wave lengths from the exciting light materially reduces the fluorescence of the material, should any fluorescent impurities be present. This fluorescence (*2*) has been troublesome to workers who attempt to obtain the Raman spectrum of hydrocarbons, especially if these compounds had petroleum as their origin. In the cases of the present investigation in which fluorescence did appear it was possible to remove the offending impurities by the simple expedient of filtering the material at ordinary temperature through a glass tube 50 cm. long and 7 mm. in diameter packed with activated alumina. The activation of this alumina was achieved by heating the material in a porcelain crucible to a bright red heat for about one hour.

The mercury arc itself provides a continuous spectrum in addition to the line spectrum. When observations are to be made of lines of such small intensity as Raman lines, and necessarily at low dispersion, the above-mentioned continuum sets the lower limit to the observable intensity of a line. As the praseodymium ammonium nitrate filter (*8*) removes a good portion of the mer-

cury continuum on the long wave length side of λ4358, particularly that part close to the line, it is possible to observe lines and measure their intensity, when they are so weak that they would be unobservable at the same dispersion if excited by means of unfiltered radiation.

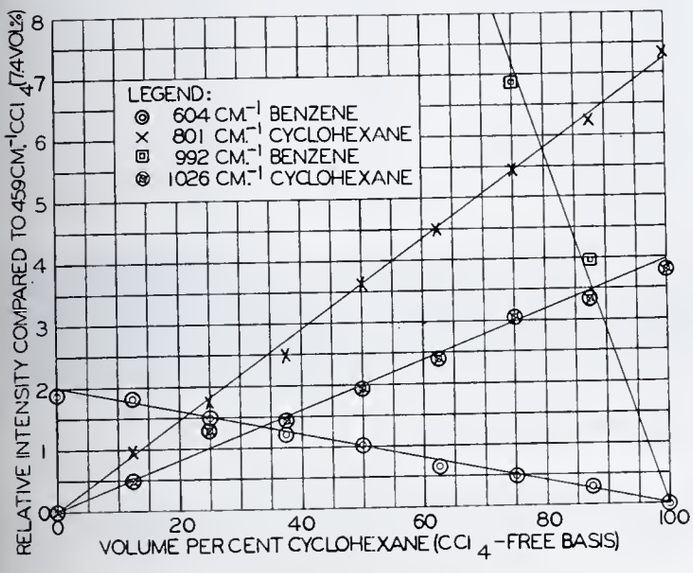

FIGURE 1. RELATIONSHIP BETWEEN RELATIVE INTENSITY AND VOLUME CONCENTRATION FOR MIXTURES OF BENZENE AND CYCLOHEXANE

The spectrograph used in this investigation was a three-prism glass instrument furnished with a Bausch & Lomb Tessar lens $f = 4.5$ as the objective. This lens had a focal length of 30 cm. The spectra produced were one third the size of the image on the slit. This instrument gave a dispersion of approximately 32 Å. per mm. at λ4500. The slit width used was 0.3 mm. which allowed the resolution of all sharp lines over 15 cm.$^{-1}$ apart. Exposure times varied from 1 to 3 hours, depending on the scattering power of the hydrocarbon under investigation. All the hydrocarbons which the authors have investigated have been obtained from the Petroleum Refining Laboratory of this college and were considered to be of exceptional purity (99.5+ per cent pure).

IDENTIFICATION OF CHARACTERISTIC FREQUENCIES. The measurement of the plates was performed in the conventional fashion using a comparator as the measuring instrument. The frequencies which were determined agreed in all cases with the measurements found in the literature within ±5 cm.$^{-1}$.

DETERMINATION OF SCATTERING COEFFICIENTS. Scattering coefficients have been determined only for the strong and moderately strong lines in the internal Raman spectrum of the hydrocarbons investigated in this paper, since these frequencies are the chief distinguishing features in their spectra.

The method which was used to determine the scattering coefficients is known as the method of internal standards. In the present case 2 cc. of carbon tetrachloride were added to 25 cc. of hydrocarbon. The intensity of the strongest hydrocarbon lines was measured relative to the 459 cm.$^{-1}$ frequency shift in the spectrum of the internal standard (carbon tetrachloride). The intensities in all cases were measured above the estimated background of the plate. The specific reasons for choosing the above given proportions of hydrocarbon and standard are as follows: (1) Carbon tetrachloride is miscible with all hydrocarbons. (2) Carbon tetrachloride is a relatively good scattering material, and thus in general the intensity of the 459 cm.$^{-1}$ line of carbon tetrachloride at the above concentration is such that in a proper exposure, it and the hydrocarbon lines will fall on the linear part of the density *vs.* log intensity curve. (3) The spectrum of carbon tetrachloride is very simple and it is well known that the 459 cm.$^{-1}$ line is very narrow (*6*), its breadth being about 4 cm.$^{-1}$. Thus the intensity of this line will be practically independent of the dispersion and the slit width employed if the spectra are photographed under small dispersion. (Incidentally, it is significant to note that with the dispersion and the slit widths which have been used, no correction need be made of the relative intensities of the Raman lines due to their dispersion breadth. This statement is true as long as the actual breadth of the lines is less than 10 cm.$^{-1}$. In the present tables of data on scattering coefficients no correction for dispersion breadth has been made. However, several cases have been indicated where lines are broader than the normal width of most of the Raman lines.)

The procedure adopted in making the intensity measurements was as follows: The plates were exposed in the spectrograph a sufficient time to obtain a proper exposure of the Raman lines. Suitable intensity marks were then impressed on the plates by means of a light source provided with an appropriate blue filter and a pair of nicol prisms. The time of exposure of the intensity marks was 2.0 seconds. Because of the disparity of the exposure time of the intensity marks and the Raman spectrum, it would seem that this procedure is not completely justified, owing to the failure of the reciprocity law for photographic plates. This failure of the reciprocity law for the present plates, Eastman 40, must have been slight, as can be seen from Figure 1. The experimental simplicity and convenience introduced by impressing the intensity marks as has been done seem to be justified by the results.

After development, fixation, washing, and drying, the plates were microphotometered. The microphotometer was designed and constructed here. It is of the photoelectric type provided with a photographic recorder. It is just able to indicate resolution of the λ doublets in the $2 \rightarrow 0$ band of the 2nd positive system of nitrogen taken in the 4th order of a 21-foot grating with a very narrow slit. More resolution could have been obtained with the microphotometer, but this expedient would have been undesirable because of the graininess of the fast plates which were of necessity used to make the exposure times reasonable. After photometering the Raman spectrum, the photometer was run over the intensity marks. In this manner a calibration was supplied for each plate.

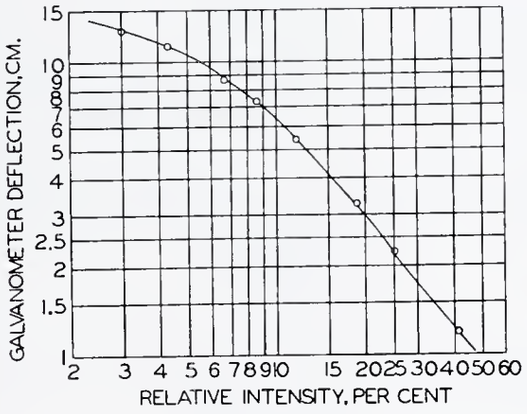

FIGURE 2. RELATIONSHIP BETWEEN GALVANOMETER DEFLECTION AND RELATIVE INTENSITY FOR *trans*-DECALIN

The calibration curve (*3*) for each plate was constructed by plotting the log of the galvanometer deflection against the log of the intensity for the calibration marks (Figure 2). The intensity varies from 0 per cent for the nicols in the crossed position to 100 per cent for the nicols in the open position. For any unknown line in the Raman spectrum the intensity corresponding to its peak on the microphotometer curve was read off the trace. The intensity of the background was determined in the same manner. The difference in the intensity of the peak minus the intensity of the background was taken as a measure of the line intensity. In a like manner the intensity of the internal standard wave length was measured.

When these measurements had been made, the scattering coefficient of the line in question could be computed, since the scattering coefficient is simply the ratio of the intensity of the Raman line to the intensity of the internal standard.

Table I. Properties of Hydrocarbons Studied

Hydrocarbon	R. I., n_D^{20}	Boiling Point at 760 mm. Hg, ° C.	Freezing Point, ° C.
Benzene	1.5012	80.1	5.53
Toluene	1.4969	110.65	....
Cyclopentane	1.4065	49.2	−93.47
Methylcyclopentane	1.4098	71.8	....
Cyclohexane	1.4263	80.8	6.44
Methylcyclohexane	1.4231	100.8	....
Ethylcyclopentane	1.4197	103.5	....
2-Methylbutane	1.3541	27.75	....
n-Pentane	1.3576	35.95	....
2,2-Dimethylbutane	1.3686	49.7	....
2,3-Dimethylbutane	1.3750	57.9	....
2-Methylpentane	1.3712	60.25	....
3-Methylpentane	1.3765	63.2	....
n-Hexane	1.3751	68.65	....
2,2,3-Trimethylbutane	1.3893	80.9	....
n-Heptane	1.3877	98.42	−90.64
2,2,4-Trimethylpentane	1.3915	99.24	−107.40
n-Octane	1.3978	125.65	....
Pentene-1	1.3713	29.95	....
2-Methylbutane-1	1.3776	31.05	....
trans-Pentene-2	1.3798	35.85	....
cis-Pentene-2	1.3814	36.65	....
2-Methylbutene-2	1.3874	38.6	....
2,4,4-Trimethylpentene-1	1.4088	101.2	....
2,4,4-Trimethylpentene-2	1.4160	104.5	....
trans-Decalin	1.4694	187.5	....
cis-Decalin	1.4810	196.0	....

Table II. Raman Frequency Shift and Scattering Coefficient

(The first number represents the Raman frequency shift and the number in parentheses immediately following is the scattering coefficient.)

Benzene

604 (2.00), 849 (0.53), 992*c* (0.78), 992*b* (2.15), 992*a* (29.4), 1178 (2.85), 1584 (2.76), 1603 (2.76)

Toluene

203 (5.42), 406 (1.42), 520 (2.31), 620 (1.15), 788 (7.61), 903 (0.62), 1003 (14.5), 1028 (3.69), 1150 (1.27), 1205 (3.88), 1375 (1.42), 1580 and 1603 (2.77 unresolved)

Cyclopentane

267 (0.17), 783 (0.45), 885*b* (0.39), 885*a* (6.67), 1027 (1.94), 1445 (1.89)

Methylcyclopentane

253 (0.38), 533 (0.52), 789 (0.29), 841 (0.43), 888 (2.76), 1010*d* (0.71), 1082 (0.38), 1304 (0.24), 1348 (0.20), 1449 (2.43)

Cyclohexane

380 (0.35), 422 (0.52), 801 (7.25), 1026 (4.00), 1154 (0.60), 1264 (3.26), 1342 (0.58), 1442 (3.26)

Methylcyclohexane

400 (0.39), 436 (1.56), 543 (0.70), 770 (4.47), 843 (1.01), 970 (0.70), 1028 (1.79), 1074 (0.78), 1158 (0.77), 1200 (0.39), 1253 (1.38), 1304 (0.23), 1343 (0.86), 1447 (2.88)

Ethylcyclopentane

392 (0.52), 423 (0.33), 757 (0.48), 851 (0.24), 891 (1.24), 931 (0.39), 1026 (1.29), 1082 (0.43), 1183 (0.24), 1288 (0.33), 1353 (0.24), 1446 (2.86)

2-Methylbutane

362 (0.23), 764 (1.42), 794 (0.85), 904 (0.20), 1024*d* (0.35), 1137 (0.23), 1341*d* (0.20), 1453*d* (1.58)

n-Pentane

263 (0.50), 398 (0.92), 764 (0.54), 837 (0.88), 863 (0.71), 1020 (0.54), 1068 (0.58), 1134 (0.42), 1296 (0.67), 1447 (2.12)

2,2-Dimethylbutane

250 (0.70), 357 (0.39), 403 (0.30), 483 (0.44), 715 (5.52), 870 (0.78), 928 (1.26), 1015 (0.57), 1072 (0.44), 1208 (1.26), 1248 (0.91), 1300 (0.43), 1447*d* (2.91)

2,3-Dimethylbutane

473 (0.64), 503 (0.50), 730 (2.43), 758 (1.14), 872 (0.50), 938 (1.79), 1030 (0.71), 1192 (0.50), 1297 (0.79), 1340 (0.79), 1380 (0.21), 1438 and 1463 (2.00 unresolved)

2-Methylpentane

318 (0.20), 444 (0.93), 811 (1.87), 884 (0.53), 945 (0.93), 1033 (1.07), 1135 (1.20), 1162 (1.07), 1300 (0.67), 1333 (0.80), 1448 (3.33)

3-Methylpentane

385 (0.27), 442 (1.24), 749 (0.81), 817 (0.59), 880 (0.32), 1037 (0.65), 1153 (0.38), 1273 (0.22), 1357 (0.27), 1453 (1.88)

n-Hexane

362 (0.35), 390 (0.26), 823 (0.44), 867 (0.52), 893*d* (0.70), 1032 (0.30), 1075 (0.57), 1132 (0.35), 1297 (0.83), 1433 (1.83), 1453 (2.26)

2,2,3-Trimethylbutene

262 (0.50), 358*d* (0.55), 523 (0.55), 634 (0.50), 692 (3.39), 917 (2.33), 1217*d* (1.22), 1251 (1.06), 1319 (0.55), 1452*d* (1.72)

n-Heptane

303 (0.88), 402 (0.35), 785 (0.41), 835 (0.53), 903 (0.65), 1073*d* (0.82), 1132 (0.41), 1302 (1.06), 1448 (2.53)

2,2,4-Trimethylpentane

258 (0.25), 509 (0.75), 742 (2.94), 820 (0.51), 893 (1.21), 923 (1.50), 1093 (0.63), 1196 (0.69), 1239 (0.75), 1277 (0.56), 1342 (0.44), 1450 (2.37)

n-Octane

282 (0.73), 837 (0.41), 867 (0.59), 884 (0.68), 962 (0.32), 1027 (0.46), 1075 (0.82), 1131 (0.55), 1298 (1.18), 1445*d* (2.91)

Pentene-1

388 (0.77), 438 (0.77), 613 (0.56), 765*d* (0.63), 815 (1.12), 879 (0.91), 911*d* (0.56), 993 (0.28), 1057 (0.77), 1091 (0.49), 1229*d* (0.63), 1293 (2.65), 1415 (1.75), 1448 (1.47), 1537 (0.21), 1581 (0.42), 1640 (5.17)

2-Methylbutene-1

258 (0.83), 394*d* (0.63), 433 (0.29), 494 (0.25), 528 (0.29), 708 (0.33), 733 (2.50), 795 (0.67), 880 (0.67), 937 (0.46), 1012 (1.04), 1080 (1.21), 1427*d* (2.29), 1649 (5.00)

trans-Pentene-2

258*d* (0.62), 409 (0.42), 488 (1.15), 748 (0.50), 796 (0.62), 859*d* (0.46), 937 (0.42), 1014 (0.69), 1059 (1.15), 1243 (0.65), 1296*d* (1.38), 1372 (0.79), 1440*d* (1.85), 1671 (2.81)

cis-Pentene-2

257*d* (0.46), 412 (0.36), 485 (0.50), 573 (0.21), 747 (0.36), 799 (0.50), 858 (1.00), 928*d* (0.32), 962 (0.29), 1020 (0.75), 1085 (0.50), 1252 (1.18), 1306 (0.93), 1367 (0.50), 1439*d* (1.97), 1640 (2.89)

2-Methylbutene-2

242 (0.70), 389*d* (0.85), 443 (0.60), 529 (1.30), 767 (3.30), 802 (0.35), 1048*d* (0.50), 1332 (1.50), 1382 (2.90), 1443 (3.35), 1677 (5.10)

2,4,4-Trimethylpentene-1

124*d* (0.22), 384 (0.30), 561 (0.78), 685 (1.61), 767 (1.74), 826 (0.39), 907*d* (1.13), 933 (1.00), 1040 (0.30), 1150 (0.57), 1193 (0.39), 1261 (0.61), 1407 (1.87), 1445*d* (2.04), 1640 (2.35)

2,4,4-Trimethylpentene-2

134 (0.76), 257 (0.20), 328 (0.63), 502 (0.24), 571 (1.00), 764 (3.81), 804 (0.43), 923 (1.43), 1022 (0.24), 1072 (0.62), 1152 (0.62), 1198 (0.91), 1383 (2.05), 1448 (3.33), 1663 (3.67)

trans-Decalin

298 (0.28), 407 (3.45), 493 (3.04), 751 (2.86), 844 (1.65), 873 (1.00), 985 (1.21), 1050 (3.79), 1178*d* (1.76), 1223 (1.90), 1249 (3.17), 1353 (2.45), 1443 (4.10)

cis-Decalin

142*d* (0.46), 257 (0.35), 318 (1.19), 361 (0.81), 377 (1.00), 445 (0.85), 594 (3.39), 741 (6.12), 792 (1.39), 848 (2.38), 883 (0.23), 928 (0.50), 974 (1.50), 1007 (1.46), 1037 (4.66), 1073 (1.69), 1111 (0.23), 1161 (0.58), 1210 (0.88), 1237 (4.00), 1267 (3.27), 1340 (1.65), 1438 (6.08)

a Strong Raman lines excited by 4358 Å. line of mercury.
b Raman line excited by 4348 Å. line of mercury.
c Raman line excited by 4339 Å. line of mercury.
d Diffuse line.

A microphotometer trace of the Raman spectrum of *trans*-decalin is reproduced in Figure 3, along with the spectrogram of this material. This spectrogram has been enlarged on such a scale that the lines correspond to the peaks on the microphotometer trace.

Results

The scattering coefficients for most of the stronger characteristic frequencies of each of the pure compounds which

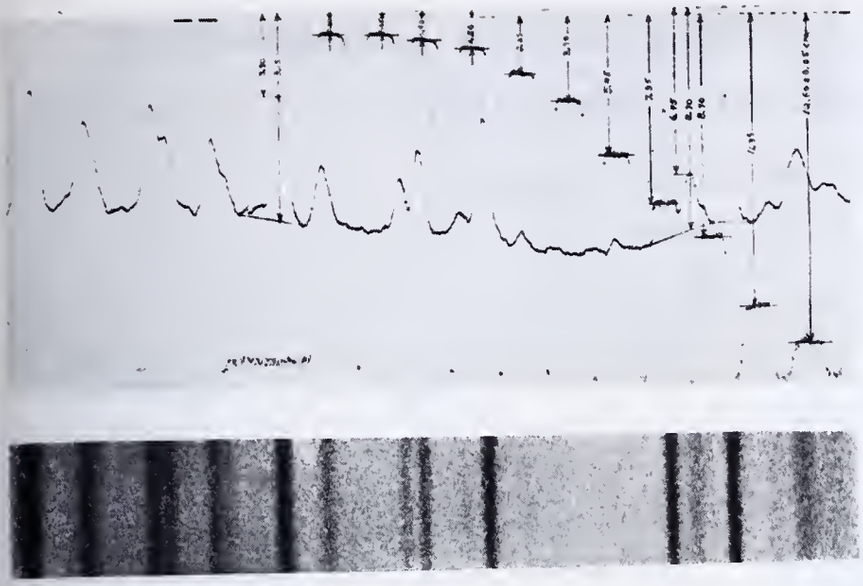

FIGURE 3. MICROPHOTOMETER TRACE AND SPECTROGRAM FOR *trans*-DECALIN

were investigated are given in Table II. The more common physical constants of these compounds are given in Table I. It seems certain that all these compounds had a purity of 99.5 per cent or better.

The following binary mixtures were examined in order to prove that there is a linear relationship between the relative intensity compared to the internal standard for the Raman lines, and the volume concentration of the compound (*1*): (1) benzene-cyclohexane, (2) *n*-heptane–2,2,4-trimethylpentane, (3) cyclopentane–2,2-dimethylbutane, (4) ethylcyclopentane-methylcyclohexane, (5) *cis*-pentene-2–*trans*-pentene-2, and (6) 2,4,4-trimethylpentene-1–2,2,4-trimethylpentane.

In each of the above-mentioned binary mixtures it was observed that the intensity of the Raman lines was proportional to the volume concentration of the compound within the limits of experimental error. In Figure 3 are plotted the results for the binary mixture benzene-cyclohexane. This pair of hydrocarbons exhibits azeotropic behavior, for they make a minimum constant-boiling mixture. However, it can be seen from Figure 3 that a linear relationship holds between the relative intensity compared to the internal standard and the volume concentration.

It seems probable from an analysis of the present measurements, that the scattering coefficients have been determined with an error not exceeding 8 per cent except in the case of the weaker lines, in which cases the error may be slightly greater. These weaker lines would, however, seldom be of any interest for analytical purposes. Successive determinations of scattering coefficients on different plates from the same compound were found to differ by an average of 6 per cent.

The lower limit of the detection of the contaminate in a binary mixture is largely determined by the magnitude of the scattering coefficient of said contaminate. In the case of benzene and cyclohexane (a particularly favorable case) it is easily possible to detect 1 per cent of benzene in cyclohexane. The normal paraffins are different and have small scattering coefficients. In many cases it might be impossible to detect less than 10 per cent of certain normal paraffins with the present Raman technique. Ternary and more complicated mixtures can be analyzed by means of this method, provided the lines of the various constituents of the mixture do not interfere with each other.

As illustrative of the utility and the necessity of the type of analyses herein described, the following examples may be cited. The usual physical properties, such as boiling point and refractive index, of 2,2,3-trimethylbutane are matched very closely by a mixture containing about 8 per cent cyclohexane and 92 per cent 2,2-dimethylpentane. This mixture must also exhibit some peculiarities in the vapor-liquid equilibria, for extensive fractional distillation was not able to disclose any appreciable shift in the composition. The Raman analytical procedure as described here readily distinguished these materials, and with its use there is no longer any reason for confusing the mentioned binary mixture with 2,2,3-trimethylbutane. The presence of 8 per cent cyclohexane in 2,2-dimethylpentane is readily determined from the Raman data.

In another example, a mixture was studied that was believed to contain both 2,2-dimethylpentane and 2,4-dimethylpentane. The Raman spectrum showed the predominance of the suspected 2,4-dimethylpentane, but the absence of 2,2-dimethylpentane. As yet it has not been possible to identify the contaminate, from either the Raman data or other physical properties.

The foregoing discussion illustrates the need for developing new analytical procedures, especially in regard to hydrocarbon analysis and identification. The usual physical data are very important in estimating the purity and in deciding, in many cases at least, what is present. But as analyses become more exact and the hydrocarbon mixtures more complex, recourse must be made to other methods such as that described here.

Conclusion

A linear relationship between the relative intensity of the Raman lines compared with an internal standard and the volume concentration has been demonstrated for six binary mixtures of hydrocarbons, one of which was an azeotropic mixture of minimum boiling point. This linear relationship is probably general for mixtures of nearly all hydrocarbons within the limits set for the determination of intensities of Raman lines by means of photographic plates.

Scattering coefficients are described and values for this constant are reported for a series of hydrocarbons.

Qualitative and quantitative analysis of hydrocarbon mixtures by means of Raman spectra could be substituted for infrared absorption analysis in cases where the components of the mixtures contain appreciable percentages of the constituents to be determined. There are conceivable cases where this method of analysis might succeed where infrared absorption would fail and vice versa.

Acknowledgment

The kind assistance of Stephen Lawroski in making available the hydrocarbons and their physical constants is gratefully acknowledged.

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Sulfur in Organic Compounds Containing Nitrogen and Halogen

Acidimetric Microdetermination

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THE disadvantages of the gravimetric microdetermination of sulfur in organic substances are so well known that many investigators (*1–4*) have sought, more or less successfully, to develop a volumetric procedure to replace it.

In the case of a sample containing only carbon, hydrogen, oxygen, and sulfur, the problem is simple: The sulfuric acid resulting from the combustion in oxygen can be titrated with standard sodium hydroxide (*5*). If the sample contains nitrogen or halogen, the problem is more difficult. The nitric or halogen acid must be evaporated without loss of sulfuric acid (*3*), or the sulfate must be titrated with barium chloride with tetrahydroxyquinone as indicator (*4*). Although the latter method has some staunch supporters, many analysts find the end point difficult. Friedrich and Watzlaweck (*3*) state that simple evaporation on a steam bath will not serve to separate nitric and halogen acids from sulfuric acid. They recommend a complicated system of titrations and countertitrations designed to hold the sulfuric acid back as sodium bisulfate while the nitric and halogen acids are evaporated.

The authors have found that the low results obtained on simple evaporation of a solution of sulfuric acid are not entirely due to volatilization of that acid, but that neutralization of the sulfuric acid by ammonia from the laboratory air accounts for part of the apparent loss. By evaporation in a stream of purified air under uniform conditions, nitric and halogen acids can be completely separated from sulfuric acid with only a small, constant loss of the latter.

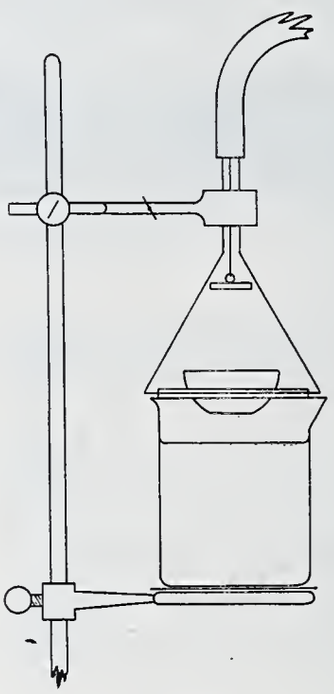

FIGURE 1

Apparatus

The combustion spiral tube is of the customary design (*5*), except that it must be made of transparent silica. Glass tubes are slightly decomposed at the temperature of combustion with resultant liberation of alkali that neutralizes some of the sulfuric acid. Opaque silica is too porous to permit satisfactory washing. The spiral of the combustion tube and the test tube used to cover the end may be of Pyrex.

The evaporating dish must also be of transparent silica. Dishes 3.75 cm. (1.5 inches) tall and 5.6 cm. (2.25 inches) in diameter, holding 65 ml., were used in this work.

The evaporation occurred in an air conditioner illustrated in Figure 1. The evaporating dish rested on a porcelain ring supported by a beaker, which served as a steam bath. A funnel was inverted over the dish. Air was purified by passage through concentrated sodium hydroxide, concentrated sulfuric acid, and a large bottle serving as a settling vessel for droplets of sulfuric acid, and was finally led into the stem of the funnel at a rate of 9 liters per minute. (Rates of 5 to 20 liters per minute were found satisfactory.) When the air stream impinged directly on the surface of the evaporating solution, excessive loss of sulfuric acid was incurred. This was avoided by employing an ordinary crucible cover as a baffle. The cover was suspended by string which was held in place by the rubber tubing over the end of the funnel.

Procedure

A sample of 4 to 6 mg. is weighed in a platinum boat and subjected to the usual combustion in the spiral tube (*5*). After combustion, the contents of the spiral tube are rinsed into the evaporating dish by five 2-ml. portions of water. If the tube is clamped vertically and the water added quickly, complete transference is achieved with this volume of wash water. Each portion of wash water is caught in the Pyrex test tube and then transferred to the dish.

The dish is then set on the steam bath (Figure 1) for 45 minutes. The evaporation appears to be complete in 30 minutes, but 40 to 45 minutes are required to drive off nitric acid completely.

After cooling, the contents of the dish are titrated with standard 0.01 *N* sodium hydroxide to the methyl red end point. The sodium hydroxide may be conveniently standardized against potassium biniodate or sulfamic acid with the same indicator.

TABLE I. RESULTS OBTAINED FROM MARCH 13 TO JUNE 21, 1940

(Blank correction = +10γ sulfur)

Compound	Formula	Sulfur Theory %	Sulfur Found %	Mean Error %	Mean Deviation %
Benzyl sulfide	$C_{14}H_{14}S$	14.97	14.92	−0.05	0.18
Sulfanilic acid	$C_6H_7O_3NS$	18.51	18.65	+0.14	0.06
Impure diphenylthiourea	$C_{13}H_{12}N_2S$	14.04[a]	14.83	+0.03	0.08
2,4 - Dichlorophenyl-4-toluenesulfonate	$C_{13}H_{10}O_3Cl_2S$	10.11	10.17	+0.06	0.01
2,4 - Dichlorophenyl-3,4-dichlorobenzenesulfonate	$C_{12}H_6O_3Cl_4S$	8.61	8.62	+0.01	0.07
Bromocresol purple	$C_{12}H_{16}O_5Br_2S$	5.96	5.85	−0.11	0.08
2,4,6-Tribromophenyl-*p*-toluenesulfonate	$C_{13}H_9O_3Br_3S$	6.61	6.79	+0.18	0.01
4 - Nitrochlorobenzene-2-sulfonic acid dihydrate	$C_6H_4O_5NClS.2H_2O$	11.71	11.49	−0.22	0.02

[a] Impure compound. Mean of several macrodeterminations with a Parr peroxide bomb was 14.80% sulfur.

TABLE II. RESULTS OBTAINED FROM JULY 11 TO JULY 18, 1941

(Blank correction = +21γ sulfur)

Compound	Formula	Sulfur Theory %	Sulfur Found %	Mean Error %	Mean Deviation %
7 - Iodo - 8 - hydroxyquinoline - 5 - sulfonic acid	$C_9H_6O_4INS$	9.13	9.22	+0.09	0.07
8 - Methyl - 2,2′ - diethylthiocarbocyanine iodide	$C_{22}H_{23}IN_2S_2$	12.66	12.56	−0.10	0.06
Methylbenzothiazoleethiodide	$C_{10}H_{12}INS$	10.51	10.59	+0.08	0.03
Ethylphenylthiohydantoin	$C_{11}H_{12}ON_2S$	14.55	14.49	−0.06	0.01

BLANK. A blank determination is performed as follows: The combustion tube is heated just as in a combustion, but no sample is taken. The tube is rinsed into the evaporating dish, and a measured volume of standard sulfuric acid (usually 2 ml. of 0.01 *N* solution) is added. The evaporation is conducted for 45 minutes, and the contents are titrated with sodium hydroxide as usual. Any difference between the sulfuric acid taken and that found by the titration is added to or subtracted from—as the case may require—the sulfur found in an actual determination.

The blank corrects for acids in the hydrogen peroxide, alkali acquired from the glass spiral, volatilization of sulfuric acid, and neutralization of sulfuric acid by ammonia from the air. The blank correction increases slightly with continued use of the combustion tube. This phenomenon, which is illustrated by a comparison of the blank correction of Table I with that of Table II, is probably due to a slow deterioration of the glass spiral during the combustion.

Results

Some typical results obtained by this method are given in Tables I and II. Each entry in the table is the mean of two or more determinations.

Discussion

This method yields accurate results in the presence of large amounts of nitrogen, chlorine, bromine, and iodine. It requires less time than the method of Friedrich and Watzlaweck (*3*) and gives a much sharper end point than the method of Hallet and Kuipers (*4*), but is not applicable to metal organic compounds. In such cases some of the sulfur is retained in the boat as metallic sulfate.

An attempt was made to apply the evaporation and titration of sulfuric acid to the determination of sulfur, following an oxidation of the organic sample by the micro-Carius method. This was not successful because alkali is extracted from the glass bomb during the oxidation. Furthermore, a longer period of evaporation is necessary to remove the large quantity of nitric acid.

Acknowledgment

The authors wish to express their indebtedness to E. B. Middleton of the Du Pont Film Manufacturing Corporation, Parlin, N. J., for several of the samples used in the analyses.

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Adaptation of an Indirect Method for Potassium to the Photoelectric Colorimeter

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The determination of potassium as potassium sodium cobaltinitrite with nitroso R salt (disodium salt of 1-nitroso-2-hydroxy-3,6-naphthalenedisulfonic acid) can be made with much greater precision using a photoelectric colorimeter with appropriate light filters instead of an optical colorimeter. The range of concentrations best suited is from 0.5 to 15 micrograms.

AN APPRECIABLE improvement was found by using a photoelectric instead of an optical colorimeter in connection with the colorimetric method of the author (*4*) for cobalt and potassium within a range of concentrations from 0.5 to 15 micrograms of potassium or from 1.0 to 30 micrograms of cobalt.

Potassium is precipitated as potassium sodium cobaltinitrite as in the original method. The precipitate is dissolved and its potassium content is determined indirectly by measuring colorimetrically with a photoelectric colorimeter (Klett or other type) the color intensities of the wine-red pigment of nitroso R salt with different amounts of cobalt which are directly proportional to those of potassium in the sodium potassium cobaltinitrite precipitate.

Reagents

NITROSO R SALT. Dissolve 1 gram of nitroso R salt in 70 ml. of water and then add 30 ml. of iron-free acetone.

SODIUM ACETATE. Place 544.3 grams of sodium acetate trihydrate in a 1000-ml. volumetric flask, add water to complete volume, heat over a steam bath, and filter.

SODIUM COBALTINITRITE. Dissolve 12.5 grams in 100 ml. of water and filter. Only immediately before using, portions of this reagent should be mixed with equal volumes of 95 per cent alcohol because the mixture is not very stable after mixing and should not be prepared in greater amounts than those needed immediately.

STANDARD COBALT SOLUTION. Dissolve 3.043 grams of cobalt chloride hexahydrate in water, add 5 ml. of 5 *M* hydrochloric acid, and dilute with water to 1 liter. One milliliter of this solution contains 0.7537 mg. of cobalt, which is equivalent to 1.000 mg. of potassium. This standard is relatively concentrated and aliquots taken for comparisons must be diluted from 100 to 500 times.

10 SODIUM HYDROXIDE. Place 400 grams of sodium hydroxide in a 1000-ml. volumetric flask and make to volume with water.

Procedure

Ash a quantity of plant tissues (0.5 to 1.0 gram) containing 0.04 to 0.10 mg. of potassium in a platinum crucible, and dissolve the ash in 5 ml. of 1 per cent hydrochloric acid. If analyses for other elements are to be made in the same material, filter to remove silica and other precipitates, and take an aliquot for the determination. If not, make the entire volume of the solution alkaline to phenolphthalein by adding a few drops of a 40 per cent solution of sodium hydroxide. Warm on a hot plate to effect flocculation of iron and of vanadium which interfere with the potassium and cobalt determinations and then either centrifuge or filter to remove the precipitate. Make to 100 ml. with water.

Take an aliquot of the centrifugate or filtrate, either 5 or 10 ml., and place in a 50-ml. beaker. Neutralize and make decidedly acid with 1 ml. of 1 per cent hydrochloric acid. Place the beaker over a steam bath and evaporate to dryness until all fumes of hydrochloric acid have disappeared. Add to the beaker after cooling about 0.5 ml. of water and then 10 ml. of the sodium cobaltinitrite–alcohol reagent. Place the mixture in a refrigerator for 2 to 4 hours and cover it to protect it from ammonia fumes. Filter through a fritted-glass crucible (Corning No. F)

Table I. Readings of Color Intensities of the Cobalt–Nitroso R Salt Pigment

Equivalent to different amounts of potassium as indicated by a Klett-Summerson photoelectric colorimeter)

Potassium γ	Photoelectric Colorimeter Readings
0.25	10
0.50	16
1.00	24
2.00	40
4.00	74
8.00	137
10.00	170
12.50	208
15.00	240
17.50	260
20.00	296
22.50	310

Table II. Variations in Potassium Recovered from Mixture

(As indicated by colorimeter readings)

Sample No.	Sodium in Reagent Mg.	Sodium Mg.	Potassium Mg.	Na: K Ratio	Potassium in Sample Analyzed Mg.	Colorimeter Reading	Deviation %
1	140	0.5	0.6	233.0 + 0.83	6.0	106	−1.85
2	140	1.0	0.6	233.0 + 1.66	6.0	113	+4.60
3	140	2.0	0.6	233.0 + 3.34	6.0	110	−1.85
4	140	4.0	0.6	233.0 + 6.67	6.0	108	0
5	140	8.0	0.6	233.0 + 13.34	6.0	112	+3.70
6	140	0	0.6	233.0 + 0	6.0	104	..
7	140	1.2	0	.....	..	4	..
6 + 7	..	..	..	.....	..	108	..

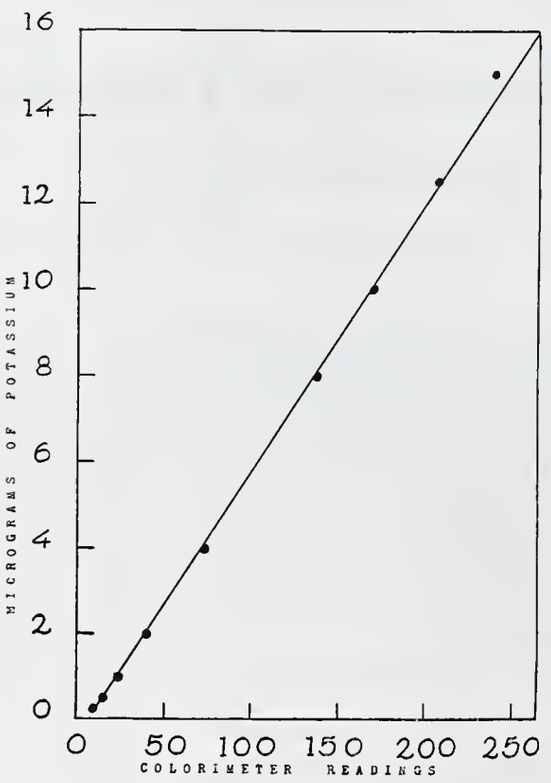

Figure 1. Determination of Potassium

and wash the precipitate repeatedly with an aqueous solution of 80 to 90 per cent iron-free acetone until the acetone solution flows free from the yellow color of the cohaltinitrite reagent. Rinse the exterior surface of the crucible with water to free it from adhering drops of the cohaltinitrite reagent and then transfer it to the heaker where the precipitation of potassium had been originally effected. Add to the precipitate in the crucihle 5 ml. of a normal solution of sulfuric acid. If the volume of the potassium sodium cohaltinitrite precipitate is very great, add 10 instead of 5 ml. of the sulfuric acid reagent.

In the space hetween the crucihle and heaker pour water until the beaker is half full. Place the heaker over a steam hath to speed solution of the precipitate. After complete solution of the precipitate raise the crucible ahove the heaker and rinse the exterior surface with a fine stream of water from a wash hottle, then transfer the crucihle to a ruhher crucible holder fitted to the mouth of a suction flask. Remove the solution in the pores of the crucihle with gentle suction and by repeated washings with small volumes of water. Transfer the solution in the heaker and in the suction flask to a 100-ml. volumetric flask and complete the volume with the washings from hoth the beaker and suction flask.

Place an aliquot of 20 ml. in a graduated test tube and add 2 ml. of an aqueous solution of 4 molar sodium acetate and 1 ml. of an aqueous acetone solution of 1 per cent of nitroso R salt. If the sample is found to contain more than 15 micrograms of potassium per ml. of solution, take a new aliquot and dilute to a convenient volume. Take 20 ml. of the diluted sample and treat with the reagents as recommended ahove.

The ratio of volumes of normal sulfuric acid to 4 molar sodium acetate should he in the final dilution less than 1 to 2; otherwise the red wine color of the complex cohalt–nitroso R salt compound will not develop. In cases where the ratio values of sulfuric acid to sodium acetate are greater and the appearance of the color is inhihited, the addition of a few drops of a 10 molar solution of sodium hydroxide will bring out the color, pH values 8 to 10 being most suitahle.

A Klett-Summerson or some other photoelectric colorimeter employing a 10-mm. cell with a 7.5-mm. plunger or a net depth of solution of 2.5 mm. can be used very satisfactorily. Light filter No. 47 was found thoroughly satisfactory and is recommended.

Data obtained by the method as recommended above are reported in Table I and Figure 1.

Effects of Different Sodium-Potassium Ratios on the Amounts of Recovered Potassium

Studies were conducted on the effects of different amounts of sodium on the formation of the sodium potassium cobaltinitrite precipitate, with results as reported in Table II.

The results show that the addition of different amounts of sodium, as stated in Table II, had no appreciable effects on the values of potassium, the deviations of −1.85 to +4.60 per cent being within the range of experimental errors. Bonneau (*1*), van Rysselberge (*3*), Peters and Van Slyke (*2*), and Snell and Snell (*5*) have presented an excellent account of the cobaltinitrite method in its application to potassium determination and also of the controversy, in the literature, of the effects of different sodium-potassium ratios on the weight of the sodium potassium cobaltinitrite precipitate. It is pointed out in the literature that variations in the weight of the precipitate are due in part to variations in the water of hydration and not in the chemical composition of the precipitate. The rest of the molecule, according to Bonneau, has the constant composition $K_2NaCo(NO_2)_6$ if, when the precipitate is formed, the sodium-potassium ratio in the solution exceeds 22, including the sodium of the reagent.

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Correction. In the article entitled "Extraction and Assay of Nicotinic Acid from Animal and Plant Tissues" [Ind. Eng. Chem., Anal. Ed., 14, 671 (1942)] the figure for nicotinic acid content of green peas digested with takadiastase should he 20 and not 35 micrograms per gram of fresh tissue.

R. R. Williams

Effect of Formaldehyde on the Volatilizations of Ammonia, Mono-, Di-, and Trimethylamines

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THE addition of formaldehyde to solutions of ammonium chloride renders the ammonia nonvolatile. Formaldehyde has no effect on the volatilization of trimethylamine. Mono- and dimethylamines give recovery curves showing points of minimum recovery, which can be explained in the case of dimethylamine on the basis of addition of methanol.

The use of formaldehyde for the separation of trimethylamine from mono- and dimethylamines and ammonia was first suggested by Delépine (*4*) and has been applied widely. Delépine separated the amines by fractional distillation at atmospheric pressure of concentrated solutions containing formaldehyde and alkali. Trimethylamine was volatilized first. The dimethylamine derivative, $CH_2[N(CH_3)_2]_2 . CH_3OH . 1/3H_2O$, contained a molecule of methanol, which was present as an impurity in the formaldehyde solution used. The monomethylamine derivative had the formula $(CH_3N{=}CH_2)_3$. He found that the corresponding amine could be regenerated easily from these compounds by treatment with alcohol in an acid solution.

Budai (*3*) tried locking up ammonia in mixtures of it and trimethylamine by the addition of formaldehyde. When he distilled the resulting solution at atmospheric pressure, he found that some (about 6 per cent) of the hexamethylenetetramine volatilized with the trimethylamine. He then devised a method based on formol titration of ammonia, followed by estimation of total base. Trimethylamine was obtained by difference.

Weber and Wilson (*8*), attempting to analyze mixtures of mono-, di-, and trimethylamines and ammonia, confirmed Budai's results but found that his formol titration method was unsatisfactory if mono- or dimethylamines were present.

In spite of the fact that no critical studies on the use of formaldehyde in this connection have appeared other than those mentioned, several workers (*1, 2, 6, 7*) have applied the technique of direct addition of formaldehyde to the amine-containing solution, followed by volatilization near room temperature, to the determination of trimethylamine in biological materials.

When a further attempt was made in the authors' laboratories to adapt distillation in the presence of formaldehyde to the separation of trimethylamine from ammonia and mono- and dimethylamines, certain anomalies that have not been reported in the literature were observed with mono- and dimethylamines.

Table I. Theoretical and Experimental Percentages of Chloride in Substituted Ammonium Chlorides

Salt	Theoretical %	Experimental %
CH_3H_3NCl	52.52	52.66
$(CH_3)_2H_2NCl$	43.48	43.67
$(CH_3)_3HNCl$	37.10	37.14

Experimental

Reagents. Ammonium chloride, Baker's C. P. grade, was used without further purification.

Mono-, di-, and trimethylammonium chlorides were prepared by distillation of Eastman's mono- and dimethylamines (33 per cent aqueous) and Commercial Solvent Corporation's trimethylamine (25 per cent aqueous) into an excess of concentrated hydrochloric acid, using tinfoil-lined stoppers. The acid solutions containing the substituted ammonium chlorides were evaporated to a small volume. The crystals obtained on cooling were dried at 110° C. for approximately 24 hours. The crystalline material was then ground to remove any mechanically trapped hydrochloric acid and was dried for 24 additional hours. Solutions of the salts prepared in this manner showed no excess acidity. When analyzed for chloride by the Volhard method, the results given in Table I were obtained.

On distillation, the trimethylamine was found to be contaminated with lower amines. A purified product was prepared by reduction of a solution of crystalline trimethylamine oxide, prepared by the method of Dunstan and Goulding (*5*). The reduction was made with Devarda's alloy in hydrochloric acid solution. The trimethylamine so produced was distilled from an alkaline solution into an excess of low-nitrogen sulfuric acid, which was then diluted. The molarity of the resulting amine solution was determined by distillation into standard acid, followed by titration of the excess acid.

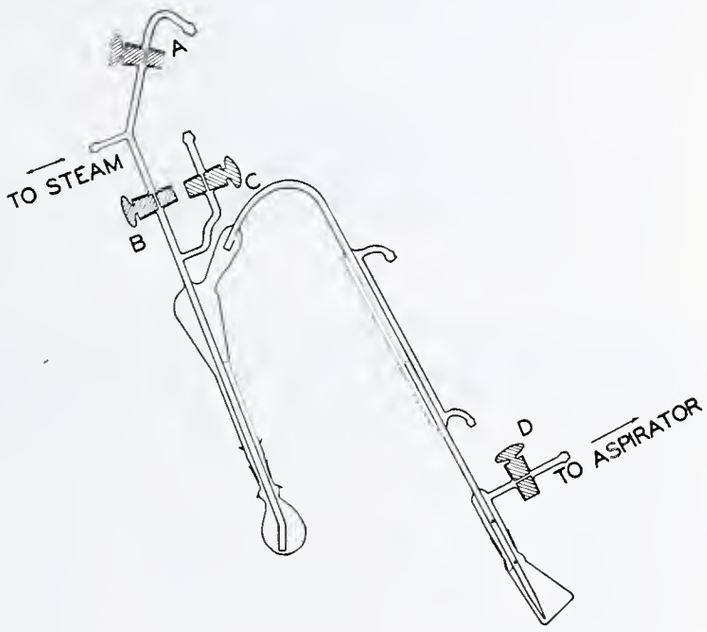

Figure 1. Distillation Apparatus

Formalin, U. S. P. grade, was used without purification; it gave a zero blank.

Sodium carbonate, Baker's C. P. grade, was used.

Solutions. Solutions of substituted ammonium salts were approximately 0.04 *M*. Sulfuric acid, 0.07 *N*, was used as the receiving acid, and the excess was back-titrated with carbonate-free sodium hydroxide solution, 0.018 *N*.

Apparatus. A slightly modified version of the Beatty and Gibbons (*2*) still was used. This was an all-glass micro-Kjeldahl distillation apparatus (Figure 1). At the start of a distillation the distillation flask and receiving flask were placed in position as shown, with the end of the condensing tube dipping into the standard acid in the receiving flask. The required amount of alkali solution was added through stopcock *C*. With stopcocks *B* and *C* closed the entire system was then evacuated with a water aspirator attached at *D*. As soon as the pressure was sufficiently reduced steam at 30° was admitted by closing *A* and cautiously opening *B*. At the end of the distillation *B* and *D* were closed

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and the system was brought nearly to atmospheric pressure by gradually opening *C*. This was then closed and the receiving flask rotated through an angle of 180°. As the neck of the receiving flask was built at an angle to the flask, its rotation placed the acid level below the tip of the capillary, so that by opening *C* again the last bit of liquid was expelled from the tip of the condensing tube. The outside of the capillary was washed after removal of the receiving flask.

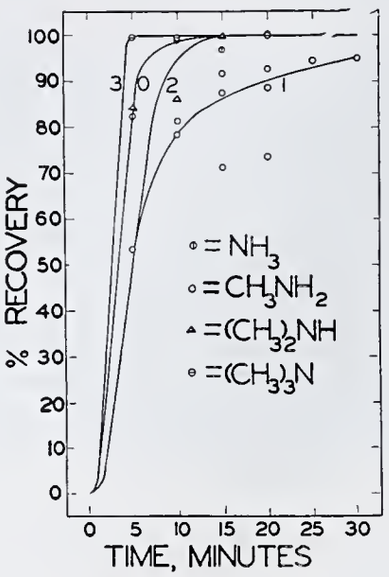

FIGURE 2. TIME-RECOVERY CURVES FOR DIFFERENT AMINES

3 ml. of 10 per cent sodium carbonate soln. used; total volume in distilling flask for each run was 12 ml., except curve 0, in which there were 10 ml.
0, 53.9 micromoles of NH_3 present. 1, 59.1 of micromoles CH_3NH_2 present. 2, 60.9 of micromoles of $(CH_3)_2NH$ present. 3, 61.7 micromoles of $(CH_3)_3N$ present

PROCEDURE. The amine solution under study was pipetted into the distillation flask of the apparatus, followed by the addition of the formaldehyde solution and sufficient distilled water to bring the total volume to an integral number of milliliters. The same volume was used for all runs in one series, since it was found that increasing the volume of liquid in the distillation flask noticeably reduced the rate of distillation. The distilling flask was then set in place on the still, followed by the insertion of the acid (receiving) flask in its proper position. The alkali solution was then added by means of a stopcock-equipped inlet. The entire system was then evacuated with a water aspirator. As soon as the pressure was sufficiently reduced, steam at 30° was passed through the system for 20 minutes except for the data of Figure 2, for which the duration of distillation was varied. At the end of this time the still was closed to the aspirator and steam supply, and air admitted; the flasks were removed and the remaining acid in the receiving flask was titrated, using bromocresol green as the indicator unless the amount of amine recovered was less than 25 micromoles, in which case chlorophenol red was used.

A few experiments showed that no significant differences in yield occurred when the formalin and amine solutions were mixed and allowed to stand overnight.

Results

The recovery of known amounts of each of the amines, in the absence of formaldehyde, as a function of the time of distillation is shown in Figure 2.

The differences in behavior of the various amines are attributed to differences in their vapor pressures above the solutions.

To test the accuracy of recovery, known amounts of the different amines were distilled for a 20-minute period as described above, except that no formaldehyde was added. The average error, excluding the data for monomethylamine, was 0.7 per cent, and the maximum error was 2.0 per cent. Deviations were usually on the lower recovery side. The error, most noticeable when larger quantities of amine were used, was traced to incomplete distillation rather than to incomplete absorption in the acid solution. Results are given in Table II.

Formaldehyde prevented the volatilization of ammonia but did not completely prevent the volatilization of mono- and dimethylamines. When the concentration of formaldehyde was varied, anomalies were observed as shown in Figure 3.

The volatilization of ammonia was nearly completely prevented with concentrations of formaldehyde above about 4 per cent, or 0.8 ml. of added formalin in the determination, and the recovery of trimethylamine was not affected by any concentration of formaldehyde. The recovery curves of mono- and dimethylamines showed minimum recoveries at concentrations of formaldehyde of approximately 0.6 and 2.0 per cent, respectively. Both values were greatly in excess of the concentration of amine.

The removal of the last traces of formaldehyde from the apparatus was found to be necessary in order not to affect recoveries in subsequent runs.

TABLE II. RECOVERY OF KNOWN AMOUNTS OF AMINES BY DISTILLATION AT 30° C.

Amine	Present	Found	Recovery	Error
	Micromoles		%	%
NH_3	53.9	54.0	100.2	+ 0.2
	92.5	92.4	99.9	− 0.1
	110.1	108.9	98.8	− 1.2
CH_3NH_2	58.9	52.7	89.4	−10.6
	59.1	54.8	92.7	− 7.3
	62.8	55.3	88.0	−12.0
	62.8	61.1	97.3	− 2.7
	120.1	109.0	90.7	− 9.3
$(CH_3)_2NH$	60.9	61.3	100.8	+ 0.8
	60.9	61.6	101.1	+ 1.1
$(CH_3)_3N$	30.2	30.4	100.7	+ 0.7
	30.4	30.4	100.0	0.0
	43.9	44.0	100.2	+ 0.2
	43.9	43.7	99.5	− 0.5
	44.0	43.5	98.7	− 1.3
	44.1	44.2	100.2	+ 0.2
	44.1	43.9	99.4	− 0.6
	61.7	61.5	99.7	− 0.3
	61.7	61.7	100.0	0.0
	88.4	88.0	99.5	− 0.5
	88.4	88.5	100.2	+ 0.2
	88.4	87.6	99.2	− 0.8
	92.1	90.2	98.0	− 2.0
	123.5[a]	121.8	98.5	− 1.5
	123.5[a]	121.0	98.0	− 2.0

[a] Distilling for 30 minutes instead of 20, recovery was 100.2%.

The anomalous behavior of mono- and dimethylamines with increasing concentration of formaldehyde must be due to the production of a more volatile basic substance by the reaction between the amine and some substance in the formaldehyde solution. Methanol is present as an impurity in commercial formaldehyde solutions; moreover, it is one of the products formed by the Cannizzaro reaction of formaldehyde in alkaline solution. Accordingly, the effect of added methanol on the recovery of mono- and dimethylamine from solutions to which had been added formaldehyde at such a concentration as to give minimum recovery was determined. The addition of methanol caused an increase in recovery of dimethylamine but only a slight increase in recovery of monomethylamine, as shown in Figure 4.

A few tests made with ethanol showed that it could be substituted for methanol with similar results. Added sodium formate was without effect on the recovery of monomethylamine.

Discussion

The curve (Figure 4) giving the increased recovery of dimethylamine with added methanol is similar to that with increasing concentration of formaldehyde (Figure 3, *D*). It is therefore probable that the methanol present as an impurity in formalin or that which resulted from the Cannizzaro reduction of formaldehyde was responsible for the increase in recovery of dimethylamine. This conclusion is completely in line with the finding by Delépine (*4*) that the volatile dimethylamine derivative contained methanol.

The steeper portion of the increase in recovery in the dimethylamine recovery curve (Figure 3, *D*, or 4) is probably due directly to the addition of alcohol to a point of saturation. The flatter portion may be due to a mass-action effect or to the influence of alcohol on the physical properties of the solution.

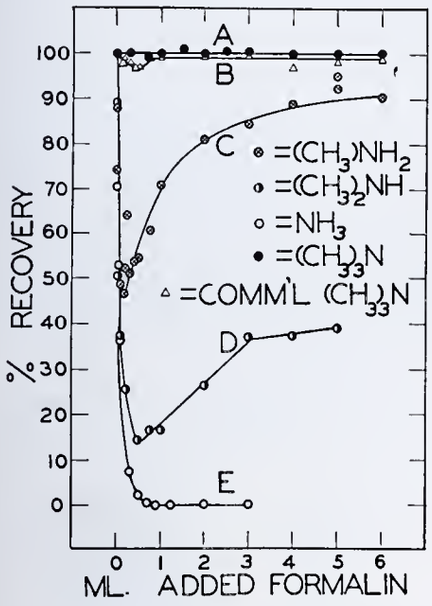

FIGURE 3. EFFECT OF ADDED FORMALIN ON AMINE RECOVERY

A. 40.2 micromoles of $(CH_3)_3N$ present, 3 ml. of 20 per cent sodium carbonate soln. used, total volume in distilling flask 10 ml.
B. 44.1 micromoles of $(CH_3)_3N$ present, 3 ml. of 20 per cent sodium carbonate soln. used, total volume in distilling flask 10 ml.
C. 62.8 micromoles of CH_3NH_2 present, 3 ml. of 20 per cent sodium carbonate soln. used; total volume in distilling flask 10 ml.
D. 98.1 micromoles of $(CH_3)_2NH$ present, 5 ml. of 10 per cent sodium carbonate soln. used; total volume in distilling flask 12 ml.
E. 92.5 micromoles of NH_3 present, 3 ml. of 20 per cent sodium carbonate soln. used; total volume in distilling flask 8 ml.

In contrast to the results obtained with dimethylamine, the reason for the increase in recovery of monomethylamine with increasing amounts of formaldehyde beyond the minimum recovery point is not found in products resulting from the Cannizzaro reaction of formaldehyde, and is still unknown.

The use of formaldehyde for the effective removal of mono- and dimethylamines requires careful control in order to achieve any semblance of the desired result. The addition of slightly more than the minimal quantity of formaldehyde required to lock up all ammonia present in a solution also containing mono-, di-, and trimethylamines will reduce the volatilities of mono- and dimethylamines (or derivatives), but will not completely eliminate them.

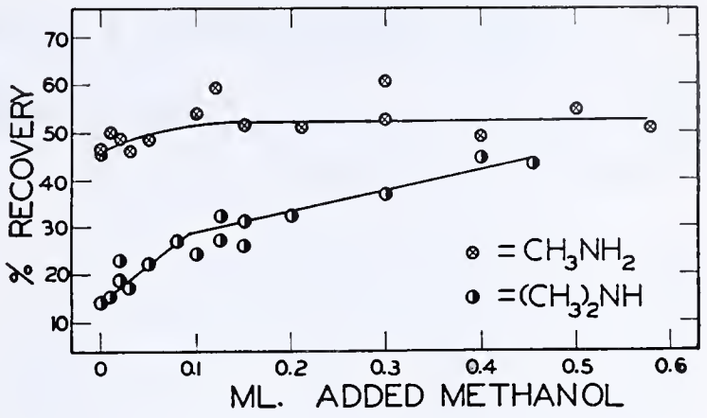

FIGURE 4. EFFECT OF METHANOL ON RECOVERY OF MONO- AND DIMETHYLAMINES FROM SOLUTIONS CONTAINING THE CONCENTRATION OF FORMALDEHYDE TO GIVE MINIMUM RECOVERY

⊗. 62.8 micromoles of CH_3NH_2 present; 3 ml. of 20 per cent sodium carbonate soln. used; total volume in distilling flask 10 ml., including 0.15 ml. of formalin
◑. 98.0 micromoles of $(CH_3)_2NH$ present; 5 ml. of 10 per cent sodium carbonate soln. used; total volume in distilling flask 12 ml., including 0.5 ml. of formalin

If the amounts of mono- and dimethylamines are small relative to those of ammonia and trimethylamine, the decreased volatilities of mono- and dimethylamines may be sufficient to bring the amounts of them recovered below the limit of detection.

Summary

Formaldehyde rendered ammonia nearly completely nonvolatile near room temperature.

Formaldehyde had no effect on the recovery of trimethylamine.

Formaldehyde did not completely prevent the volatilization of mono- and dimethylamines or products from them.

When mono- and dimethylamines were distilled in the presence of formaldehyde, anomalies were observed which in the case of dimethylamine are probably attributable to the methanol present in the formaldehyde solution.

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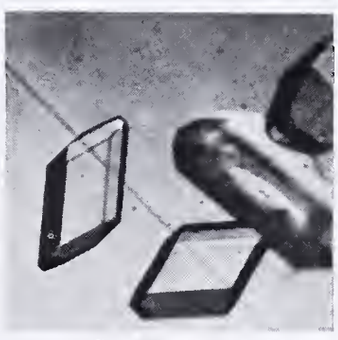

Courtesy, Mary L. Willard

COPPER SULFATE CRYSTALS

An Improved Photometric Method for Ascorbic Acid

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MOST of the chemical methods for the estimation of ascorbic acid are based upon the rapid reduction by this substance of the dye, 2,6-dichlorophenolindophenol. Greater specificity is gained if the reduction is carried out at a pH of between 2 and 3 (*2*). The use of a photoelectric colorimeter has resulted in increased accuracy, since compensation for reducing substances other than ascorbic acid can be accomplished by measuring the rate of fading of the dye (*3*, *4*, *7*). With these newer techniques, an accurately standardized dye solution is not necessary, and the method can even be applied to colored or turbid solutions (*3*).

In the author's modification, the objectionable reduction of the 2,6-dichlorophenolindophenol by other reducing substances is much retarded by the addition of mercuric chloride. The dye is dissolved in a phosphate solution which buffers it at pH 6.5 to 6.6. To a measured volume of this blue dye solution, a small amount of an equimolar (0.001 *M*) solution of a mixture of mercuric and cadmium chlorides is added. After mixing the above, the extract of the biological material (in 2 per cent metaphosphoric acid) is also added. This lowers the pH to 2.5 to 2.7. Part of the dye will be reduced immediately, varying naturally with the amount of ascorbic acid present. Then phosphate buffer (pH 8.0) is added, which restores the pH of the dye solution to 6.5 to 6.6, and any unreduced dye is reconverted to the blue form. From the difference in per cent transmittance before and after reduction of the dye by the ascorbic acid, the concentration of this latter substance is easily calculated.

Apparatus and Solutions

Coleman spectrophotometer, or an equivalent instrument.

Dibasic sodium phosphate and monopotassium phosphate buffers, 0.066 *M* of pH 6.4 and 8.0, respectively.

Mixed solution of mercuric and cadmium chlorides, 0.001 *M* with respect to each metal ion.

Sodium thiosulfate solution, 0.01 *N*.

Metaphosphoric acid (4 per cent) stock solution, made by dissolving pulverized glacial metaphosphoric acid sticks in distilled water and filtering. The stock solution is stable at 0° C. (*3*). The 2 per cent acid is prepared as required.

The stock solution of 2,6-dichlorophenolindophenol (LaMotte) is made by extracting 50 to 60 mg. of the dye on a filter with successive small portions of hot water until the filtrate is colorless. After cooling to room temperature, the solution is diluted to exactly 100 cc.

Procedure

The stock dye solution is standardized by the method of Menaker and Guerrant (*6*) to obtain the ascorbic acid equivalency. One cubic centimeter of this solution should be equivalent to 0.25 to 0.30 mg. For a convenient working concentration, 5 cc. of this stock solution may be added to 50 cc. of phosphate buffer (pH 6.4) in a 250-cc. glass-stoppered volumetric flask and made up to volume with distilled water. The ascorbic acid equivalency of this buffered solution (pH 6.5 to 6.6 as measured by glass electrode) will average about 5 micrograms per cc. The solution absorbs light maximally at 605 mμ. Both stock and buffered dye solutions should be stored at 0° C.

To obtain the transmittance-concentration curve, accurately measured amounts of the buffered dye solution are made to volume in glass-stoppered volumetric flasks with distilled water after adding proportionate amounts of phosphate buffer (pH 6.4), so that the pH is identical (6.5 to 6.6) in all samples. This is essential, since the color of the dye varies also with the hydrogen-ion concentration. Standards are made containing the equivalent of 0.5 to 5.0 micrograms of ascorbic acid per cc. When the per cent transmittance is plotted against the ascorbic acid equivalency per cubic centimeter of dye, a straight line results, showing that the dye obeys Beer's law at the dilutions needed.

Technique for pure ascorbic acid dissolved in 2 per cent metaphosphoric acid: 5 cc. of the buffered dye solution are delivered from a pipet to the dry sample cuvette of the spectrophotometer (the cuvette is dried by rinsing with reagent grade acetone and removing the last traces of solvent by aspiration). The transmittance of the dye solution alone is first determined. From this the ascorbic acid equivalency can be read from the per cent transmittance–concentration curve. Now the cuvette is removed, 1 cc. of the mercuric-cadmium chloride solution is added, and the mixture is stirred. Then 1 or 2 cc. of standard or sample solution containing 5 to 20 micrograms of ascorbic acid in 2 per cent metaphosphoric is pipetted in and the mixture is again stirred. The pH drops to 2.5 to 2.7, at which point some of the dye (depending on the amount of ascorbic acid present) is reduced immediately. Then 3 or 6 cc. of phosphate buffer (pH 8.0) are added, and the mixture is agitated. The pH returns to 6.5 to 6.6. (For each cubic centimeter of 2 per cent metaphosphoric acid, 3 cc. of phosphate buffer are used.) The per cent transmittance of the resultant blue solution is determined and from this the amount of unreduced dye in terms of ascorbic acid can be read directly from the per cent transmittance–concentration curve. Keeping in mind the volume changes, the amount of ascorbic acid present is readily calculated. The results of many determinations for the recovery of pure ascorbic acid in 2 per cent metaphosphoric acid solution are shown in Table I.

Table I. Recovery of Pure Ascorbic Acid

Added		Found	Recovered
Micrograms/cc.	*Micrograms/2 cc.*	*Micrograms*	*%*
..	5	4.8– 5.3 (5.1)[a]	102
..	10	10.0–10.1 (10.1)	101
..	20	19.4–20.2 (19.7)	98
10	..	9.4–10.1 (9.8)	98
20	..	18.5–20.1 (19.3)	97
40	..	38.1–40.7 (39.6)	99

[a] Average figures in parentheses.

When the same procedure was applied to the metaphosphoric-acid extract of tissues without the addition of mercuric chloride, the dye was rapidly reoxidized at pH 6.5 to 6.6 after reduction by ascorbic acid at pH 2.7. Experimentation revealed that a solution of 0.001 *M* mercuric chloride prevented the reoxidation of the dye at that higher pH. To prevent the slight oxidation of ascorbic acid by mercuric chloride, cadmium chloride was included as recommended by Kassell and Brand (*5*).

Extracts of animal tissues were prepared by the method of Bessey (*3*) using 2 per cent metaphosphoric acid and a small amount of acid washed sand to facilitate maceration. The extracts were filtered (Munktell's OA) and made up to volume. With the small amounts of tissue used in most of this work, the filtrates were colorless. The remainder of the procedure for the determination of ascorbic acid in the extracts is the same as that described above for a pure solution. Usually 2 cc. of extract sufficed for a single determination.

Sample Calculation. 2-cc. portions of a metaphosphoric acid extract of 0.218 gram of carcinoma. Total volume of extract 5 cc. 5 cc. of the dye solution had per cent transmittance of 17.0, equivalent to 5.28 × 5 or 26.5 micrograms of ascorbic acid. After addition of 1 cc. of mercuric-cadmium chloride solution, 2 cc. of carcinoma extract, and 6 cc. of phosphate buffer (pH 8.0), per cent transmittance was 63.3. This was equivalent to 1.08 × 14 (total volume) or 15.1 micrograms of ascorbic acid left as unreduced dye, and 25.5 − 15.1 = 10.4 micrograms of ascorbic acid per 2 cc. of extract.

The ascorbic acid contents of liver, epidermal squamous cell carcinoma, whole skin, 2-day-old embryonic whole skin and isolated epidermis are shown in Table II. The epidermi

Table II. Ascorbic Acid Content of Tissues of Albino Mice

Tissue	No. of Mice Represented	Weight	Ascorbic Acid Found
		Grams	*Mg./g.*
Liver	1	1.632	0.169
	1	0.887	0.247
	1	1.666	0.231
	1	1.273	0.243
	1	1.563	0.256
Carcinoma[a]	1	0.181	0.162
	1	0.218	0.130
	1	0.327	0.141
	1	0.358	0.138
	1	0.320	0.127
Epidermis[b]	3	0.054	0.180
	3	0.055	0.254
	3	0.051	0.319
	3	0.074	0.246
Whole skin[c]	1	1.273	0.163
	1	0.809	0.215
2-day embryonic whole skin	8	0.400	0.312

[a] Epidermal squamous cell carcinoma.
[b] Isolated epidermis.
[c] Dermis plus epidermis.

was separated from the dermis at 50° C. by the method of Baumberger, Suntzeff, and Cowdry (*1*).

The recovery of ascorbic acid from metaphosphoric acid extracts of liver and of squamous cell carcinomas of mice is given in Table III. Glutathione and cysteine (0.4 millimolar) proved without effect upon the assay of pure ascorbic acid. This observation alone definitely indicates the effectiveness of the mercuric ion in inhibiting the reducing action of other tissue substances on 2,6-dichlorophenolindophenol.

Table III. Recovery of Ascorbic Acid from Biological Material

Ascorbic Acid Present per Cc. of Metaphosphoric Acid Extract	Ascorbic Acid Added per Cc. of Metaphosphoric Acid	Ascorbic Acid Recovered	Recovery
Micrograms			%
8.3[a]	10.0	18.1	99
16.5[a]	10.0	25.0	94
12.8[a]	10.0	22.5	98
12.6[a]	10.0	22.7	99
15.4[a]	10.0	23.9	94
11.0[a]	10.0	20.1	96
8.6[a]	10.0	18.0	96
12.6[a]	20.0	32.0	98
15.4[a]	20.0	34.1	96
15.4[a]	40.0	53.7	97
4.6[b]	5.0	9.5	99
3.9[b]	10.0	13.7	98
15.0[b]	10.0	25.6	102
3.9[b]	40.0	42.4	96

[a] Livers; [b] Epidermal squamous cell carcinomas.

Urine

The method can also be used for the determination of ascorbic acid in urine. Urine samples (freshly voided) are diluted with 4 per cent metaphosphoric acid, so that the pH is 2.5 to 2.7. The subsequent addition of dye, mercuric-cadmium chloride solution, and phosphate buffer to aliquots of the acidified urine will satisfy the conditions described. By diluting 2 volumes of urine with 1 volume of 4 per cent metaphosphoric acid, the requirements for adequate pH changes were met with the particular samples used here.

Because of the many reducing substances occurring in urine, there is a slight reoxidation of the dye at pH 6.5 to 6.6 after reduction by ascorbic acid at pH 2.5 to 2.7. If the readings are not delayed for over 30 to 60 seconds after the addition of phosphate buffer, no difficulty is encountered, since the rate of reoxidation amounts to only 0.5 to 1.0 unit per cent transmittance per minute. The recovery of pure ascorbic acid from acidified urine was as good as that from tissues, a fact which further supports the effectiveness of the mercuric ion for inactivating other reducing substances.

With colored solutions such as urine, the reference cuvette should contain the same aliquot of sample as is used in the actual determination. For urine, a reference solution is used which contains 5 cc. of distilled water, 1 cc. of 0.001 *M* mercuric-cadmium chloride solution, and 1 or 2 cc. of urine with either 3 or 6 cc., respectively, of phosphate buffer.

Stability of Stock and Buffered Solutions of 2,6-Dichlorophenolindophenol

To determine the stability of stock solutions of the dye, solutions containing 50 to 60 mg. of dye per 100 cc. of water were stored at 0° C. and the ascorbic acid equivalency was determined at intervals by titrating portions with 0.01 *N* sodium thiosulfate. The results (Table IV) show that the dye is stable for at least one month at that temperature.

The ascorbic acid equivalency of the buffered dye solution, calculated from accurately standardized stock solutions and determined by reading the per cent transmittance, agreed to within 2 per cent (Table V). The titration values given in the table were calculated from standardized stock solutions. The transmittance values were obtained from a per cent transmittance–concentration curve previously made from a standardized solution of the dye. Therefore, standardization of new lots of dye is not necessary, since the agreement between the calculated value and the per cent transmittance value is good.

Although there is a slow change in the per cent transmittance of the buffered dye solution (Table V, samples 4, 5, and 6 at 28 days), this change is paralleled by an actual change in the ascorbic acid equivalency of the dye (with a maximum error of about 2 per cent) as determined by titration with 0.005 *N* sodium thiosulfate. Further proof that this change in the dye was actually accompanied by a change in its ascorbic acid equivalency was given by excellent recoveries of pure ascorbic acid from older dye solutions. Buffered solutions of the dye exhibit a slight change during the first few days and then remain stable. Since the actual loss in ascorbic acid equivalency of the buffered dye solution is measured by the per cent transmittance, the advantages of this solution are obvious.

Table IV. Stability of Stock Solutions of 2,6-Dichlorophenolindophenol

Sample No.	Time	Ascorbic Acid Equivalent
	Days	*Mg./cc.*
1	0	0.273
1	22	0.272
1	36	0.270
2	0	0.264
2	14	0.267
2	28	0.265
3	0	0.304
3	17	0.305
4	0	0.251
4	13	0.255

Table V. Ascorbic Acid Equivalent of Buffered Dye

Sample No.	Time	Ascorbic Acid Equivalent Titrimetric	Photometric	Difference
	Days	*Micrograms/5 cc. dye*		%
1	0	24.3	24.8	2.0
2	0	30.4	30.6	0.9
3	0	24.3	24.7	1.6
4	0	24.6	...	...
4	5	23.7	23.9	0.8
4	28	23.8	23.5	1.2
5	0	27.4	...	...
5	22	26.2	26.5	1.1
6	0	26.4	26.9	1.9
6	14	25.7	26.3	2.3
6	28	25.8	26.4	2.3
7	0	25.1	25.6	2.0

Summary

An improved microphotometric method for ascorbic acid based upon the difference in transmission of buffered 2,6-dichlorophenolindophenol before and after reduction is described. Reduction is carried out at pH 2.5 to 2.7 and transmission is measured at 6.5 to 6.6.

Interference due to other reducing substances such as glutathione and cysteine is greatly inhibited by the addition of mercuric chloride.

Frequent restandardization is unnecessary and changes in the ascorbic acid equivalency can be read directly from the transmittance-concentration curve.

Acknowledgment

The writer is indebted to V. Suntzeff for the carcinomas and for the samples of isolated epidermis used in this study.

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End Point of Microtitrations with Color Indicators

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Solutions of the concentrations customary in macroanalysis are proposed for use in microtitrimetry.

A discussion of the limitations resulting from the use of color indicators in microtitrations must consider whether the color change occurs throughout the titrated solution or whether the color effect is localized in a small portion of a mixture. In the first instance it can be shown that in microtitrations the light must travel approximately the same distance through the titrated solution as in macroanalysis, if the indicator concentration is identical with that used in the standard procedure. In spite of the necessity of working with volumes of the order of 0.1 ml., a 4-cm. thickness of layer can be obtained in microprocedures by observing the end point in a coloriscopic capillary which is part of the titration vessel. This capillary may further be used to reserve some of the titrated solution for the final adjustment of the end point.

Observation of color changes taking place in a portion of the titrated system is discussed with reference to the use of organic solvents for the indication of the end point in iodometric titrations. A mathematical investigation shows that this particular principle will fail when applied on a very small scale. Iodometric titrations employing a droplet of chloroform as indicator, as well as argentometric titrations with adsorption indicators, may be successfully performed on a milligram scale.

CLOSE adherence to the provisions of a well-established analytical procedure is desirable, for it permits interpretation of the results in the light of the experience gathered in study and application of the method. For the same reason it appears desirable to reproduce the conditions of a standard procedure when it is tried on a different scale. If no heterogeneous equilibria or other surface phenomena are involved and time elements, such as rate of adding reagents or rate of stirring, have little influence, the rest of the more generally considered factors—concentration, temperature, and pressure—are easily reproduced on a smaller scale.

Maintenance of the concentrations used in the standard procedure needs continuous attention, because of the universal validity of the law of chemical equilibrium. Since concentration $= \frac{\text{mass}}{\text{volume}}$, it is sufficient to change in the same proportion all masses and volumes specified in the standard procedure. This detail of the directions of a micromethod is simply derived from the standard procedure by multiplying all specified masses and volumes by the reduction factor

$$f = \frac{\text{size of microsample}}{\text{size of macrosample}}$$

Concentrations and purity specifications of all reagents, standard solutions, diluents, and wash liquids must be left unchanged.

Application of the outlined principle to the transposition of a standard procedure from the gram to the milligram scale is shown in Table I. As in the macroprocedure, the carbon dioxide will be eliminated by boiling after addition of a small excess of standard acid. When the titration is finished with 0.5 *N* sodium hydroxide, there is no reason why the color change at the end point should not occur with a fraction of the volume of standard solution listed as drop error in Table I.

For the titration of milligram samples with standard solutions of the customary concentrations, a number of satisfactory burets of approximately 50-cu. mm. total capacity are available (*2*, *4–7*, *11*, *12*). The same cannot be said of titration vessels, and for this reason the

Table I. Titration of Sodium Carbonate with 0.5 *N* Hydrochloric Acid

	Mass of Sample	Reduction Factor	Volume of Titrated Solution: At start	Volume of Titrated Solution: At end point	Capacity of Buret	Methyl Red: Volume of 0.3 per cent solution taken	Methyl Red: Mass taken	Drop Error
Macro	1 gram	..	25 ml.	60 ml.	50 ml.	0.03 ml.	90 μg.	0.03 ml.
Micro	1 mg.	0.001	25 cu. mm.	60 cu. mm.	50 cu. mm.	0.03 cu. mm.	0.09 μg.	0.03 cu. mm.

special requirements are outlined below, and ways of filling them are indicated.

Color Change throughout Titrated Solution

It is obvious that the color change will take place throughout the entire solution, if the coloring matter is dissolved in the titrated solution. The design of a vessel for the titration of small samples must then satisfy the following requirements:

Its capacity must be in accord with the volume of the titrated solution, and its shape must permit recognition of the color change and efficient execution of the necessary operations.

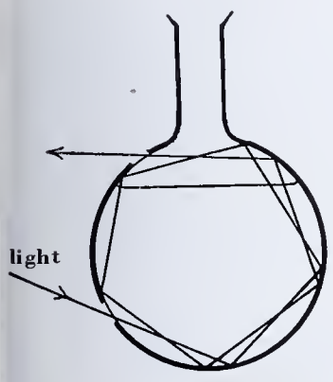

FIGURE 1. LENGTHENING OF PATH OF LIGHT BY REPEATED REFLECTION

These requirements are contradictory in part. Mixing, boiling, and adding reagents call for a container of compact shape. The perception of color in spite of the low concentration of coloring matter calls for a depth of liquid which, because of the small volume, can be attained only by spreading the solution in a thin film or by collecting it into a long, fine thread. Neither proposal appears satisfactory from a mechanical point of view.

The requirements for the perception of the color change at the end point may be stated more definitely. Beer's law implies that the intensity of a coloration is determined by the areal concentration of the matter causing selective absorption in the visible range of the spectrum. In other words, the intensity of color depends upon the mass of coloring matter acting on the light per unit of the area exhibiting the coloration (*1*). Mika (*9*, *10*) has determined the following limits of areal concentration that assure satisfactory observation of color (micrograms per sq. cm.): methyl red, 6.5; bromothymol blue, 80; phenol red or phenolphthalein, 26; thymolphthalein, 5; alizarin yellow R, 13; and potassium permanganate, 2 (or 0.012 microgram-mole).

One may assume that the titrated solution containing m units of mass of the coloring matter has a volume v at the end point and fills length l of a cylindrical tube with inner diameter d. If the light used for the observation of color travels parallel to the axis of the tube, the areal concentration of the coloring matter is given by

$$C_a = \frac{4m}{\pi d^2} \text{ units of mass per units of area}$$

The concentration of coloring matter is obviously equal to

$$c = \frac{4m}{\pi d^2 l} \text{ units of mass per units of volume}$$

and division of the first equation by the second gives

$$l = \frac{C_a}{c} \text{ units of length}$$

The length of the column of titrated solution is determined by the ratio of the areal concentration to the concentration by volume of coloring matter. Recalling that the concentration, c, is fixed by the choice of the standard procedure, it follows that the minimum depth of the column of titrated solution is determined by the smallest areal concentration assuring perception of the color change. The limiting areal concentration is essentially a constant of the indicator, and, consequently, the minimum length of the column of titrated solution becomes a constant of the standard procedure and must be retained, no matter how small the scale on which the titration is performed. If the standard procedure does not employ an unnecessary excess of coloring matter for the indication of the end point, this minimum length will be approximately the same as the thickness of layer of the titrated solution of the macroprocedure. The volumes of the solutions of microprocedures, however, are reduced in proportion to the decrease of the size of sample.

Application to the example illustrated by Table I gives

$$l = \frac{6.5 \ \mu\text{g./sq. cm.}}{0.09 \ \mu\text{g./0.06 cu. cm.}} = 4.3 \text{ cm.}$$

In order to see the color change, the titrated solution must be given a depth of at least 43 mm. Table I also shows that even samples as large as 1 mg. require reduction of the volume of the titrated solution to 60 cu. mm. The solution will fill a sphere of 5-mm. diameter, but the light must travel through it for a distance of not less than 43 mm.

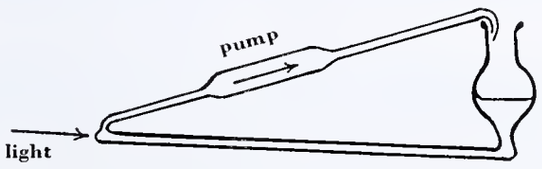

FIGURE 2. CIRCULATION OF TITRATED SOLUTION THROUGH COLORISCOPIC CAPILLARY

The standard procedure should be transposed without change of the essential conditions. Obviously, the indicated difficulties disappear at once when this premise is waived. It is not necessary, however, to accept the disadvantages of such a short cut (flattening of titration curve, poorly defined end points, necessity of using color standards, and corrections for the blank), for it is possible to fill the contradictory requirements for the transposing of standard procedures in at least two ways:

A titration vessel of simple compact shape may be used, and the path of light through the solution may be given the required length by passing the light back and forth through the solution by repeated reflection (Figure 1).

The titration vessel may be designed to provide two compartments, one for the observation of color and the other for the performance of such mechanical operations as stirring, adding of reagent, etc. The titrated solution may be made to circulate (Figure 2) or to oscillate from one compartment to the other (Figure 3).

Color Change Localized in Part of Titrated System

The use of organic solvents for the detection of a small excess of iodine at the end point of iodometric titrations (*3*) provides an interesting example.

Assuming that the organic solvent is able to extract practically all of the iodine added in excess, one is inclined to think that the sensitivity of the end-point indication would be equal to that of the macroprocedure, if the cross-section area of the drop of organic solvent were reduced in the same proportion as the size of the sample. Then the amount of iodine corresponding to the drop error would be divided by the same number as the area showing the coloration, and the areal concentration of iodine in the organic solvent would remain unchanged. The assumption of a practically complete extraction of the iodine is, however, not permissible. Even for the distribution of the iodine between water and chloroform the partition ratio

$$k_p = \frac{[I_2]_{\text{org. solvent}}}{[I_2]_{\text{water}}}$$

has too low a value to support the premise. Furthermore, the titrated solution contains an appreciable amount of iodide ion at the end point of an iodometric determination, and the equilibrium $I_2 + I^- \rightleftharpoons I_3^-$ must be taken into consideration

$$\frac{[I_2]\,[I^-]}{[I_3^-]} = 0.00137$$

It becomes obvious that only a few per cent of the available iodine can be taken up by the organic solvent.

The areal concentration of iodine in the drop of organic solvent is a somewhat involved function of the total amount of iodine available, the volumes of aqueous and organic phases, the partition ratio, and the iodide concentration. The organic solvent is assumed to take the shape of a sphere of volume v_0, cross-section area A, and diameter d; v_a represents the volume of the aqueous solution at the end point. The total mass, m, of iodine added in excess corresponds to the "drop error" or more correctly to the amount of iodine consumed in a blank titration. The calculation begins with the statement that the excess of iodine added at the end point is partly dissolved in the organic solvent, partly dissolved in the aqueous solution, and partly converted to triiodide ion

$$m = v_0\,[I_2]_{\text{org.}} + v_a[I_2]_{\text{aqu.}} + v_a\,[I_3^-]$$

Using the equations for the partition ratio and for the instability constant of the triiodide ion, one calculates $[I_2]_{\text{aqu.}}$ and $[I_3^-]$ as functions of $[I_2]_{\text{org.}}$ and substitutes in the above equation, which is then solved for $[I_2]_{\text{org.}}$ Multiplication of the concentration of iodine in the organic solvent by the volume, v_0, of the organic solvent gives the mass, m_0, of iodine dissolved in the organic solvent, and division by the area, A, of the organic solvent furnishes the areal concentration of iodine in the organic liquid:

$$\frac{m_0}{A} = \frac{m_0}{1.21\,v_0^{2/3}} = \frac{0.828\,mk_pv_0^{1/3}}{v_0k_p + v_a\left(1 + \dfrac{[I^-]}{0.00137}\right)} = \frac{a\,x}{bx^3 + c}$$

The form of the equation indicates that the areal concentration of the iodine in the organic solvent, m_0/A, must arrive at a maximum value for a certain volume, v_0, of the organic solvent, provided that the other conditions are kept constant. Differentiation is made simple by setting v_0 equal to x^3. The areal concentration of the iodine in the organic solvent becomes a maximum for

$$v_0 = \left(1 + \frac{[I^-]}{0.00137}\right)\frac{v_a}{2\,k_p}$$

When a standard procedure has been selected, the quantities m, k_p, v_a, v_o, and $[I^-]$ have been assigned definite values, and the equation for the areal concentration may be simplified to

$$\left(\frac{m_0}{A}\right)_{\text{macro}} = \frac{k_1mv_0^{1/3}}{k_2\,v_0 + k_3\,v_a}$$

In transposing to the microscale, the quantities m, v_0, and v_a are decreased in the same proportion by multiplication by the reduction factor, f, and the areal concentration of the iodine in the organic solvent becomes

$$\left(\frac{m_0}{A}\right)_{\text{micro}} = f^{1/3}\left(\frac{m_0}{A}\right)_{\text{macro}}$$

This means that the areal concentration of the iodine decreases in proportion to the third root of the reduction factor. Provided that the standard procedure employs the most favorable volume of organic solvent and does not use an unnecessary excess, m, of iodine at the end point, it follows that transposition to a smaller scale lessens the sensitivity of the end-point indication. When proceeding from the gram scale to the milligram scale, the areal concentration of the iodine in the organic solvent is reduced ten times.

Table II and Figure 4 apply to a microprocedure in which chloroform is taken for the organic solvent.

Thirty cubic millimeters of 0.5 N thiosulfate are titrated with approximately the same volume of 0.5 N iodine standard solution, which is assumed to be 0.12 molar with respect to potassium iodide. At the end point the titrated solution has a volume of 60 cu. mm., and its iodide concentration is 0.11 mole per liter because of the reaction

$$I_2 + 2\,S_2O_3^{--} \longrightarrow 2\,I^- + S_4O_6^{--}$$

The areal concentration of iodine has been calculated for various volumes, v_0, of the droplet of chloroform from the equation

$$\frac{m_0}{A} = \frac{0.108\,v_0^{1/3}}{130\,v_0 + 4.88}\,m$$

The areal concentration of iodine reaches a maximum when the spherical drop of chloroform is given a volume of 18.7 cu. mm., corresponding to a diameter of 3.3 mm. Table II and Figure 4 show that the diameter of the drop of chloroform may vary approximately from 1 to 8 mm. without reducing the areal concentration of iodine and, consequently, the intensity of the coloration to much more than one half of the obtainable maximum. Table and graph show that the areal concentration of iodine decreases directly with the diameter of the chloroform drop when its volume becomes very small (diameter less than 2 mm.). The areal concentration increases in inverse proportion to the cross-section area of the drop when the volume of the chloroform becomes more than ten times larger than the volume of the titrated solution (A = 1 sq. cm. and more, or d = 11 mm. or more). Either the former or the latter will occur, depending upon which of the two items 130 v_0 or 4.88 can be neglected in the denominator of the equation.

The corresponding macroprocedure would give a volume of 60 ml. at the end point of the titration, and 5 ml. of carbon tetrachloride are customarily used for the extraction of the iodine (k_p = 85). Assuming approximately spherical shape for the carbon tetrachloride layer, the areal concentration of iodine at the end point will be 0.0227 m—i. e., nearly six times higher than in the microprocedure which employs the most favorable volume of a solvent giving a higher partition ratio.

The appearance of the color of iodine in the organic solvent can be used without difficulty for the detection of the end

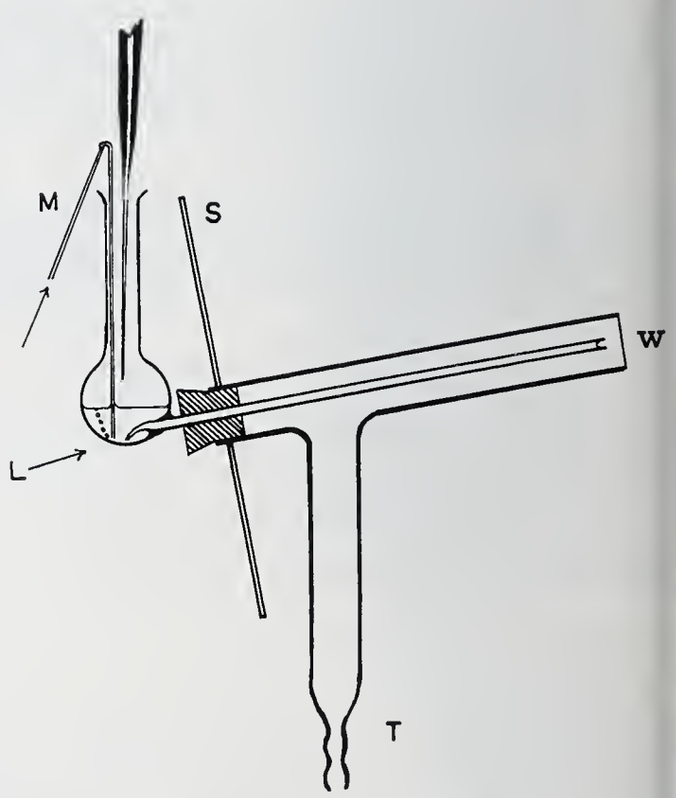

Figure 3. Titration Bulb with Attached Coloriscopic Capillary

point in the titration of milligram samples. However, there are serious limitations to the application of this principle on a much smaller scale. The difficulties may be lessened by reducing the iodide-ion concentration of the titrated solution, selecting a solvent giving a better partition ratio, and increasing the distance which the light travels through the organic solvent.

Experimental

U-shaped burets operated by remote control (*8*) were employed. Millimeter scales of 30-cm. length were placed behind the vertical capillaries of approximately 0.8-mm. bore, so as to make the total capacities of the calibrated portions of the burets equal to approximately 0.15 ml. The control of the outflow of standard solutions was sufficiently fine to warrant estimation of tenths of the millimeter scale, and 1 mm. of the scale corresponded to a volume of approximately 0.5 cu. mm. In the tables the amounts of standard solutions are given in centimeters. Conversion to units of volume would not be helpful, since only ratios were determined. A significant variation in the bore of the authors' capillary tubes has not been discovered so far.

Table II. Areal Concentration of Iodine

As a function of volume, cross-section area, and diameter of the drop of chloroform. $v_a = 60$ cu. mm.; $k_p = 130$; $[I^-] = 0.11$ mole per liter; m(micro) = 0.001 m

v_0	A	d	m_0/A
Cu. mm.	*Sq. cm.*	*Mm.*	*Mass/sq. cm.*
2120	2.0	16.0	0.00049 m
750	1.0	11.3	0.00096 m
266	0.50	8.0	0.00176 m
94	0.25	5.6	0.00288 m
33.5	..	4.0	0.00378 m
27.0	..	3.7	0.00385 m
18.7	0.085	3.30	0.00391 m
10.9	..	2.75	0.00380 m
5.0	0.037	2.16	0.00333 m
4.2	..	2.00	0.00321 m
0.52	..	1.00	0.00175 m
0.17	0.00365	0.70	0.00123 m
0.066	..	0.50	0.00089 m
0.0082	..	0.25	0.00045 m
Zero	Zero	Zero	Zero

For stirring, a stream of small gas bubbles was passed through the titrated solutions by means of a capillary of 0.1- to 0.2-mm. bore. A satisfactory supply of gas may be obtained by compressing air in a 2.5-liter bottle by blowing with the mouth. Since 16,000 bubbles of 0.5-mm. diameter have a total volume of only 1 ml., one filling of the bottle should suffice for a number of titrations. The outlet of the bottle is connected through a U-tube with Ascarite to the fine capillary, and a screw clamp on the rubber tubing permits regulation of the rate of the gas stream.

Most of the titrations were performed in centrifuge cones of 2-ml. capacity and of the shape commonly used in qualitative work. The bulb with attached coloriscopic capillary (Figure 3) facilitates recognition of the end point. The capillary is given a length of 4 to 5 cm. Its capacity should not exceed 0.25 c, if c is the total capacity of the buret used. This then always leaves a sufficient volume of liquid in the bulb, which is given a capacity of 2 c or more. A T-tube with a window of thin glass at W is placed over the coloriscopic capillary. Its open arm, T, is connected by means of flexible rubber tubing to an Ascarite tube provided with a mouthpiece. Light is sent in the direction of the arrow, L, through the bulb into the coloriscopic capillary. A shield, S, made of black cardboard, may be placed around the T-tube.

For use with burets operated by remote control, titration vessel and fine capillary, M, are mounted on an elevating stand, so that the tip of the buret can be touched with the meniscus of the titrated solution by raising the titration vessel by means of the rack-and-pinion motion of the elevating stand.

A slow stream of gas through M is first started, and then the solution to be titrated is introduced into the bulb. Part of the solution enters the coloriscopic capillary, and, if necessary, slight suction with the mouth may be applied through T to draw the meniscus up into the opening which faces W. This portion of the solution is kept in the capillary for use in the final adjustment of the end point. The flow of gas is adjusted to give a steady stream of bubbles from the opening of M, and then standard solution is added from the buret. By observation of the color of the liquid

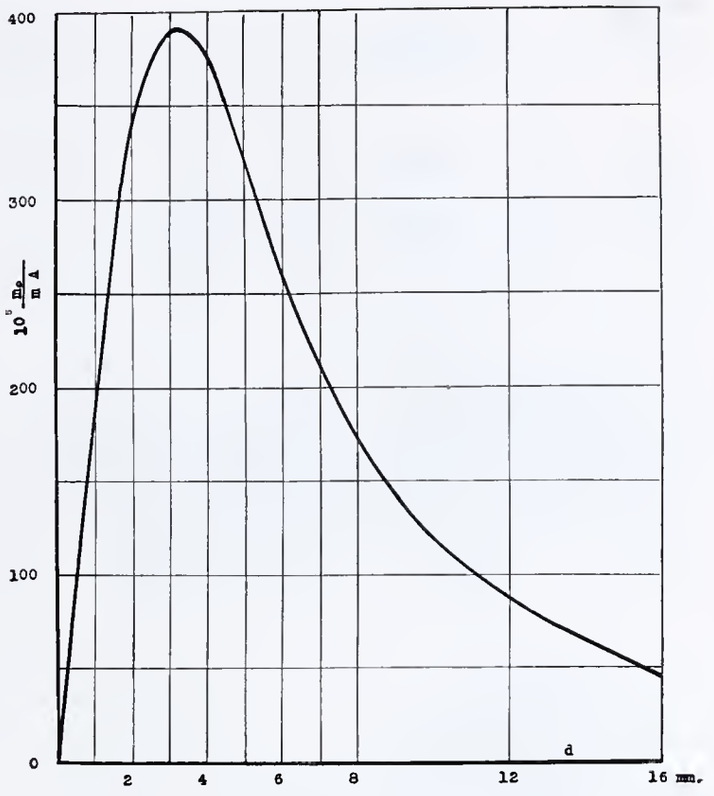

Figure 4. Areal Concentration of Iodine as a Function of the Diameter of the Drop of Chloroform

in the bulb it is possible to shut off the flow of standard solution soon after the end point has been overstepped. The solution in the coloriscopic capillary is now transferred to the bulb by cautiously blowing with the mouth through T. The tip of the coloriscopic capillary cannot be drawn out very fine, since this would delay the back-and-forth transfer of the titrated solution. Thus, care must be exercised, since a violent stream of large air bubbles may throw some of the solution out of the bulb. As soon as the contents of the bulb have become homogeneous, the original color of the titrated solution is restored, and then the coloriscopic capillary is made to fill up. Standard solution is now added more slowly until the end point is again overstepped in the bulb. The contents of the bulb are once more back-titrated as described, and the whole procedure may be repeated, if the ratio of the capacity of the coloriscopic capillary to the total volume of titrated solution permits.

The final adjustment of the end point requires observation of the color change by means of the coloriscopic capillary. The buret is adjusted to give a low rate of outflow. The standard solution is added in small portions, and after each addition the contents of coloriscopic capillary and bulb are mixed. The color of the resulting solution is observed after it has again filled the whole length of the capillary. The opening of the coloriscopic capillary is viewed through W with the aid of a magnifying glass. Thus, the end point is recognized before a change of color can be seen in the bulb of the titration vessel.

After some practice has been acquired, a titration can be performed within 10 minutes. This time will even allow the elimination of carbon dioxide by blowing steam on the outside of the titration vessel while a stream of gas is bubbled through the titrated solution.

Neutralimetric Titration with Methyl Red. Half normal sodium hydroxide was titrated with hydrochloric acid of approximately the same normality. The customary concentration of indicator was obtained by adding 1 drop of methyl red solution to 200 ml. of each standard solution. The base was run from a buret into the titration vessel, and acid was added from another buret until the color changed to pink. Carbon dioxide was expelled by heating before the final adjustment of the end point.

Table III indicates the superiority of the titrating bulb with attached coloriscopic capillary. Use of centrifuge

Table III. Determination of Base-Acid Ratio with 0.5 *N* Solutions

In Microcone			In Bulb with Attached Coloriscopic Capillary		
NaOH *Cm.*	HCl *Cm.*	Cm. NaOH / Cm. HCl	NaOH *Cm.*	HCl *Cm.*	Cm. NaOH / Cm. HCl
11.99	12.00	0.9993	10.12	10.15	0.9970
10.94	11.10	0.9858	12.23	12.26	0.9974
10.02	10.15	0.9872	18.21	18.21	1.0000
			21.35	21.43	0.9962
			25.99	26.08	0.9965
	Mean	0.991 ±0.004			0.9974 ±0.0005

Table IV. Permanganate Titration with 0.1 *N* Solutions

In Microcone			In Bulb with Attached Coloriscopic Capillary		
$KMnO_4$ *Cm.*	$(NH_4)_2Fe(SO_4)_2$ *Cm.*	Cm. $KMnO_4$ / Cm. $FeSO_4$	$KMnO_4$ *Cm.*	$(NH_4)_2Fe(SO_4)_2$ *Cm.*	Cm. $KMnO_4$ / Cm. $FeSO_4$
12.14	10.41	1.166	12.12	10.57	1.147
14.30	12.26	1.166	15.59	13.62	1.145
18.55	15.96	1.162	19.15	16.77	1.142
22.98	19.76	1.163	22.27	19.30	1.154
25.75	22.08	1.166	24.22	20.97	1.155
	Mean	1.165 ±0.001			1.149 ±0.002

cones cannot be considered by any experimenter who is unable to recognize slight differences of hue in faint colorations.

Titration with Permanganate. Tenth normal acid ferrous ammonium sulfate was measured from a buret into the titration vessel and permanganate solution of approximately the same normality was added from another buret until a pink coloration of the titrated solution could be perceived.

The results are compiled in Table IV. Again it is obvious that the end points were not recognized in proper time when the titrations were performed in centrifuge cones.

Iodometric Titration Using Chloroform as Indicator. Tenth normal thiosulfate solution was titrated with iodine solution of approximately the same strength in a centrifuge cone of 2-ml. capacity. The approximate volumes of standard solutions required were found by a preliminary titration, and then the exact ratios were determined as follows: The thiosulfate solution in the centrifuge cone was continuously stirred by a stream of small air bubbles and treated with such a volume of iodine solution as to approach the end point closely. A small drop of propyl alcohol was then added to the titrated solution, and a small drop of chloroform was allowed to fall into the titration vessel from a height of about 5 cm. above the surface of the titrated solution. This procedure and the lowering of the surface tension by the addition of propyl alcohol enabled the drop of chloroform to penetrate the surface of the aqueous solution and to sink to the bottom of the container. In spite of its greater density, the chloroform would otherwise have floated on the surface. The titration was finished by adding small portions of iodine solution until the end point was indicated by the appearance of a yellow coloration in the drop of chloroform.

Since a stream of gas bubbles serves for agitation, the chloroform evaporates rapidly. It is not practical, therefore, to use a very small drop of chloroform or to add the chloroform at an early stage of the titration. The rate of evaporation may be considerably decreased, however, by passing the gas used for agitation through a washing bottle with chloroform before it is led to the fine capillary.

Drops of chloroform of 1- to 2-mm. diameter have been found most satisfactory for the indication of the end point. If the droplet never gets close to the tip of the buret, iodine will not be absorbed as long as thiosulfate is present in the solution. Even drops of 3- to 4-mm. diameter may be used, if the opening of the buret is kept far away from the chloroform.

Whenever an excess of iodine solution is added, back-titration is best performed by adding an excess of thiosulfate and then increasing the rate of the gas stream so that the chloroform droplet is completely evaporated. This does not require much time, whereas the extraction of iodine from a chloroform drop of more than 2-mm. diameter is extremely slow. The excess of thiosulfate takes care of the iodine liberated during the evaporation of the chloroform. Finally, a fresh droplet of chloroform is added, and the end point is reached by adding iodine standard solution.

The ratio of centimeter of iodine to centimeter of thiosulfate calculated as the mean of six determinations was 1.119 ± 0.002. The average deviation of the single determinations was equal to ±0.005 or ±5 parts per thousand. When the titration was carried out in the same cones with the customary concentration of starch for the indication of the end point, the ratio 1.130 ± 0.003 was obtained as the mean of three determinations.

Argentometric Titration Using Adsorption Indicator. The color change takes place at the surface of the precipitate formed during the titration. If it sufficed to watch the color shown by one particle of the precipitate, no matter how small, this method of end-point indication should be suitable for titrations on an extremely small scale. There is, however, reason to assume that a satisfactory color effect is obtained by a repeated reflection of the light from the surfaces of a large number of particles. This view is supported by the experience in microtitrations, which showed that it is preferable to prevent flocculation of the silver chloride by adding the standard solution rather fast while agitating by a moderate stream of air bubbles.

Tenth normal sodium chloride was run from the microburet into a centrifuge cone of 2-ml. capacity. There the standard solution was treated with one tenth of its volume of 1 *M* acetic acid, two tenths of its volume of 1 *M* ammonium acetate, and a small drop of dichlorofluorescein indicator solution. Then 0.1 *N* silver nitrate was added in a rather rapid stream until the end point was near. Most of the precipitate remained suspended in the solution. The titration was finished by adding small portions of silver nitrate until the color change took place throughout the mixture. Back-titration was not feasible, since the pink coloration of the precipitate was not completely discharged on adding sodium chloride. Thus, the approximate location of the end point had to be established by a preliminary titration.

The ratio centimeter of silver nitrate to centimeter of sodium chloride was found as the mean of 4 titrations to be 1.051 ± 0.003. The average deviation of the single determination was equal to ±0.005 or ±5 parts per thousand.

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A Fluorescent Microscope Lamp

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An inexpensive and versatile fluorescent microscope lamp is described. Sufficient light can be obtained for use with high-power microscopy and low-magnification photomicrography. Daylight-type bulbs give illumination comparable to that obtained from the ordinary microscope lamp using a daylight filter, but without an uncomfortable radiation of dissipated heat.

THE microscope lamp described herein has been used by the author for the past year and has proved very satisfactory. The purpose in designing and building it was to overcome the uncomfortable heat output of the ordinary microscope lamp when used over extended periods. Every microscopist is well aware of the discomforts of working with a lamp whose housing is too hot to handle, owing to the intense heat radiated from the incandescent wires of the bulb.

The fluorescent-bulb lamp shown in Figures 1 to 4 gives off little heat. It can be used for hours and still not be uncomfortable to touch. The lamp accommodates three 6-watt fluorescent bulbs, each independently operated by a switch, so that any combination may be used. Daylight-type bulbs are used, and their light is practically the same as that obtained from the ordinary microscope lamp using a daylight filter. Enough light can be obtained for use with high-power microscopy and low-magnification photomicrography.

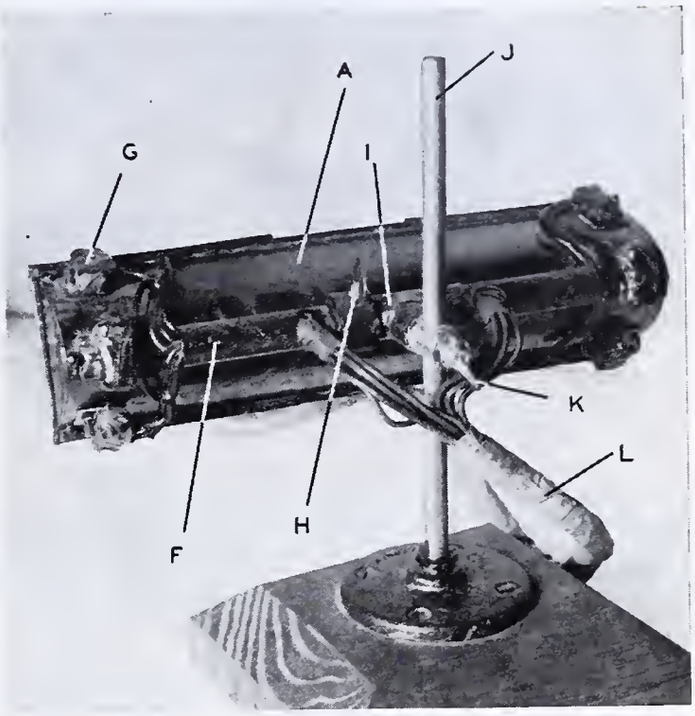

FIGURE 2. REAR VIEW OF MICROSCOPE LAMP SHOWING CONSTRUCTION AND PARTS

The lamp (Figures 1 and 2) consists of a parabolically shaped, troughlike reflector, *A*, of sheet brass, with Bakelite composition ends, *B*. The reflector is flanged to accommodate sheet-iron sliding covers, *C*, which allow for a variation in reflector opening. A sheet of polished aluminum, *D*, is held in place inside the reflector by small soldered-on clips, *E*. A brass tube, *F*, is soldered to the back of the reflector. This holds the wiring from the lamp sockets, *G*, which are fastened to and project through the reflec-

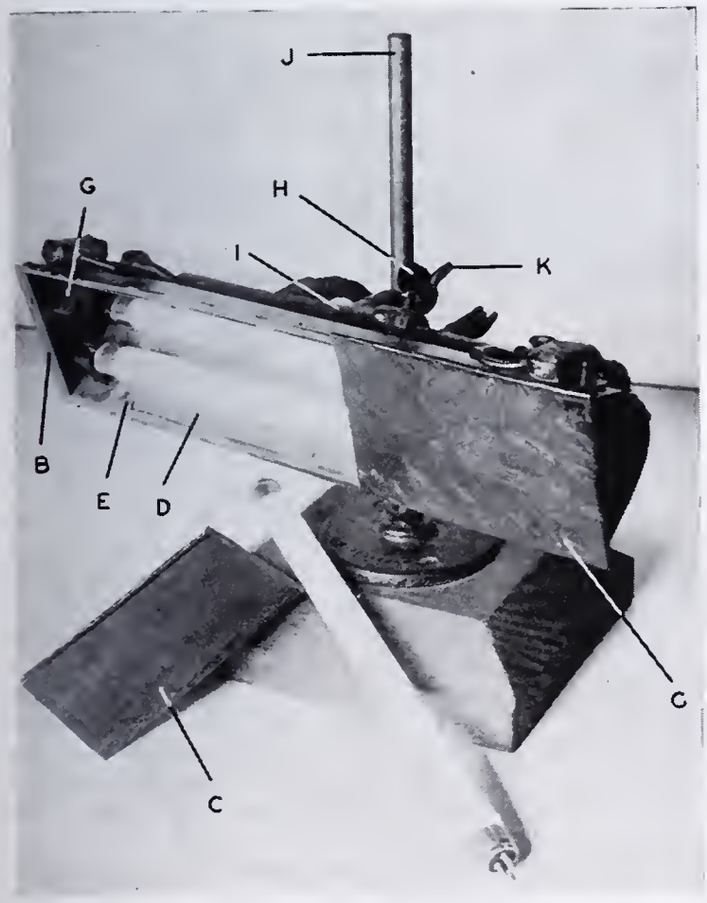

FIGURE 1. FRONT VIEW OF MICROSCOPE LAMP, SHOWING CONSTRUCTION AND PARTS

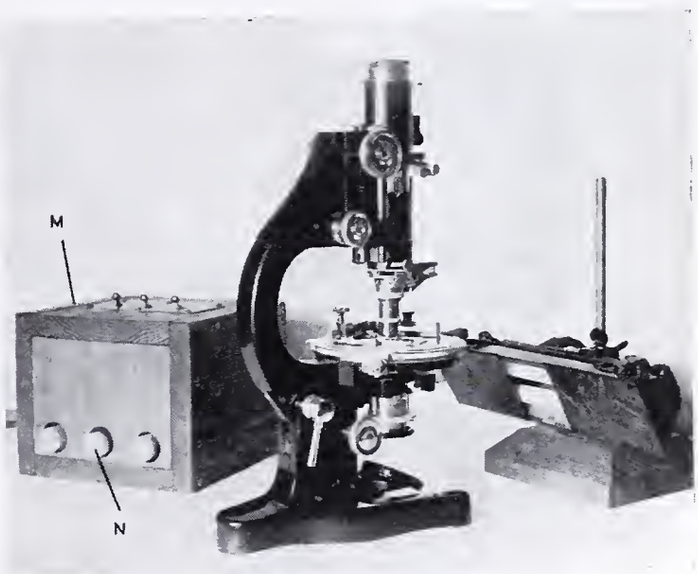

FIGURE 3. MICROSCOPE LAMP AND CONTROL BOX, SHOWING USE WITH PETROGRAPHIC MICROSCOPE

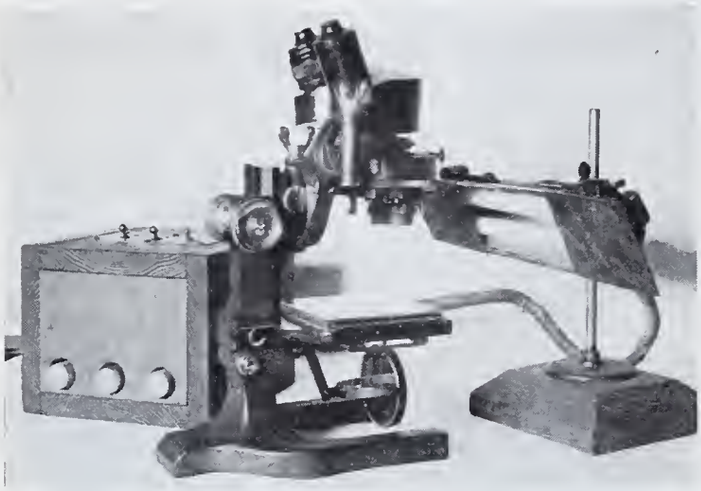

FIGURE 4. MICROSCOPE LAMP AND CONTROL BOX, SHOWING USE WITH BINOCULAR MICROSCOPE

tor. The brass tube also allows the reflector to be partly rotated about a horizontal axis. A wing nut, *H*, in a T-clamp, *I*, locks the reflector at any point in this rotation. The T-clamp slides on a vertical rod, *J*, and a wing nut, *K*, locks the reflector at any position along the rod. Cable *L* from the reflector enters a switch box, *M* (Figure 3), which also houses the three ballasts and attached lamp-starters, *N*, necessary for individual operation of the three fluorescent bulbs. The lamp-starters project through the switch box and can be replaced without opening the box. A cord from the switch box leads directly to a 110-volt alternating current outlet.

Dimensions and construction details are not included here because they are not rigidly fixed but depend on materials at hand in the laboratory and shop. The following electrical equipment is necessary:

3 G. E. Mazda (F) fluorescent T-5 lamps, 6 watts, daylight, 0.625-inch diameter, 9-inch length	$0.90 each
3 G. E. fluorescent lamp ballasts, catalog No. 58G800	0.79 each
3 G. E. fluoroescent lamp-starter FS-5, for use with ballast	0.37 each
3 pairs of fluorescent lamp sockets, G. E. type T-5	0.54 pair

The lamp is relatively inexpensive compared with other good microscope lamps, and its only disadvantage seems to be inadequate intensity of light for high-magnification photomicroscopy. The illustrations show how compactly the lamp can be built (Figure 4) and its versatility in use.

Acknowledgment

Appreciation is expressed to R. D. Spencer, undermechanic in the shop, who did the construction work.

THIS report represents the results of cooperative work between the Bureau of Mines, United States Department of the Interior, and the University of Alabama. Published by permission of the Director, Bureau of Mines.

A Heating Device for Microbeakers, Flasks, and Centrifuge Tubes

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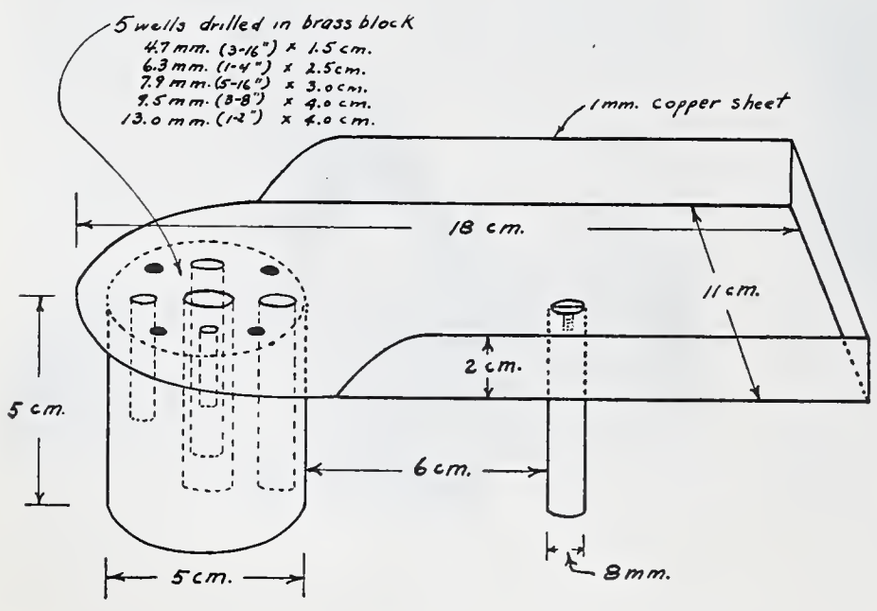
THE heating of small beakers, Erlenmeyer flasks, and centrifuge tubes for the purpose of causing solution of a compound or evaporation of a solvent is often attended by inconvenience and annoyance. If a free flame is used, the solvent may catch fire, while steam may contaminate the solution with water or be insufficiently hot. An ordinary hot plate does not give adequate contact with centrifuge tubes. For beakers and flasks the hot plate is almost never at the desired temperature, which results in too slow a rate of heating if it is too low or violent bumping if the plate is too hot.



The device illustrated has been in use in the author's laboratory for several years and has proved very satisfactory. Wells are bored in a brass block to accommodate the standard sizes of small centrifuge tubes. To this is bolted a copper tray which carries a foot to prevent tipping. For use the block and tray are placed on a 6-inch hot plate. When temperature equilibrium between the hot plate, tray, and air is reached, one has a gradual change in temperature from the block to the end of the tray, and beakers or flasks need only be shifted from front to back to have the contents boil, simmer, or just keep warm. If metals other than brass and copper are used or the dimensions are changed, it may be necessary to alter the length of the tray or the thickness of the metal sheet in order to get the desired temperature change from front to back. The brass block may, of course, be wrapped with resistance wire and heated electrically, but the author prefers the simpler device.

Determination of Carbon in Low-Carbon Iron and Steel

Low-Pressure Combustion Method

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An improved apparatus and method for the determination of carbon in low-carbon iron and iron alloys are described. The method consists of combustion of the sample in an all-glass apparatus, collection of the carbon dioxide by condensation in a liquid nitrogen trap, and, after pumping out the excess oxygen, determination of the carbon dioxide by a pressure measurement in a standard volume. The sample is heated by a high-frequency induction furnace. The precision of the method is within ±10 per cent on samples containing 0.003 per cent carbon.

IN CONNECTION with the analysis of hydrogen-treated iron some years ago (*3*) it became necessary to devise a method of high sensitivity for the determination of carbon. This was an application of techniques described by Cioffi (*2*) for heat-treating metals in vacuum in conjunction with the low-pressure method of gas analysis (*8*). It consisted of combustion of the sample in an all-glass apparatus, collection of the carbon dioxide by condensation in a low-temperature trap (liquid air or nitrogen), and, after pumping out the excess oxygen, determination of the carbon dioxide by a pressure measurement in a calibrated volume. The sample was heated by means of a high-frequency induction furnace. More recently the method has been improved and applied with satisfactory results to iron-silicon and iron-nickel alloys of low carbon content. This paper describes the improved method and some of its applications.

Somewhat similar methods for the determination of carbon in low-carbon iron have been described previously (*9*, *10*, *11*), but these methods do not employ high-frequency heating; hence they cannot utilize the advantages of an all-glass system. The induction furnace has been applied (*7*) to the determination of carbon in tungsten and chromium steels and other alloys difficult to burn by the conventional method, but in this case the carbon dioxide was determined by the usual gravimetric method.

Apparatus

The apparatus developed for the determination of carbon is shown in Figure 1. It consists essentially of a purification system, a combustion chamber, and an analysis system, approximately 200 cc. in volume, connected to a mercury diffusion pump backed by a mechanical oil pump (Cenco Megavac).

PURIFICATION OF OXYGEN. The oxygen, admitted from a tank connected at A through a flexible copper tubing, first passes through a liquid nitrogen trap, then over a palladium catalyst at 800° to 900° C. (Evidence of the presence of hydrocarbon impurity, probably methane, in some tanks of commercial oxygen made this step necessary. A temperature of 400° C. has been found satisfactory for oxygen now available), and finally through two more traps at liquid nitrogen temperature before being admitted to the combustion chamber. The palladium catalyst is contained in a quartz tube connected to the glass by graded seals. The oxygen is purified by condensing approximately 10 ml. in trap T_1 and then evaporating a portion of this and recondensing it in traps T_2 and T_3. These traps collect any oxidation products formed by passing the oxygen over the palladium catalyst and also hold the liquid oxygen until ready to be admitted to the system.

At liquid nitrogen temperature the vapor pressure of oxygen is approximately 16 cm., so that it is possible, with the cutoff at I closed, to hold a supply of pure oxygen in trap T_3 while evacuating and outgassing the analysis and combustion systems. The mercury cutoff at I is employed as a manometer. The capillary by-pass (1-mm. bore) in I is a device for admitting oxygen at a relatively high pressure of about 16 cm. to the evacuated system. The capillary at K serves a similar purpose in exhausting the excess oxygen subsequent to the combustion of the sample.

COMBUSTION SYSTEM. The combustion system is sealed on at D and is shown in the upper left of Figure 1. It consists of a Pyrex tubular chamber in which is suspended coaxially a platinum crucible which contains the refractory oxide crucible. The sample, in the form of millings or thin turnings, is introduced into the side tube, Q, which is then sealed off. The sample is held in the horizontal portion of the loading tube, Q, at point a, until the system has been exhausted and a final blank run on the crucible, after which the sample is introduced into the combustion crucible by means of a magnet. With additional horizontal branches on the loading tube, provision can be made for admitting several samples at one time, thus reducing by a considerable factor the average time required for a determination. This technique also permits a blank to be run on the crucible immediately before introduction of the sample, thus eliminating any gas adsorbed on the crucible.

The platinum crucible, which serves as the heating element of the high-frequency induction furnace, is of standard form with reinforced rim and attached bail, of approximately 15-ml. capacity, and was obtained from Baker & Co. The refractory oxide crucible which fits snugly in the platinum crucible is made of alumina or magnesia and was supplied by Norton and Company. (For suggesting the use of magnesia the authors are indebted to S. E. Q. Ashley, General Electric Company, Pittsfield, Mass., in whose laboratory multiple loading was first employed.) The blank on these crucibles which were specially prepared for the analysis of low-carbon iron is readily reduced to a low and constant value by prefiring in oxygen. Both alumina and magnesia crucibles, however, will adsorb a small quantity of carbon dioxide when exposed to air at room temperature and this will introduce an error unless removed by heating in vacuum or in oxygen before introducing the sample.

For the combustion of nickel or iron-nickel alloys which require a higher temperature, the sample was placed directly in a small platinum crucible. The rapid formation of an oxide film on the sample prevents alloying with the crucible.

The high-frequency unit used in this work was a vacuum tube oscillator designed and constructed by the Western Electric Company. A coil of 15 turns with an inside diameter of 6.25 cm. (2.5 inch) was used. A current of about 35 amperes at a frequency of approximately 500 kc. was required to give a temperature of 1100° C. under the conditions specified in the method. The coil was air-cooled by means of a small blower. Temperature was measured by means of an optical pyrometer.

Stopcocks S_1 and S_7 are lubricated with Apiezon L, which is a very low vapor pressure grease formerly supplied by Technical Products, Ltd. Traps T_1 and T_6, which are held at liquid nitrogen (or liquid air) temperature, serve to protect the system from condensable gases originating in the stopcock grease as a result of oxidation.

Analysis System. The analysis system consists simply of a McLeod gage and the double trap, T_5, separated from the other sections of the apparatus by the mercury cutoffs, J and K. [Contrary to a prevalent opinion, the McLeod gage can be satisfactorily employed to measure carbon dioxide and other condensable gases. The accuracy and precision of the gage applied to carbon dioxide are well within the requirements of the present method—for example, the maximum error due to nonideality under the working conditions is less than 0.3 per cent (*5*).] The McLeod gage, M, has a sensitivity of 5×10^{-5} mm. and a range up to 1 mm. of mercury. The gage was calibrated by the usual procedure (*4*), and the volume was calibrated by expanding measured quantities of gas from the McLeod gage.

Procedure

The first step in the procedure is to exhaust and degas the entire system, by heating the glassware with a Bunsen flame with all cutoffs lowered and the pumps in operation. Holding the glassware at a temperature of approximately 425° C. for a period of 30 minutes is sufficient to condition the system. During this period furnace F is heated to 900° C., and the crucible is heated at 1150° C. by means of high-frequency induced current for a 10-minute period. During this and subsequent heating of the crucible, the combustion chamber is cooled by a stream of air from a small blower. A final degassing of the analysis and combustion sections of the apparatus is made with cutoff I closed. After the initial degassing of the apparatus it is unnecessary to give it more than a few minutes' flaming in subsequent runs, provided the system is kept under vacuum. For routine analysis of samples of higher carbon content, the outgassing procedure can be shortened considerably.

When the system has been satisfactorily conditioned (as indicated by ability to maintain a vacuum of approximately 5×10^{-5} mm. of mercury for at least 1 hour with cutoffs I and K closed), traps T_1, T_2, T_3, and T_6 are surrounded by liquid nitrogen, oxygen is admitted, and purified, and blank determinations are run under the conditions used in the combustion of a sample.

An analysis is run as follows: The sample (0.5 gram) is introduced into Q, the tube is resealed, and the system is immediately exhausted. Oxygen is introduced and purified as described above. It is important first to collect liquid oxygen in T_1 and then to redistill it to T_2-T_3, where an excess of the liquid oxygen (over that required for the combustion of the sample) is collected and held until ready to be introduced into the combustion chamber. This procedure of filling the combustion system by evaporation of oxygen from T_2-T_3 prevents condensable impurities from being swept through the traps mechanically.

Having a supply of liquid oxygen in T_2 and T_3, K is closed, and the McLeod gage is filled with mercury by admitting air through

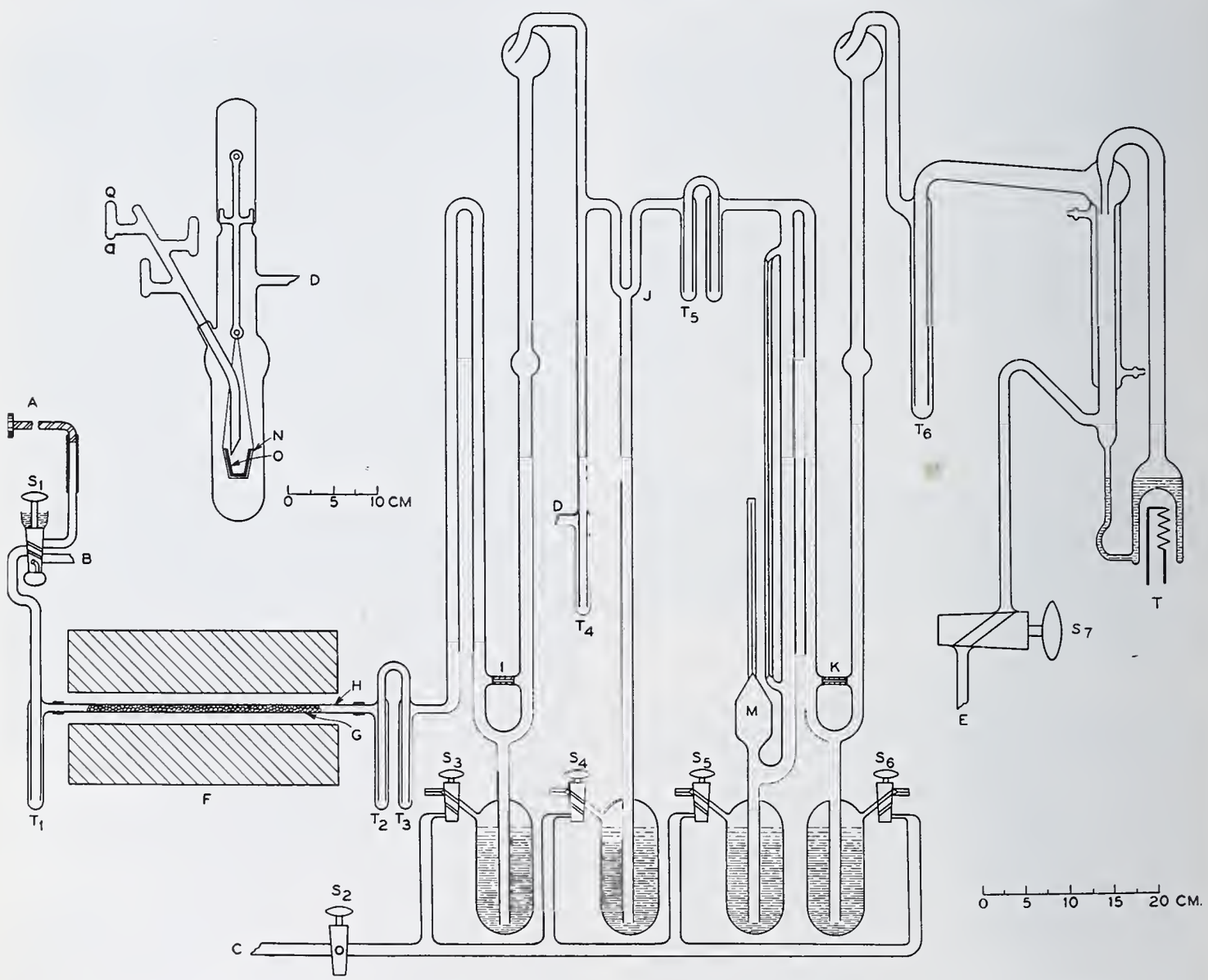

Figure 1. Apparatus for Determination of Carbon in Iron by the Low-Pressure Combustion Method

A. Flexible copper tube with copper-to-glass seal
B, C. Outlet to rough vacuum (Cenco Hyvac)
D. Connection to analysis system
E. Outlet to oil pump (Cenco Megavac)
F. Electric furnace
G. Palladium catalyst on asbestos
H. Clear quartz tube
I, J, K. Mercury cutoffs
M. McLeod gage
N. Platinum crucible with reinforced rim supplied by Baker & Co.
O. Magnesia crucible supplied by Norton and Co.
Q. Side tube for admitting sample
S_1–S_7. Stopcocks
T. Heater
T_1–T_6. Traps

stopcocks S_6 and S_5, respectively. Assuming the pressure in the system has been tested and found satisfactory, oxygen is admitted to the combustion chamber by lowering the mercury in I (by applying suction to stopcock S_3) to a point where the oxygen will just pass through the capillary by-pass and bubble up through the mercury column in the right arm of I. When the pressure differential between the purification and combustion systems is somewhat reduced, I is opened completely to allow the pressure in the two sections to equalize more quickly. I is then closed to a point just above the capillary by-pass and held there during the heating of the crucible.

Having admitted the oxygen as described above, a liquid nitrogen (or liquid air) bath is placed on T_5 and a solid carbon dioxide-Cellosolve acetate bath on T_4. The platinum crucible is now heated by high-frequency induced current to a temperature of 1150° C. Heating is continued for 10 minutes.

At the end of this period the excess oxygen is evacuated in the following manner: The main stopcock, S_7, is closed and then K is lowered very slowly to a point where the oxygen can escape slowly through the capillary at K. When the pressure has equalized in the sections separated by K, S_7 is opened just enough to allow the oxygen to be slowly pumped from the system through the capillary at K. When a pressure in the system of less than 1 mm. is reached, K is opened completely and the system is pumped down to a vacuum of about 5×10^{-5} mm. The elapsed time from the escape of the first bubble of oxygen until K is opened completely is about 10 minutes. An additional 10 minutes is usually required to reduce the pressure to a value of approximately 5×10^{-5} mm.

As soon as the evacuation of the system is completed, K and J are closed and the carbon dioxide condensed in T_5 is released in the calibrated volume and allowed to attain thermal equilibrium at room temperature. If the blank is too high—i. e., greater than 10 per cent of the expected value on the sample—the blank run is repeated until satisfactory.

When a satisfactory blank has been established a sample is transferred from the loading tube to the crucible by means of a magnet, while the system is maintained under vacuum. Oxygen is admitted and the sample is burned according to the procedure described above. As the combustion proceeds and the oxygen pressure decreases in the combustion chamber, I acts as a valve, allowing more oxygen to enter the combustion system, thus tending to keep the pressure constant at slightly below 16 cm. of mercury while not allowing any of the gaseous combustion products to escape.

The same crucible may be employed for the combustion of several samples one after the other. A high blank value is never obtained after one sample has been burned in a crucible.

Knowing the volume of the system and the pressure, the carbon content of the sample may be computed. One cubic centimeter of carbon dioxide at 1 mm. of mercury and 25° C. (1 cc. mm.) is equivalent to 0.6454 microgram of carbon. Therefore

$$\frac{\text{Pressure (mm.)} \times \text{volume (cc.)} \times 0.6454 \times 10^{-6}}{0.5000} \times 100 = \text{per cent carbon}$$

Sensitivity of the Method. The sensitivity of the method as described is of the order of 0.02 cc. mm. of carbon dioxide. This is equivalent to less than 0.02 microgram of carbon. Practically, however, it is not possible to realize the full sensitivity of the method because of the difficulty of reducing the blank on the apparatus and oxygen used as reagent. With care, however, it is possible to hold the blank to the equivalent of 1 microgram or less of carbon.

Experimental

In the method described above it is assumed that all the carbon in the sample is converted quantitatively to carbon dioxide and as such is condensed at low temperature in trap T_5; and that the gas so collected after passing through trap T_4 at −80° C. is substantially pure carbon dioxide. It is of particular interest to determine the influence of sulfur on the purity of the carbon dioxide obtained, since sulfur is usually present in small quantities in the iron samples to be analyzed. In the following paragraphs data are presented on the completeness of combustion of the sample, the composition of condensable gas obtained, and the influence of sulfur upon the results obtained by the method as applied to some typical samples.

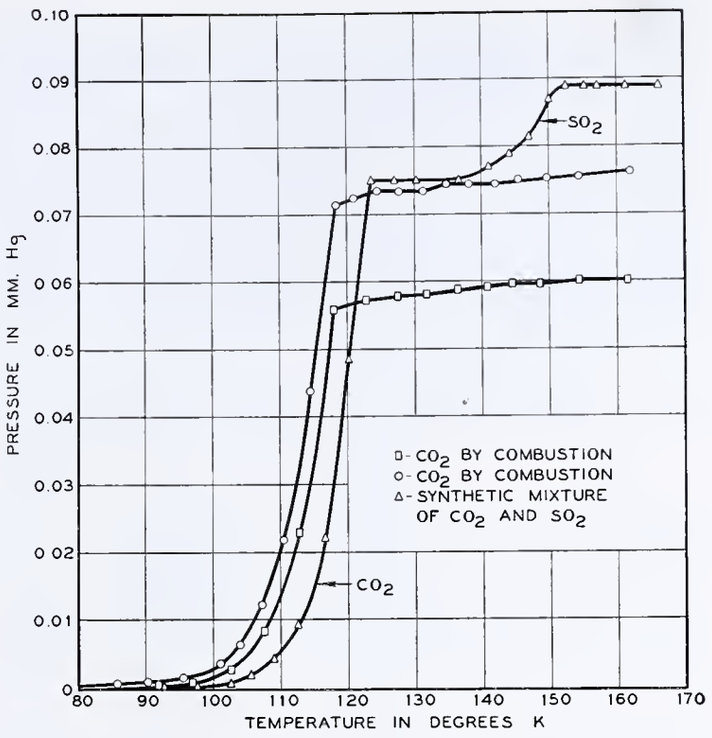

Figure 2. Analysis of Gases

Combustion of the Sample. In order to determine whether the carbon is completely removed by this method, the fused iron oxide obtained from the combustion of thirteen 0.5-gram samples was collected, pulverized, and reanalyzed for carbon by firing in oxygen for a 30-minute period at 1200° C. The value obtained was equivalent to 0.0001 per cent carbon on material which originally contained approximately 0.01 per cent carbon.

The presence of the platinum crucible, heated to 1100° C., and of the platinum oxide which forms and evaporates to the walls of the combustion chamber, catalyzes the oxidation to carbon dioxide of any carbon monoxide formed in the combustion reaction, thus making possible the elimination of the copper oxide reagent previously employed (*9, 11*).

Condensation of Carbon Dioxide. By inserting an additional cutoff and trap between T_5 and K it was established that the condensation of carbon dioxide in T_5 under the conditions employed is substantially complete. No carbon dioxide was detected in the added trap after making a run in the usual way.

Composition of Condensable Gas. The gas collected in T_5 on combustion of typical samples was identified as carbon dioxide by the following tests: The gas obtained from a typical sample was completely absorbed when exposed to soda-lime. Portions of the gas obtained from the combustion of two different samples of iron, containing approximately 0.02 per cent carbon and 0.02 per cent sulfur, were analyzed by the low-temperature distillation method (*1, 8*). The vapor pressure curves obtained were characteristic of carbon dioxide. No evidence of sulfur dioxide was found in either case. A plot of the vapor pressure curves obtained on these two samples is shown in Figure 2 in comparison with a curve on a synthetic mixture of carbon dioxide and sulfur dioxide. It is clear that the presence of sulfur dioxide can be readily detected by this method.

In an attempt to determine the effect of sulfur, if present in larger quantities, 5 mg. of ferrous sulfide were added to a 0.5-gram sample of Bureau of Standards Sample No. 55A and the mixture was analyzed by the procedure described. The carbon dioxide obtained was equivalent to 0.011 per cent carbon, the same value as obtained without the addition of ferrous sulfide. Sulfur dioxide was not detected in the condensable gas. The apparatus was then opened and the walls of the combustion chamber and T_4 (the dry ice trap) were extracted with a solution of hydrochloric and nitric acids. These solutions, analyzed separately for sulfur by a microgravimetric procedure, contained 1.1 and 0.1 mg. of sulfur for the combustion chamber and trap, respectively. A portion of the magnesia crucible used was also analyzed and was found to have increased in sulfur content. The total sulfur recovered from all sources was roughly equivalent to that added. However, when two consecutive runs were made on samples to which ferrous sulfide had been added, without changing the crucible or cleaning the combustion chamber be-

tween runs, a high result was obtained on the second sample burned and sulfur dioxide was found in the carbon dioxide trap, indicating incomplete oxidation of the sulfur to sulfur trioxide.

Effect of Preheating Sample in Vacuum. In the early work the sample was preheated in vacuum for a short period at 500° C. to remove adsorbed carbon dioxide (*9*, *11*). It was found, however, that such a preheating treatment is unnecessary if the crucible is preheated and the sample transferred to it in vacuum. Furthermore, it appears to be unsound practice to preheat the sample in vacuum because of the probable loss of carbon as carbon monoxide. Some carbon dioxide is no doubt adsorbed on the magnesia crucible when exposed to air and is later evolved when the crucible is heated, but this can be eliminated by heating the crucible in vacuum before the sample is transferred.

Table I. Determination of Carbon by the Low-Pressure Method

Sample		Carbon (Corrected for Blank) %	Deviation %	Blank Correction %
Armco iron		0.0231	+0.0008	
		0.0229	+0.0006	
		0.0216	−0.0007	
		0.0210	−0.0013	
		0.0220	−0.0003	
	Av.	0.0223	±0.0007	0.0003
Armco iron H_2 treated		0.0031	+0.0001	
		0.0031	+0.0001	
		0.0030	0.0000	
		0.0030	0.0000	
	Av.	0.0030	±0.0001	0.0002
Carbonyl iron		0.0087	+0.0002	
		0.0078	−0.0007	
		0.0090	+0.0005	
	Av.	0.0085	±0.0005	0.0003
Iron-silicon alloy		0.0060	0.0000	
		0.0059	−0.0001	
	Av.	0.0060	±0.0001	0.0002

Precision of the Method

The precision of the method applied to typical samples is shown in Table I.

In order further to test the method Bureau of Standards Sample No. 55A, which has a certificated value of 0.014 per cent carbon, was analyzed. Results are listed in Table II.

Discussion

The data presented in Table I show that the precision of the method is well within the limits of ±10 per cent of the quantity of carbon determined in the range of 0.003 to 0.02 per cent carbon. The precision obtained on hydrogen-treated samples of very low carbon content is generally higher than that obtained on samples of higher carbon content. This indicates a more uniform distribution of the residual carbon in the hydrogen-treated samples of low carbon content.

The data in Table II show that the method gives a value of 0.011 per cent carbon as compared with the certificated value of 0.014 per cent. Further work showed that the low-pressure method, when vacuum loading is employed, consistently gives slightly lower results than are obtained with the standard carbon combustion method (*6*). This difference may be due to the elimination of adsorbed carbon dioxide from the crucible by heating in vacuum or in oxygen immediately before the sample is introduced, a procedure not possible with the standard high-pressure method. It was found, for example, that the crucible employed in this work would adsorb carbon dioxide equivalent to 0.002 to 0.003 per cent (0.5-gram sample basis) on exposure to air for 30 minutes.

The absence of sulfur dioxide in the condensable gas obtained on combustion of samples containing small quantities of sulfur suggests that this gas, in contact with the crucible and the platinum oxide observed to deposit on the walls of the combustion chamber, is oxidized to sulfur trioxide and is adsorbed on the ceramic crucible and the glass walls of the apparatus or condensed in trap T_4 at −80° C. The presence of sulfur on the walls of the combustion tube and in T_4 following the combustion of a sample containing larger quantities of sulfur supports this explanation. The evidence indicates, however, that the method is not suitable for the analysis of samples containing greater than 0.1 per cent sulfur, unless modified to ensure complete oxidation of sulfur to sulfur trioxide. Most samples of low carbon content, however, are also low in sulfur, and on these samples no significant error is introduced. The method deserves wide application not only to low-carbon iron and iron alloys but to higher carbon alloys where only very small samples are available—for example, the authors have successfully applied it to samples of vacuum tube filament core made of nickel and nickel alloys, weighing only a few milligrams. The method is generally applicable to the determination of microquantities of carbon. For application to samples of higher carbon content the procedure may be simplified and the time for a determination correspondingly reduced.

Table II. Data on Bureau of Standards Sample 55A

Date of Analysis		Carbon %	Deviation %
11/26/41		0.0106	−0.0002
12/4/41		0.0109	+0.0001
12/4/41		0.0108	0.0000
12/30/41		0.0108	0.0000
12/30/41		0.0105	−0.0003
1/2/42		0.0109	+0.0001
1/5/42		0.0109	+0.0001
2/3/42		0.0109	+0.0001
2/3/42		0.0107	−0.0001
	Av.	0.0108	±0.0001

Summary

An improved apparatus for the determination of carbon in iron by the low-pressure combustion method is described and applied to samples of very low carbon content. The method is inherently of very high sensitivity. To realize the advantages offered by the high sensitivity of the method, however, it is necessary to exercise care in the purification of the oxygen and in the selection and conditioning of the combustion crucible. Methods for the purification of the oxygen and crucible and for minimizing other sources of error are described and precision data on the method are presented.

Acknowledgment

The authors are indebted to James Morrison for the data in Table II and for performing the experiments on the influence of sulfur.

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Volumetric Determination of Sulfates

Tetrahydroxyquinone as an External Indicator

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SULFATE ion may be determined volumetrically, using tetrahydroxyquinone as an internal indicator (*2*). Earlier workers point out:

> The unpracticed eye may have severe difficulty in accurately detecting the end point of the titration. False indications of an end point are often given by the color change to pink in the bulk of the solution and on the surface of any sodium chloride precipitate (*2*).
>
> When using solutions low in sulfate (less than 0.1 per cent sulfur in coal) more care must be exercised, for, owing to a rather indistinct color change, there is a tendency to overrun (*1*).

The various precipitates in the solution make it cloudy, and there is great difficulty in seeing the slight color change in the transmitted light, or upon the surface of the silver chloride precipitate, if silver nitrate is added. Figure 1 shows the change in color which a solution containing tetrahydroxyquinone undergoes at the end point as determined spectrophotometrically.

Peabody (*3*) recommended the use of tetrahydroxyquinone externally in microtitrations of sulfate, but the spot-plate technique which he used does not have some of the advantages of the method described here. However, a method similar to the one which the authors have used, but employing sodium rhodizonate as the indicator, has recently been noted (*4*).

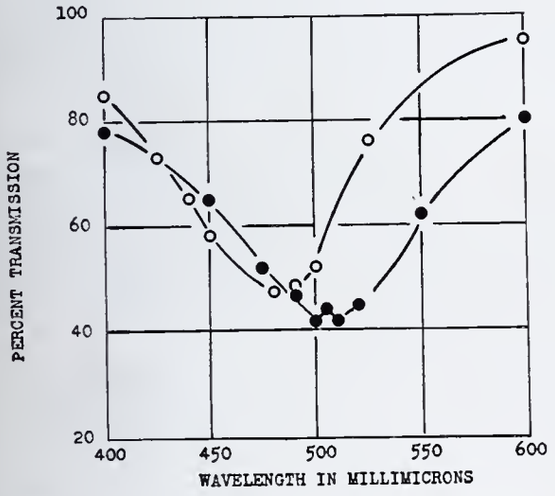

FIGURE 1. ABSORPTION SPECTRA OF SODIUM AND BARIUM SALTS OF TETRAHYDROXYQUINONE IN WATER-ALCOHOL SOLUTION

○ Sodium
● Barium

It was found that there were several advantages in using the end-point reaction between barium ions and tetrahydroxyquinone as a spot test on filter paper. One unfamiliar with this method has no trouble in finding the correct end point. This type of outside indicator is fairly rapid and offers a means of eliminating interfering substances. The method gave good results in titrations employing 0.1 *N* barium chloride, or for sulfate-ion concentration greater than about 150 p. p. m.

Spectrophotometric absorption curves of the water-alcohol solutions of the barium and sodium salts of tetrahydroxyquinone were run (Figure 1). The curves are similar in shape, as one would expect. The absorption maxima of the barium compound are at approximately 500 and 510 mμ. The barium salt has a higher tinctorial value at the same molar concentration. It can be seen from the two curves that the spectrophotometer can be used to check the visually determined end point, and that 525 mμ is the best wave length for this purpose. When two drops of an end-point solution were added to a cuvette containing a water-alcohol solution of the sodium salt of tetrahydroxyquinone, a sharp drop in the per cent transmission occurred.

Procedure

A 10 per cent (approximately) aqueous solution of the sodium salt of tetrahydroxyquinone was used as the indicator. Only a few drops of this solution were used for each determination, thus effecting a saving over the internal method. It was most satisfactory when made up in small quantities immediately before use. However, the addition of a small amount of mineral oil to prevent contact of the indicator solution with the air stabilized the indicator for several hours.

TABLE I. SULFATE DETERMINATIONS USING TETRAHYDROXYQUINONE EXTERNALLY

(All determinations in duplicate)

Source of Sulfate	Sulfate Added	Net Volume of Barium Chloride Solution Used	Sulfate Found
	Gram	*Ml.*	*Gram*
Na_2SO_4 (C. P.)	0.0045	1.03 (0.0946 *N*)	0.0047
	0.0157	3.40 (0.0946 *N*)	0.0155
	0.390	7.19 (1.126 *N*)	0.389
	1.052	19.41 (1.126 *N*)	1.049
$Al_2(SO_4)_3.18H_2O$ (C. P.)	0.0226	4.85 (0.0946 *N*)	0.0220
H_2SO_4 (Standard Solution)	0.422	7.75 (1.126 *N*)	0.419
Na_2SO_4 (C. P.) (Solution saturated with NaCl)	0.0366	8.04 (0.0946 *N*)	0.0365

A known weight of sulfate, in an aqueous solution, was titrated with standard barium chloride solution. A microburet calibrated to 0.01 ml. was used when less than 5 ml. of barium chloride solution were added. The volumes were chosen so that at the end point there was a total of about 30 ml. As the end point (determined approximately in a preliminary titration) was approached, a drop of the solution being titrated was added to a wet spot of indicator solution on a piece of filter paper. The color which formed was observed by transmitted light. The indicator spot was conveniently made with an eye-dropper. The first immediate (2 or 3 seconds) change from yellow to pink in the indicator spot indicated the end point. Only the changes of color which occurred immediately were of significance. The colors of older spots (15 seconds or longer) were found to be misleading. The indicator blank was found to be 0.20 ml. for titrations with 0.1 *N* barium chloride, and 0.04 ml. for titrations with 1 *N* barium chloride. The value of this blank, of course, depends on the final volume of the titrated solution. If the final volume differed greatly from 30 ml., the indicator blank was multiplied by the factor obtained by dividing the volume by 30, since the concentration of the slight excess of barium ion was being measured. Table I shows the range of the amounts of sulfate determined.

Elimination of Interfering Substances

The substances which ordinarily interfere in the tetrahydroxyquinone titration of sulfate are hydrogen and aluminum ions and sodium chloride, when present in large amounts. Solutions from peroxide fusions always contain excessive amounts of salt after they are neutralized.

The sulfuric acid solutions were first neutralized with ammonia, and then titrated as described above.

The aluminum ion was precipitated as the hydroxide with ammonia, heated to boiling, filtered, and washed. The filtrate was concentrated to 30 ml., and titrated as described.

The titration of sulfate solutions which were saturated with sodium chloride was best carried out in 50 per cent alcohol. The total volume was kept to 30 to 40 ml. The following procedure for the indicator spot was found to be more satisfactory in this case. A drop of the indicator solution was placed near a drop of 15 to 20 per cent silver nitrate, so that the spreading boundaries just met. The drop from the titration flask was put inside the silver nitrate spot, close to the indicator spot, and the color change was observed at the boundary. A second silver nitrate spot–indicator spot pair was used as a control.

Use of Spectrophotometer in Confirmation of the End Point

Qualitative absorption curves of the sodium salt of tetrahydroxyquinone in 50 per cent alcohol (in such concentration that maximum absorption in 2-cm. cuvettes was approximately 50 per cent) and of the barium salt were run (Figure 1). It is clear from the curves that the optimum wave length for the detection of the end-point color change is about 525 mμ. Accordingly, the end point was checked with the aid of a Coleman Universal spectrophotometer.

The spectrophotometer wave-length dial was set at 525 mμ, and the per cent transmission of the indicator solution was determined. The titration was then carried out in the manner described above. When a drop was taken from the titration flask just prior to the end point and added to the cuvette, no change in the per cent transmission occurred. However, a drop of the end-point solution lowered the per cent transmission from 52 to 45. This method could be used for confirmatory purposes only, for the addition of pre-end point solution to the cuvette would build up a sulfate-ion concentration and lead to a false value.

Summary

A method of using tetrahydroxyquinone as an outside indicator in the determination of sulfate is described, which can be conveniently and rapidly used, and with considerable accuracy. It offers a means of removing interfering substances such as aluminum ion and a large excess of sodium chloride. The possibility of using a spectrophotometer in confirming the end point in this titration is pointed out.

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Analytical Use of Sodium Rhodizonate[1]

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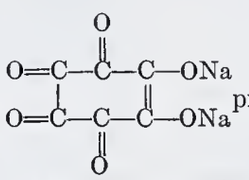
SODIUM rhodizonate  produces colored precipitates when added to solutions of barium or strontium salts. Feigl (*2*) was the first to apply this fact as a basis for spot tests for barium and strontium. Barium rhodizonate is stable toward acetic acid and dilute hydrochloric acid but is decomposed by sulfate ion, and accordingly Gutzeit (*5*) used it in his spot-test method of detecting sulfate. The formation and decomposition of barium rhodizonate have also made it possible to use sodium rhodizonate as indicator in the volumetric determination of barium or sulfate. A considerable number of papers have dealt with this method of determining the equivalence point of the sulfate-barium reaction (*1*, *4*, *6*, *8*, *9*, *10*).

This laboratory has been studying the possibilities of further analytical uses of sodium rhodizonate and the behavior of this reagent toward various metal ions has been examined. It has been found that this salt exhibits a selective action. With certain univalent and divalent metal ions it forms colored precipitates, whereas it produces no visible reaction with trivalent or quadrivalent cations. Bismuth is an exception, presumably because it can function in its salts as the bismuthyl radical, BiO^+.

Table I exhibits the findings obtained with 1 per cent neutral and acidified salt solutions. The behavior of the respective hydroxides and oxides is also recorded.

The behavior of iron salts is to be noted. Fe^{++} salts in neutral solution form a red-brown precipitate, which quickly becomes black-blue, probably through oxidation. In solutions of pH 2.8 there is no reaction. Fe^{+++} salts form no precipitate but produce a blue-green color; rhodizonic acid is a phenol and therefore reacts with Fe^{+++} salts like other phenols. In the presence of fluoride no color reaction occurs, owing to the formation of the complex FeF_6^{---} ion.

In the presence of fluoride ion the behavior of Fe^{++} salts is remarkable. The yellow of the rhodizonate solution is immediately discharged. No explanation can be given for this phenomenon, though it may be due to the increase of the reduction potential of Fe^{++} salts in the presence of fluoride ion, so that the oxidation of rhodizonic acid is induced by autoxidation of Fe^{++} salt.

From the analytical viewpoint, the formation of the slightly soluble red and blue-violet lead rhodizonates is particularly interesting. Under certain well-defined conditions, specified below, these precipitates can be utilized as a sure test for lead. These new tests are so sensitive that positive results are immediately given by even such slightly soluble materials as lead sulfide, lead sulfate, lead chromate, alloys, and a wide variety of other lead-bearing materials.

Rhodizonic acid can be used only as its sodium salt. This is a black-green solid; its water solutions are orange to yellow, depending on the concentration, and are stable for only a few days, even if stored in a refrigerator. The color changes and finally disappears, owing to a gradual decomposition arising from the ease with which rhodizonic acid is oxidized to

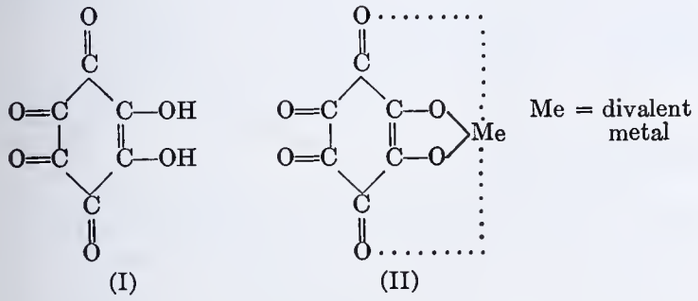
Table I. Reactions with Sodium Rhodizonate

Metal Ion	Metal Salt Solution: Neutral	pH = 2.8	Hydroxide	Oxide
Ag^+	Black	Black	.....	.....
Hg^+	Brown-red	Brown-red (disappears on standing)		
Tl^+	Dark brown	Dark brown	.....	.....
Pb^{++}	Blue-violet	Scarlet	Blue-violet	Blue-violet
Cu^{++}	Orange-red	.....	.....	.....
Hg^{++}	Red-orange	.....	.....	.....
Cd^{++}	Brown-red	Brown-red	Gray-brown	.....
Bi^{+++}	Brown-red	.....	Brown-red	.....
Ni^{++}	.....	.....	.....	.....
Co^{++}	.....	.....	.....	.....
Zn^{++}	Brown-violet	.....	Brown-violet	Brown-violet
Mn^{++}	.....	.....	.....	.....
UO_2^{++}	Brown	.....	.....	.....
Be^{++}	.....	.....	.....	.....
Mg^{++}	.....	.....	.....	.....
Ca^{++}	.....	.....	Brown-red	Brown-red
Ba^{++}	Red-brown	Red-brown	Red-brown	Red-brown
Sr^{++}	Red-brown	.....	Red-brown	Red-brown
Sn^{++}	.....	Violet	Violet	.....
Sn^{++++}	.....	.....	.....	.....
Al^{+++}	.....	.....	.....	.....
Zr^{++++}	.....	.....	.....	.....

[1] Translated by Ralph E. Oesper, University of Cincinnati.

croconic and leuconic acids. The decomposition of sodium rhodizonate is hastened by ultraviolet light, particularly if the salt is finely divided in the capillaries of paper.

The intense color of all water-insoluble rhodizonates and also of the soluble alkali salts in the solid state is worthy of consideration, as it indicates that these compounds may be inner-complex salts. This conception is justified. In rhodizonic acid (I) the two acid hydrogen atoms are so situated with respect to the adjacent CO-groups, which are capable of developing auxiliary valences, that the 5-membered rings, characteristic of inner-complex salts, can form. Accordingly, the inner-complex salts of rhodizonic acid can be formulated as shown in (II):



(I) (II)

The two rhodizonates of barium, and the two lead rhodizonates that have been isolated and are described below, are basic salts. It may well be that the color of these four compounds is likewise due to inner-complex rings of the type pictured above.

Preparation of Lead Salts of Rhodizonic Acid

Two rhodizonates, each with a distinctive color, can be precipitated from lead solutions by sodium rhodizonate. A blue-violet amorphous precipitate is thrown down from neutral lead solutions; whereas a crystalline, scarlet-red product is precipitated from weakly acidified lead solutions. Digestion with a dilute acid transforms the amorphous form into the crystalline.

Blue-Violet Lead Rhodizonate, $PbC_6O_6.Pb(OH)_2.H_2O$. Solutions containing 0.825 gram of lead nitrate and 0.55 gram of sodium rhodizonate, respectively, are mixed, brought to boiling and filtered, and the precipitate is washed with water and dried at 105° C. Weighed samples of the dried product were fumed several times with concentrated nitric acid, the residue ignited gently, and then taken to dryness with concentrated sulfuric acid. The resulting lead sulfate was weighed. An average lead content of 65.24 per cent was found; this agrees well with 65.3 per cent demanded by the formula $PbC_6O_6.Pb(OH)_2.H_2O$.

A basic lead rhodizonate corresponding to the formula $PbC_6O_6.Pb(OH)_2.5H_2O$ is obtained if the product precipitated by the foregoing procedure is not heated, but instead is freed of moisture by successive treatments with alcohol and ether. The calculated lead content is 58.64 per cent; found, 58.30 per cent.

Scarlet-Red Lead Rhodizonate, $2Pb(C_6O_6).Pb(OH)_2.H_2O$. Lead nitrate solution (1 per cent) was treated with an excess of buffer solution and heated to boiling, and any lead tartrate that had precipitated was removed by filtration. The buffer solution (pH = 2.79) contained 15 grams of tartaric acid and 19 grams of sodium bitartrate per liter (7). A saturated solution of sodium rhodizonate was added to the clear filtrate; the resulting red precipitate was washed free of excess lead with water, and then dried at 105° C. Analysis showed the compound to contain 61.69 per cent lead; the foregoing formula demands 61.60 per cent.

Analytical Uses of Sodium Rhodizonate

This laboratory has made a study of the use of sodium rhodizonate for spot-test analysis. This reagent can now be recommended for detection of (1) barium; (2) strontium; (3) barium in the presence of strontium; (4) strontium in the presence of barium; (5) sulfate; (6) barium and strontium in the carbonate precipitate of the alkaline earth metals; (7) lead in dilute solutions; (8) lead in the presence of ions that otherwise interfere; (9) lead in ores and minerals; (10) lead in alloys; (11) lead in pigments, glass, etc.

Cases 1 to 5 have been discussed elsewhere (*3*). Accordingly, only the newer possibilities of using sodium rhodizonate are described here.

Detection of Barium and Strontium in Carbonate Precipitate of Alkaline Earth Metals

Barium carbonate is more soluble than calcium or strontium carbonate and if it is thoroughly washed and then spot-tested directly with sodium rhodizonate a brown-red precipitate appears. Calcium and strontium carbonate do not react under these conditions. However, this differential behavior cannot be used indiscriminately to detect barium in a mixed carbonate precipitate, because barium rhodizonate is formed only if a very large excess of barium carbonate is present. When a mixture of the carbonates is thrown down, the barium carbonate may be enveloped by calcium or strontium carbonate, and thus shielded from the action of the sodium rhodizonate.

The alkaline earth metals are precipitated together as carbonates by the addition of ammonium carbonate to the solution obtained in the course of the usual qualitative scheme of analysis. To detect barium, or strontium, or both in this precipitate it suffices to place about 1 mg. of the thoroughly washed precipitate in the depression of a spot plate, add one drop of 0.2 per cent solution of sodium rhodizonate, and stir. If a positive reaction is obtained barium is present. If no reaction is observed, it is acidified with one drop of 1 *N* acetic acid. A red precipitate or coloration will indicate the presence of barium, strontium or both.

Detection of Lead

The formation of either the blue-violet or the scarlet-red lead rhodizonate can be used to detect lead. When testing for lead in neutral solutions that contain no other significant solutes, the conditions should be so chosen that the blue-violet salt is formed. Insoluble compounds should be treated directly with the tartaric acid–bitartrate buffer solution, whose composition has been stated above, because it also serves to dissolve sufficient lead to give the test.

In Solutions. A drop of the test solution is placed on filter paper. After the solution has been soaked up, the spot is touched with a drop of 0.2 per cent solution of sodium rhodizonate. A blue-violet fleck or ring is formed if lead is present. Identification limit: 0.1 microgram of lead; concentration limit: 1 to 500,000.

The lead in extremely dilute solutions can be concentrated by the following procedure:

Ten milliliters of the test solution are treated with 1 ml. of 0.2 *N* mercuric chloride, the mixture acidified, and the mercury, together with the lead, then precipitated by hydrogen sulfide (not ammonium sulfide). The precipitate is collected by filtering or centrifuging, washed slightly, dried, separated from the filter paper, placed in a crucible, carefully heated at first, and then ignited to volatilize the mercuric sulfide. The cooled residue is treated with 3 drops of buffer solution and mixed, and then 1 drop of 0.2 per cent freshly prepared solution of sodium rhodizonate is added. If lead is present, a red precipitate or coloration will appear. The identification limit is 5 micrograms lead in 10 ml.; the concentration limit is 1 to 2,000,000. It has been suggested that the lead might also be concentrated by electrolytic deposition. The microelectrode can be fumed in nitric acid and dried and the deposit then taken up in a drop of the buffer solution.

In the Presence of Other Materials. Table I shows that only thallium, silver, cadmium, barium, and stannous tin also react at pH = 2.8. However, if certain conditions are maintained, it is possible to detect lead with certainty, even though these interfering ions are present.

In the Presence of Insoluble Chlorides. Lead can easily be identified in the ordinary qualitative scheme if it is present along with silver chloride, mercurous chloride, and thallous chloride.

The chlorides thrown down by hydrochloric acid are not washed but are transferred directly to a crucible, dried by gentle warming, and then carefully heated to redness. Thallous and mercurous chloride are thus removed by volatilization. The cold residue is digested with 4 drops of strong ammonia water to dissolve silver chloride, and the contents of the crucible are then evaporated to dryness. Three drops of buffer solution and 1 drop of sodium rhodizonate are added. If the chloride precipitate contained lead, a red precipitate or coloration will appear. It is necessary to dissolve the silver chloride in ammonia because this salt melts and encloses lead chloride, which may thus be shielded from the sodium rhodizonate.

Table II. Alloys Tested

Pb	Cu	Sn	Zn	Ni	Fe	Mn	Al	Result of Test
19.77	72.15	6.85	1.03	..	0.20	..	..	+++
2.41	60.52	0.13	36.54	..	0.28	..	0.12	+++
1.81	59.74	...	28.58	9.87	..	..	..	++
1.08	85.73	12.28	0.97	1.34	..	..	..	+
0.89	57.26	0.77	37.85	..	1.45	1.82	..	+
0.56	85.34	11.87	0.74	1.48	..	..	..	+
...	60.13	0.23	39.56	..	..	..	..	−
...	89.17	9.54	...	1.44	..	..	..	−

This procedure, which can also be used to detect lead in the presence of all other metals, is far more sensitive than the usual method of dissolving the lead chloride in hot water and then adding a suitable reagent. The limiting proportion was determined in about 5 mg. of the mixed chlorides; the value is Pb:Ag = 1:5000.

In the Presence of Barium. The test solution is treated with concentrated sulfuric acid and then taken to definite fuming.

The mixed sulfates are brought onto a filter by means of alcohol and washed with this solvent until the sulfate test is no longer given. About 50 mg. of the washed precipitate are transferred to a spot plate, thoroughly mixed with 5 drops of a saturated solution of sodium acetate in 6 *N* acetic acid, and then dried by a current of heated air. One drop of water and 1 drop of sodium rhodizonate solution are then added. On stirring, the mass becomes violet if lead is present. If only minute quantities of lead are suspected, it is well to run a parallel test with pure barium sulfate. Limiting proportion, Pb:Ba = 1:10,000.

In Solid Products. *In Ores and Minerals.* A small quantity (not more than 1 mg.) of the finely pulverized specimen is placed on a spot plate. Three drops of buffer solution are added and, after 2 minutes, 1 drop of sodium rhodizonate. A red precipitate or coloration signals the presence of lead.

The following minerals gave a positive reaction: galena (PbS), cerussite ($PbCO_3$), stolzite ($PbWO_4$), anglesite ($PbSO_4$), crocoite ($PbCrO_4$), and lead-bearing zinc blende (ZnS-PbS).

In Alloys. A few fragments of the alloy are rasped off by means of a fine file, the grease is removed with ether, and the alloy is treated on a spot plate with 3 drops of the buffer solution. The solid and liquid are kept in agitation for 2 minutes by blowing into the suspension through a pipet. A drop of sodium rhodizonate is then added, but is not stirred into the system. If the alloy contains lead, a red precipitate or coloration appears. A blank test employing 3 drops of the buffer solution and 1 drop of the reagent should be run when testing alloys that are very low in lead. The blank becomes completely colorless after a few minutes.

The relative lead content of a series of alloys can be judged by the intensity of the red coloration, provided that the parallel tests are run under like conditions, such as equal quantities and reaction times of the buffer and reagent solution.

Although stannous tin gives a positive reaction in the buffer solution, no significant quantities of this metal ion were derived from treatment of the alloys tested with the buffer solution. Other types of alloys may not yield the same noninterference, and consequently with such samples it may be well to apply the test with caution and reserve.

The alloys tested are listed in Table II.

If the lead content of the alloy is above 1 per cent, the specimen can be drawn several times across an unglazed porcelain plate, and the streaks tested with 1 drop each of the buffer and reagent. The red lead salt is clearly visible when formed by this simple procedure.

Metallic lead will obviously give positive results, by either of these procedures. If a lead bullet has passed through wood, for instance, the fact can be established by spot-testing the hole with rhodizonic acid.

In Pigments, Glass, etc. Several milligrams of the pulverized specimen are treated with 3 drops of the buffer solution and 1 drop of the reagent. The details of the procedure are the same as in testing for lead in alloys. Materials tested are given in Table III.

If oil colors or paints are being examined for lead, a small sample should be ashed and the residue carried through the procedure as outlined.

The foregoing studies demonstrate that sodium rhodizonate can be utilized for the speedy solution of certain analytical problems which are far more difficult and tedious when attacked by the earlier methods. Preliminary work in this laboratory indicates that sodium tetrahydroxyquinone (*5*) can be substituted for sodium rhodizonate in these new procedures. This is not surprising, since the two reagents have similar constitutions.

Table III. Materials Tested

Material	Formula or Lead Content	Result of Test
White lead	$2PbCO_3.Pb(OH)_2$	+++
Chrome red	$2PbCrO_4.Pb(OH)_2$	+++
Chrome yellow	$PbCrO_4$	+++
Red lead	Pb_3O_4	+++
Lead dioxide	PbO_2	+++
Naples yellow	Lead antimonate	+++
Lead glass	About 30% Pb (as silicate)	++
Lead glass	About 0.3% Pb (as silicate)	+
Zinc oxide	About 0.9% Pb (as PbO)	+
Lead arsenate	$PbHAsO_4$ (insecticide)	+++

Acknowledgment

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Determination of Moisture in Starch and Its Modifications

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MANY extensive investigations have been made on the determination of moisture in cereals, starch and its modifications, proteins, and other biological products. These studies have indicated that empirical or relative moisture is possible rather than true moisture (*8*, *9*, *10*, *12*, *13*, *15*) and have led to the general conclusion that part of the water is so "bound" that it cannot be differentiated from water of constitution (*7*). Consequently, workers requiring a knowledge of the true dry substance in a cereal, or in its constituents, are at loss to know which moisture method, if any, to employ. In the wheat milling industry, empirical or relative moisture methods have proved adequate in computation of factory yields or losses, for any method suitable for ground wheat would be expected to be equally satisfactory for the milled products, since the milling process involves only the physical separation of wheat into flour, shorts, bran, and germ.

In the corn wet-milling industry an entirely different set of conditions arises. The corn is steeped with dilute sulfurous acid which produces changes in the character of the constituents. The separation is then carried out in the wet state. The final product, starch, is either dried or converted by acid hydrolysis to corn sirup or corn sugar. The starch may be modified before or during drying.

The industry has long recognized that no one moisture method is applicable to all products, and different methods have been developed by the laboratories of the individual companies. In order to unify and standardize these methods, the Corn Industries Research Foundation established a fellowship with the objective of developing methods which would give true moisture for all products of the wet-milling industry. Moisture methods for corn, corn gluten meal, corn gluten feed, corn oil meal, steep water, corn sirup, and corn sugar have been developed and are fully discussed elsewhere (*4*, *6*, *14*). Although the results of many studies have been published on the determination of moisture in starch, there is still apparently considerable question as to which method, if any, yields the true value. Little attention has been paid to the determination of moisture in commercial modified starches.

Block (*3*) showed that under atmospheric conditions temperatures as high as 160° C. are necessary for removing the moisture from starch. Maquenne (*11*) compared data obtained in dry air at 120° C. to atmospheric air in an oven at 110° C. The results indicated that the former procedure was capable of removing the total moisture. Zerewitinoff (*16*) applied the use of magnesium methyl iodide to the determination of moisture in starch, and found that results from this method agreed with the values obtained in a vacuum oven at 100° C. Other investigators (*5*) have made use of distillation procedures.

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Experimental

To the average person, starch means pearl or pure-food starch, which includes not only corn but also tapioca, potato, sago, etc. Commercial starches include the wet modified starches, principally those in which the character of the original starch has changed by a treatment with acid. There is also a group of oxidized starches, chlorinated or hypochlorite-treated, with different properties. Finally, there is the group of roasted starches or dextrins whose properties vary over a considerable range. The purpose of this research was to determine methods for true moisture, since it was considered unlikely that all these products could be covered by a single method.

The study covered 22 different commercial starches. These samples, if lumpy, were ground to pass a 40-mesh sieve. After thorough mixing, the starches were transferred to 120-ml. (4-ounce) screw-top bottles, and stored in the refrigerator.

METHODS. *Distillation.* The assembly was a modified Bidwell Sterling, fully described elsewhere (*2*, *4*). Twenty-five to 35 grams of starch (depending on the moisture content) were weighed into the tared distillation flask, containing 5 to 8 grams of asbestos, both of which had been previously dried in an air oven at 100° C. The purpose of the asbestos is to prevent bumping during distillation. Sufficient toluene (usually 100 ml.) is added to fill the traps and cover the sample. The flask is then placed in an oil bath, and distillation continued until an additional 24 hours showed no increase in recovered water.

Vacuum-Oven Methods. A Weber vacuum oven and a Hyvac pump were employed, with oven pressures ranging from 0.5 to 2.5 mm. and temperatures of 100° ± 1° C. Standard A. A. C. C. moisture dishes, 55 × 15 mm., were used (*1*). Tests were in duplicate (3 to 5 grams), and included sampling error, since the samples for each determination were taken from different storage bottles. The oven vacuum was restored by air passing through a drying train which included concentrated sulfuric acid, Drierite, and phosphorus pentoxide. The samples were cooled in desiccators over calcium carbide for 15 to 20 minutes.

Air-Oven Methods. The air oven was an Elconap set at 100° ± 1° C.

MOISTURE RESULTS. The data obtained for the first samples of pearl cornstarch are shown in Table I.

Table I. Moisture of Pearl Cornstarch

Length of Drying	Oven Methods, 100° C. Air oven	Vacuum oven	Distillation Methods[a] Benzene	Xylene
Hours	%	%	%	%
2	...	...	7.9	10.0
5	8.96	10.50	9.7	10.2
20	9.19	10.52	10.33	10.34
40	9.33	10.54	10.65	10.63
65	...	...	10.63	10.60
			10.67	10.66

[a] All values except final are approximate readings of traps.

Table II. Open Versus Partially Closed Dishes

(Vacuum oven, 100° C.)

Drying Conditions	Moisture	Moisture Change
	%	%
24 hours, open dishes	10.54	...
Plus 24 hours, partially open lids	10.66	+0.12
	10.64	...
Plus 24 hours, open dishes	10.52	−0.12

The difference between the final values obtained by the air- and vacuum-oven methods is 1.21 per cent. This may be explained on the basis that the values obtained from an air oven depend upon the moisture content of the air passing through the oven and upon the hygroscopic properties of the material being dried. The difference between values employing the vacuum oven and the distillation methods amounts to 0.10 per cent. It was thought that this difference resulted from moisture picked up during the time the lids were removed from the bottom of the dish and placed on the top. This hypothesis was tested by the following experiment: On placing the samples in the oven the dishes were set on the lids for the first 24-hour drying period, for the next two 24-hour periods the lids were tilted on the dishes so that they were only slightly open, after which for another 24 hours the dishes were again placed on the lids. The results are given in Table II.

The data on pearl starch were confirmed by many other tests made in like manner. Table III gives the results on a number of starches, natural and modified. In these tests the oven temperature was raised to 135° C. after completing 101 hours at 100° C., to see if any further moisture was lost.

Although not included in the table, eight other starch varieties were analyzed with results which gave as the maximum 0.04 per cent between a vacuum oven at 100° C. and at 135° C. The acid-modified starches—20, 40, and 60 fluidity—as well as British gum and chlorinated starch, can be treated as straight starch for determination of moisture. However, in the case of 90 fluidity starch and white and canary corn dextrins, a definite loss in weight of 0.12 to 0.08 per cent occurred at the higher drying temperature. With the hypochlorite-oxidized starch, the weight loss was 0.45 per cent and there was visual evidence of decomposition, in that the starch changed to a dark brown color. The distillation results were in agreement with vacuum-oven procedures.

Reversibility of Vacuum-Dried Starches

A full discussion of the use of the reversibility method for the determination of moisture in cereals has been given in moisture studies covering corn and gluten meal (*14*). This procedure was applied to the vacuum-dried starches (135° C.) in order to determine whether any volatiles had been lost along with the adsorbed water. The starch samples dried in vacuo at 100° and 135° C. (Table III) were allowed to readsorb moisture at room conditions. These samples, together with unheated samples of the same starch, were then placed in desiccators which served as conditioning chambers. Through the desiccators was passed a stream of air which had been passed previously through a large quantity of concentrated sulfuric acid. Equilibrium required approximately 14 days. If no volatiles are lost during vacuum drying at 135° C., the vacuum-heated and the control starch samples should reach the same equilibrium value, with respect to moisture retained, provided that the moisture-retaining property of the starch has not been changed during heating.

The results obtained are shown in Table IV. The data show that the weight loss which occurs upon transfer of the samples from the desiccator over sulfuric acid to the vacuum oven at 135° C. is recovered in most cases when the samples are replaced in their original condition. The two samples which clearly do not regain the total weight lost are the white corn dextrin and the hypochlorite-oxidized starch, and in both these cases it was found that the 135° C. vacuum-oven procedure removed volatiles other than water, as indicated by the 100° C. *vs.* the 135° C. vacuum-oven results and the toluene distillation data.

Of interest in these results is the amount of water each product retains over concentrated sulfuric acid. Under these conditions, the amount of moisture still held by the various products varied from 1.00 to 1.65 per cent. These results demonstrate the danger inherent in empirical methods.

Table III. Determination of Moisture in Starch Products

Starch Product	Vacuum Oven, 100° C. 5 hours	Plus 16 hours	Plus 40 hours	Plus 40 hours	Vacuum Oven, 135° C., Plus 20 Hours	Additional Loss at 135° C. *vs.* 100° C.	Distillation with Toluene, 48 Hours
	%	%	%	%	%	%	%
Corn	9.70	9.76	9.82	9.80	9.85	+0.05	9.84
				9.80			9.80
Acid modification, 60 fluidity	12.44	12.49	12.57	12.55	12.54	+0.01	...
				12.51			
Acid modification, 90 fluidity	11.16	11.22	11.30	11.30	11.42	+0.12	11.30
				11.30			11.21
White dextrin	5.60	5.67	5.69	5.70	5.82	+0.12	5.62
				5.69			
Canary dextrin	2.92	2.96	3.06	3.04	3.13	+0.09	...
				3.03			
British gum	3.40	3.44	3.50	3.48	3.50	+0.02	...
				3.47			
Chlorinated starch	7.12	7.22	7.24	7.22	7.25	+0.01	...
				7.25			
Hypochlorite-treated starch	12.76	12.84	12.90	12.91	13.38	+0.45	12.85
				12.95			12.91
Tapioca	14.67	14.72	14.80	14.78	14.82	+0.04	...
				14.78			

Tenacity of Water Retention of Starch Products

It is well known that at the same humidity different starch products retain different amounts of water. Little thought has been given to the possible significance of this fact, with respect to the properties of starches. A preliminary study was conducted for the purpose of determining the relation of degree of modification to the sorptive capacity of the starches. Vacuum-dried samples (100° C.) were allowed to readsorb moisture under room conditions. The rate of moisture uptake and the final equilibrium values obtained are given in Table V.

Table IV. Weight Loss and Gain between Vacuum Drying at 135° C. and Drying over Concentrated Sulfuric Acid in Desiccators

Product	Loss in Weight on Transfer of Starch Dried over Concentrated H_2SO_4 to Vacuum Oven at 135° C.	Gain in Weight on Replacement of Vacuum-Dried Samples (135° C.) in Desiccators over Concentrated H_2SO_4	Loss in Recovery
	%	%	%
Corn	1.43	1.36	0.07
Acid-modification, 60 fluidity	1.40	1.49	0.09[a]
Acid-modification, 90 fluidity	1.21	1.15	0.06
White corn dextrin	1.65	1.19	0.46
Canary dextrin	1.03	1.01	0.02
British gum	1.41	1.37	0.04
Chlorinated starch	1.41	1.43	0.02[a]
Hypochlorite-oxidized starch	1.65	0.84	0.81
Tapioca	1.00	0.99	0.01

[a] Gain.

Table V. Adsorption of Water by Vacuum-Dried Starch Products

Product	Moisture Adsorbed by Vacuum-Dried Samples (Wet Basis) 1 hour	2 hours	3 hours	6 hours	24 hours
	%	%	%	%	%
Cornstarch	5.4	7.4	9.0	10.8	11.8
90 fluidity starch	4.5	6.2	7.7	9.3	10.3
White corn dextrin	5.1	6.8	8.0	9.0	9.8
Canary dextrin	4.5	5.8	6.8	7.9	9.2

With increasing modification the sorptive capacity of starch decreases—that is, the modified products are less hygroscopic. As shown in Table VI, although the modified products retain less water, the water that is held is retained with equal or greater tenacity than the water held by the original cornstarch. These data show that canary dextrin holds the least moisture and retains it with the weakest tenacity. The white corn dextrin retains its moisture with the greatest tenacity. More critical studies are now under way to determine the relation of the sorptive capacity of starches to their properties.

Table VI. Tenacity with Which Starches Retain Moisture

Product	Moisture Retained (Vacuum at 100° C. = 0%) 24 hours, air oven, 70° C.	Plus 24 hours, air oven, 100° C.	Plus 100 hours, 70° C. in dry air
	%	%	%
Cornstarch	32.8	16.5	7.6
90 fluidity starch	34.8	17.2	8.6
White corn dextrin	34.8	17.7	9.1
Canary dextrin	30.2	13.8	6.9

Conclusions

Either of two moisture methods is suitable as a reference method for determining moisture in starch and its modifications. These two methods are the toluene distillation procedure, and the vacuum-oven method at 100° C. Rapid oven procedures using temperatures as high as 140° C., based on these methods, should prove reliable for most starch products. Care must be exercised, however, in drying highly acid modified starch and the hypochlorite-oxidized products at temperatures higher than 100° C.

The official Corn Industries Research Foundation toluene distillation method for corn and its feed products (*14*) is equally suitable for starch products. The recommended official C. I. R. F. reference 100° C. vacuum method for starch products is essentially similar to the A. O. A. C. vacuum-oven method for wheat flour, with the following modifications:

Starch is extremely hygroscopic. For this reason, the air used to replace the vacuum in the oven should be passed through a train as follows:

Two 19-liter (5-gallon) bottles, in series, each containing 2.5 to 5 cm. (1 to 2 inches) of concentrated sulfuric acid; drying tower filled with glass wool; and preferably another tower filled with Drierite.

The A. O. A. C. oven tolerance of a pressure of 25 mm. or less of mercury is too great. The oven should have a well-seated door which will retain the vacuum for several hours after the pump is stopped. Otherwise, a slight leak of atmospheric air will occur, resulting in low moisture data.

The period of drying should be continued until constancy is obtained.

Acknowledgment

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Insulation for Necks of Wash Bottles

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IN THE Subgrade Laboratory, Division of Tests, Public Roads Administration, necks of wash bottles containing hot liquids had been insulated by a wrapping of thin sheet asbestos. A serious drawback is the tendency of the asbestos to crumble with use, leading to the danger of asbestos particles falling into precipitates being washed.

Bottlenecks insulated by the following procedure have been used daily for over two years without sign of deterioration.

The neck of the wash bottle is snugly wound with a wetted thin asbestos strip about 2 inches wide, and the bottle is set aside to dry overnight. Bakelite lacquer (BL 3128, B 57) is then brushed on the dry asbestos, allowed to air-dry for an hour, and baked at 110° C. for 4 hours.

Ascorbic Acid

Rapid Determination in Fresh, Frozen, or Dehydrated Fruits and Vegetables

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PLANT tissues must be finely divided and uniformly mixed as a preliminary step in analysis. These objectives, formerly accomplished only by laborious hand grinding after cutting up the sample or passing it through a food chopper, may now be attained easily by use of a commercial household food-preparing device described as a liquefier or blender. This was first pointed out by Davis in 1939 (*2*). Morell (*16*) has found that in 3 per cent metaphosphoric acid plant tissues may be disintegrated in these devices, such as the Waring Blendor (made by Waring Corporation, 1697 Broadway, New York, N. Y.), without causing any loss of ascorbic acid. Davis (*2*) has also used such a blender for ascorbic determination using an extraction and titration mixture of trichloroacetic and metaphosphoric acids.

Morell's adaptation (*16*) of the photometric method for determining ascorbic acid by comparing photoelectric colorimeter readings on specially prepared plant extracts with a standard curve obtained with regularly increasing increments of pure ascorbic acid is satisfactory where only one type of material is to be tested, but the research programs on dehydrated and frozen foods under way in the Western Regional Research Laboratory have necessitated a method equally adaptable to many kinds and varieties of fruits and vegetables that may be fresh, frozen, or dehydrated (partially or completely), and in some cases highly pigmented. This requirement is met by combining Morell's technique of preparing plant extracts with a modification of the Evelyn *et al.* (*4*) colorimetric method for determining ascorbic acid. This combination method is an extension of that used by Loeffler (*10*) for orange juice and utilizes a rate reaction in a photoelectric colorimeter. It is somewhat simpler in operation than the excellent Bessey (*1*) modification of the Mindlin and Butler technique (*15*) which also utilizes the photoelectric colorimeter.

Simplified Method

Blend 25 or 50 grams of fresh or frozen fruit or vegetable tissue with 350 ml. of 1 per cent metaphosphoric acid in a blending machine operated for 5 minutes at high speed. If the material is of high ascorbic acid content, such as leafy vegetables, raspberries, strawberries, or asparagus, use the smaller quantity. Fifty grams are used with foods containing less ascorbic acid, such as stored potatoes, carrots, yams, peaches, plums, and apricots. If a dehydrated fruit or vegetable is being analyzed, 5 or 10 grams of sample are sufficient, depending upon this same classification. Some thoroughly dehydrated vegetables, such as sweet potatoes or carrots, may need 0.5 hour of soaking in the acid before blending. Frozen foods may be blended without preliminary thawing.

Filter the extract through coarse, fluted filter paper. Extracts of starchy vegetables, such as potatoes and corn, filter better through a Büchner funnel. They can also be cleared by centrifugation. Moderate turbidities do not interfere, since the instrument is calibrated with proper blanks.

Pipet 1-ml. portions of the filtrate into three matched tubes from the Evelyn photoelectric colorimeter.

Add 9 ml. of distilled water to one tube and adjust the colorimeter to read 100 with this tube, using filter No. 520.

To each of the other tubes add 9 ml. of the previously standardized indophenol dye solution from a calibrated rapid delivery pipet. Take a reading in the photoelectric colorimeter, using filter No. 520, 15 seconds after the start of the dye addition. This reading is G_2, from which the corresponding L_2 value is obtained, from the calibration table provided with the instrument, and substituted in the following equation of Evelyn:

$$\text{Ascorbic acid, mg. per 100 ml. of filtrate} = 10.8\ (L_1 - L_2)$$

The equation for fruit and vegetable tissue becomes:

$$\text{Ascorbic acid, mg. per 100 grams of tissue} = 10.8\ (L_1 - L_2)\ \frac{[\text{ml. of acid extractant} + (\%\ \text{liquid in sample})\ (\text{wt. of sample})]}{\text{wt. of sample}}$$

The authors use a reservoir-type automatic pipet for the acid and nonreservoir types for the 1-ml. portions of filtrate and 9-ml. portions of dye, so that they can achieve a rate of 20 samples per hour (two testers and a helper). Unlike Evelyn, they have found it more suitable to add the dye to the tube outside the colorimeter and agitate the tube slightly before putting it in the instrument. The automatic 9-ml. pipet must extend to near the surface of the liquid in the tube to avoid splashing and must be calibrated to drain uniformly in less than 5 seconds.

Standardization of Dye

The dye is standardized by noting the 15-second reading with filter 520 (when the instrument is calibrated to 100 with distilled water) given by a tube containing 1 ml. of 1 per cent metaphosphoric acid and 9 ml. of the dye solution. This value is G_1, from which L_1 is obtained on the calibration table. This standardization is very much easier and faster than titrimetric procedures.

The dye solution is prepared simply by dissolving enough of the dye in water so that a G_1 reading of about "30" is given with the Evelyn photoelectric colorimeter. The concentration of dye to give such a reading is roughly 13 mg. per liter. The reaction between ascorbic acid and stronger dye solutions is not a linear relationship, so that a calibration curve rather than a constant factor must be used with such solutions.

Volume of Acid and Extraction Calculation

Because of the thorough disintegration of the sample in the blender, the ascorbic acid is distributed uniformly through the entire liquid phase present. By determining the ascorbic acid concentration in the filtrate (per milliliter of liquid phase) and by knowing the total volume of liquid, the amount of ascorbic acid in a sample can be determined from a single extraction. Only a few milliliters of filtrate are necessary. The total amount of liquid is simply the sum of the volume of metaphosphoric acid solution added plus the liquid originally present in the sample.

The authors have found a ratio of seven parts of metaphosphoric acid solution to one of vegetable or fruit tissue satisfactory. Under such circumstances the liquid originally present in the sample (water plus soluble solids) may be approximated from food tables, since a variation of 7 per cent causes less than 1 per cent variation in the ascorbic acid calculation. The results are more accurate and simpler to obtain than those after three or more extractions with small volumes of extractant (usually 1 to 1 ratio). Most of the previous workers [Mack and Tressler (*13*), Kirk and Tressler (*7*), McHenry and Graham (*12*), and Rolf (*18*)] have disintegrated the tissue in a mortar and then used such multiple extractions.

Bessey (*1*) uses but one extraction and suggests that the volume of the solids may be neglected. He recommends a sample of only 0.5 to 2.0 grams; but a much larger sample, 25 to 50 grams, is necessary with fruit and vegetable tissues to approach uniformity. Under such circumstances the results approach the accuracy of the method, which is about 1 per cent.

Type and Concentration of Extracting Acid

Ascorbic acid usually is extracted by strong acid to inhibit enzymic and autoxidation and is determined by oxidation with 2,6-dichlorophenolindophenol or less specifically with iodine.

Lyman, Schultze, and King (*11*) have shown that metaphosphoric acid prevents the autoxidation of pure ascorbic acid solutions, while Morell (*16*) found that 3 per cent metaphosphoric acid prevents loss of ascorbic acid during blending of vegetable tissue. Harris and Ray (*6*) recommend trichloroacetic acid, but Fujita and Iwatake (*5*) disagree. Though workers describing photoelectric methods [Mindlin and Butler (*15*), Bessey (*1*), Morell (*16*), etc.] have noted the fading of the dye in strong acid solutions and buffer their extracts to a pH of 3.6 before testing, some analysts still use the erratic titration with an unbuffered acid, such as the 5 per cent sulfuric acid plus 2 per cent metaphosphoric acid recommended by Mack and Tressler (*13*). Kreuther and Roe (*9*) suggest that the final pH after addition of dye should be no greater than 3.0, since at higher values the effect of interfering substances, particularly sulfhydryl compounds, was more pronounced.

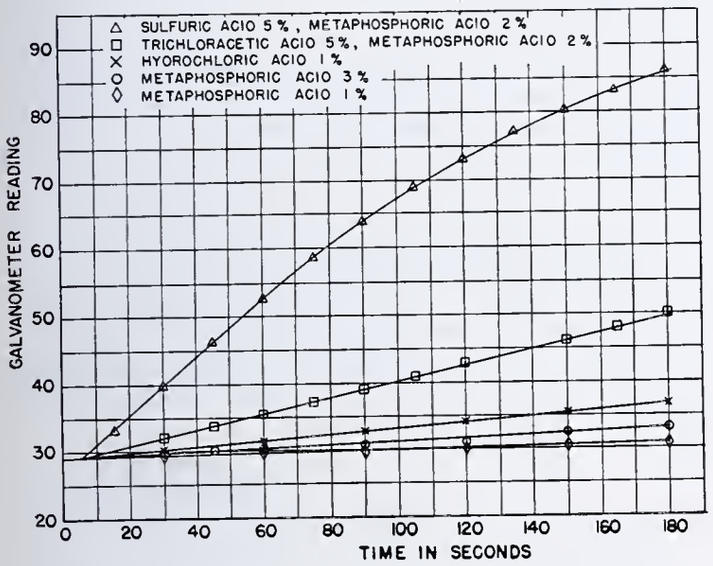

FIGURE 1. FADING OF INDOPHENOL REAGENT

The authors have found that the buffering can be eliminated, since the use of a large proportion of 1 per cent metaphosphoric acid yields a pH sufficiently low to prevent losses during blending and yet sufficiently high to prevent fading during the reaction with the dye. The extracts with 1 per cent metaphosphoric acid of all vegetables and fruits tested, including strongly buffered soy and lima beans, have ranged between pH 2.4 and pH 3.1 before addition of the dye. The pH may increase as much as 0.5 during the dye addition. Extracts with 3 per cent metaphosphoric acid have ranged from pH 1.8 to 2.2.

The extremely unstable end points in strong acid are indicated in Figure 1, where the rate of fading of the dye is plotted in the absence of ascorbic acid. It is evident that in a mixture of 5 per cent sulfuric and 2 per cent metaphosphoric acids a high ascorbic acid content would be indicated when none is present. Similar errors were observed in tests with vegetable tissue filtrates. Spinach extracted with 1 per cent metaphosphoric acid or 1 per cent metaphosphoric acid plus 5 per cent trichloroacetic acid tested 12 to 14 mg. of ascorbic acid per 100 grams. When 5 per cent sulfuric plus 1 per cent metaphosphoric acid was used, a 30-second end point indicated 27.3 mg., a 1-minute end point indicated 41.0 mg., and a 3-minute end point indicated 372 mg. per 100 grams. Titration of the unknown and standard under identical circumstances in the same time interval is not satisfactory, since the buffering action of vegetable tissue greatly reduces the fading from that found with the standard alone. With sulfuric acid, however, fading continues even at higher pH values. Figure 1 indicates that with a 15-second end point the fading in 1 per cent metaphosphoric acid is negligible. (Because of present war conditions it is difficult to obtain metaphosphoric acid. Preliminary experiments have shown 0.4 per cent oxalic acid to be the best substitute for 1 per cent metaphosphoric acid. No loss was incurred during blending of pure solutions; vegetable filtrates lost no more ascorbic acid in oxalic than in metaphosphoric acid when stored at room temperature in the presence of added copper; and the fading of the dye reagent was about the same in either acid of the indicated concentration.)

The dye fades at a significant rate in 3 per cent metaphosphoric acid, but only one tenth as fast after reaction with ascorbic acid. Hence the dye itself should be standardized with 1 per cent metaphosphoric acid, but the test material, if strongly buffered may be extracted and tested using 2 per cent metaphosphoric acid.

One per cent metaphosphoric acid added to cabbage in a 7 to 1 ratio protects ascorbic acid during blending, as shown in Table I. Fifty grams of shredded cabbage were blended at high speed and small samples were removed every 2 minutes. (Stone, *19*, has shown that ascorbic acid is lost very rapidly from shredded cabbage.) Even the heating that results from the blending does not cause a significant loss. Essentially similar results were obtained with 3 per cent metaphosphoric acid plus a different cabbage sample.

Filtrates from raw cabbage extractions were tested before and after 3 days' storage at room temperature in stoppered flasks. The results, given in Table II, show that the actual loss in ascorbic acid is no greater in 1 per cent metaphosphoric acid than in 3 per cent metaphosphoric or 2 per cent metaphosphoric plus 5 per cent trichloroacetic acid, under the conditions of this test. The authors' many previous experiments are confirmed, since the actual amount of ascorbic acid obtained in two extractions is shown to check very closely with

TABLE I. ASCORBIC ACID RETENTION DURING BLENDING OF CABBAGE

(Metaphosphoric acid solution and cabbage mixed in ratio of 7 to 1)

Blending Time	Temperature	Ascorbic Acid 1% HPO_3	Ascorbic Acid 1% HPO_3	Ascorbic Acid 3% HPO_3
Min.	*° C.*	*Mg./100 cc. filtrate*	*Mg./100 g. cabbage*	
2	27	3.00, 3.03	23.8, 24.0	23.5
4	34	3.00, 3.05	23.8, 24.2	23.9
6	38	3.08, 3.09	24.4, 24.5	24.4
8	42	3.03, 3.06	24.0, 24.2	24.4
10	45	3.00, 3.05	23.8, 24.2	24.3
12	49	2.97, 3.00	23.5, 23.8	..

TABLE II. EFFECT OF THREE DAYS' STORAGE ON ASCORBIC ACID CONTENT OF CABBAGE EXTRACTS

Sample	Extractant	Ascorbic Acid Total in 2 extractions	Ascorbic Acid Calculated from 1st extraction (90% liquid assumed)	Ascorbic Acid After 3 days' storage	Loss
		Mg./100 grams		*Mg./100 grams*	
I	1% HPO_3		23.3	15.1	8.2
IA	1% HPO_3	23.1, 23.3	23.3, 23.3	9.4	13.9
II	3% HPO_3	24.8	24.8, 25.0	11.2	13.7
IIA	3% HPO_3	21.3	21.8, 21.2	7.4	14.1
III	2% HPO_3 + 5% Cl_3HAc		25.1, 25.0	11.1	13.9
IIIA	2% HPO_3 + 5% Cl_3HAc	25.6	25.4, 25.4	11.9	13.5

Table III. Ascorbic Acid in Frozen Crushed Raspberries

Sample	Weight of Sample	Volume of 1% HPO_3	Ascorbic Acid Recovered (Assuming 100% Liquid Phase)
	Grams	*Ml.*	*Mg./100 cc.*
1	25	175	21.9
2	25	105	21.2
3	25	105	21.3
4	5	105	20.2
5	5	105	20.9

the calculation based on a few milliliters of filtrate and the assumption of 90 per cent liquid content.

Application to Fruits and Berries

The method has been applied successfully to fresh and frozen fruits and berries, particularly peaches, nectarines, raspberries, loganberries, strawberries, boysenberries, and youngberries.

Color in the sample is not an interfering factor, since the photoelectric colorimeter, when properly adjusted to allow for color in the sample, measures the added red color of the dye in acid solution. The dye is standardized with the colorimeter adjusted to 100 with distilled water alone; but for the extract being analyzed the colorimeter is adjusted to 100 with a tube containing 1 ml. of the extract plus 9 ml. of distilled water.

With most fruits the volume of the solids can be neglected, since the distribution of ascorbic acid is in the liquid phase present—that is, water plus soluble solids—rather than in the water alone. Most fruits and berries do not possess sufficient insoluble solids to alter the results materially. The formula then becomes:

$$\text{Mg. of ascorbic acid per 100 grams} = 10.8\,(L_1 - L_2)\left(\frac{\text{volume of acid} + \text{wt. of sample}}{\text{wt. of sample}}\right)$$

If the insoluble solids can be neglected, the formula above should hold despite wide variations in extracting volumes and ratios. Table III demonstrates that such conditions are met and that very consistent results can be obtained. The berries had been crushed before freezing; hence they were uniform in character.

Table III, together with unpublished results obtained at the Western Regional Research Laboratory, demonstrates that many berries, besides strawberries, are good sources of ascorbic acid. The statement of Morgan (*17*) that berries are a poor source of ascorbic acid, averaging 3 to 10 mg. per 100 grams, has not been confirmed. Fresh California berries purchased commercially, have averaged in ascorbic acid content approximately as follows: raspberries 25 mg., boysenberries 15 mg., loganberries 35 mg., and blackberries 15 mg. per 100 grams. Hence, berries may provide ascorbic acid or vitamin C for farm and other communities whose principal fruit consumption has been apples, pears, apricots, and peaches, which have relatively lower ascorbic acid contents, averaging about 5 mg. per 100 grams.

Application to Other Products

Since most food products can be disintegrated with a blender, especially after a preliminary soaking, and since interfering colors or turbidities can be blanked out in the photometric method, almost any food can be tested by these techniques.

Some products may require slightly different treatment—for example, clear filtrates from extractions of certain varieties of fresh lima beans may become turbid when the dye is added. Apparently protein fractions soluble in 1 per cent metaphosphoric acid solution are precipitated because of the reduction of ionic strength on dilution with dye solution. A preliminary dilution of the clear filtrate with an equal volume of saturated sodium chloride solution maintains the ionic strength and prevents the turbidities.

Filtrates from extractions of dehydrated vegetables will occasionally cause fading of the indophenol dye to an appreciable extent other than by reduction with ascorbic acid, because interfering substances have been concentrated by the dehydration. Under such circumstances the approximate correction suggested by Bessey (*1*)—that is, to subtract from the 15-second reading the extent of fading that occurs between a 15- and a 30-second reading—has been satisfactory. Elliott, Sklar, and Acree (*3*) have shown that blood plasma reduces the rate of reaction between ascorbic acid and the indophenol dye; hence a more careful extrapolation to zero time as suggested by Evelyn may not always be desirable.

Preliminary experiments on dehydrated vegetables treated with sulfur dioxide prior to drying indicated that, somewhat contrary to the results of Kirkpatrick (*8*), sulfur dioxide does interfere with the indophenol reaction by supplementing the reducing effect of the ascorbic acid. However, the rate of reaction is sufficiently slow to give a rapidly drifting reading with the photoelectric colorimeter. The suggestion of Mapson (*14*), that the sulfur dioxide can be removed by evacuation or sweeping with nitrogen, was not substantiated on strongly acid bisulfite solutions. However, Mapson's other suggestion of binding the sulfur dioxide with an excess of acetone has proved successful in preventing interference in pure ascorbic acid solutions. Ten per cent of acetone in the ascorbic acid solution prevented the interference of 500 p. p. m. of sulfur dioxide, provided the indophenol dye was standardized with 1 per cent metaphosphoric acid solution containing the same acetone concentration.

With dehydrated vegetables, the moisture content (if below 5 per cent) can usually be neglected. The formula then becomes:

$$\text{Mg. of ascorbic acid per 100 grams} = 10.8\,(L_1 - L_2)\left(\frac{\text{volume of acid added}}{\text{wt. of sample}}\right)$$

Additional dilution may be necessary for some foods, such as citrus concentrates, strawberries, and brussels sprouts. A small portion of the filtrate can be quickly diluted to desired volume with 1 per cent metaphosphoric acid when necessary.

Summary

Ascorbic acid can be determined quickly in fruits and vegetables, whether fresh, frozen, or dehydrated, by disintegrating the sample with dilute metaphosphoric acid in a high speed cutter and measuring the decolorizing effect of the extracted ascorbic acid on indophenol dye with a photoelectric colorimeter. The ascorbic acid is distributed within the total liquid phase present, which includes the water and dissolved solids originally in the sample. Hence by using a large proportion of extractant and knowing the approximate amount of liquid in the sample, the ascorbic acid can be determined from as little as 3 ml. of filtrate after only one extraction. By using a large amount of 1 per cent metaphosphoric acid as the extractant the buffering step is avoided, since the pH obtained is sufficiently low to prevent losses during blending, yet sufficiently high to prevent fading of the dye reagent. The rate of fading is high with many previously recommended extraction mixtures. Rapid ascorbic acid determinations can be made easily on highly pigmented berries and tough dehydrated vegetables.

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Agar-Agar as a Coagulant for Barium Sulfate

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THE precipitate of barium sulfate in the determination of sulfate usually consists of very fine particles. Standard procedures recommend digestion for several hours and preferably overnight in the hot solution before filtration. Numerous methods have been proposed to improve the character of the precipitate.

Lindsly (*6*) proposed the addition of a saturated solution of picric acid as a means of increasing crystal size. Krak (*5*) recommended digestion in an ammonium acetate solution after decanting the mother liquor through the filter. Ziegeler (*8*) suggested coagulation of the barium sulfate by precipitating silver chloride in the suspension and subsequent removal of the silver chloride with concentrated ammonium hydroxide. Orlow (*7*) precipitated a known amount of aluminum hydroxide with the barium sulfate and made a correction for the weight of aluminum oxide in the ignited precipitate. None of these methods has proved entirely satisfactory.

Caldwell and Moyer (*3*) proposed the use of the sensitization phenomenon as a means of coagulating analytical precipitates. They showed that finely divided zinc sulfide can be coagulated by the addition of minute quantities of gelatin.

It has been found that positively charged barium sulfate which is obtained when sulfate is precipitated by an excess of barium ions can be coagulated by the addition of a trace of agar-agar. Approximately 1 mg. of agar-agar will cause the flocculation of an ordinary analytical precipitate of barium sulfate obtained in the determination of sulfate. On the other hand, negatively charged barium sulfate obtained in the determination of barium is not appreciably improved by the addition of agar-agar. However, this precipitate, as a rule, causes little difficulty in filtration.

Another troublesome property of finely divided barium sulfate is its tendency to creep up the sides of the funnel or filtering crucible. Creeping seems to be completely eliminated from precipitates coagulated with agar-agar.

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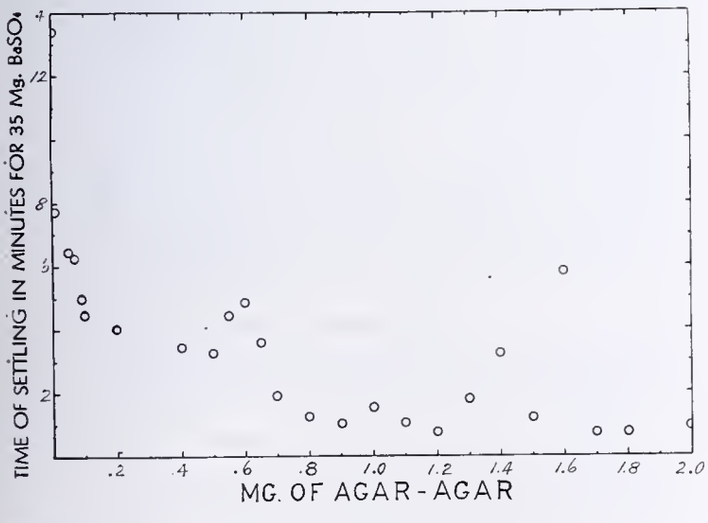

Figure 1. Effect of Agar-Agar on Time of Settling of Precipitates of Barium Sulfate
Each point represents separate sample

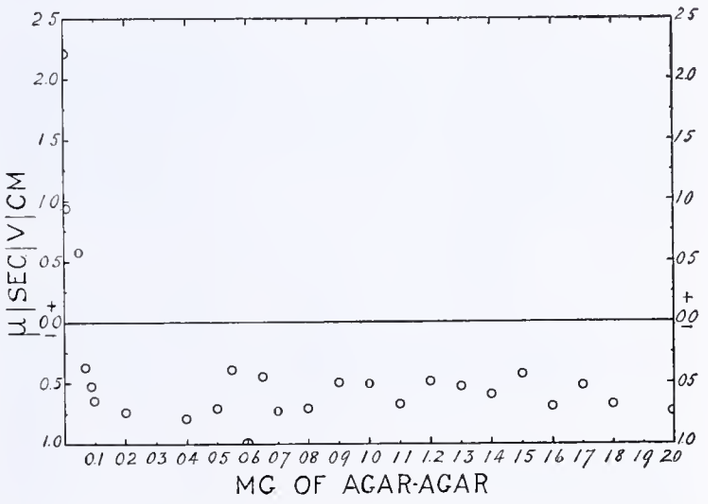

Figure 2. Mobility of Particles of Barium Sulfate in Abramson Electrophoresis Cell
Measurements on same solutions as in Figure 1

Procedure

The sulfate is precipitated as usual in a hot solution containing 1 ml. of 1 *N* hydrochloric acid by the slow addition of 5 per cent barium chloride solution. After precipitation, from 0.5 to 1 ml. of a solution of agar-agar containing 1 mg. per ml. is added a drop at a time. After each drop the suspension is stirred rapidly for about 10 seconds. Some coagulation can be observed after the introduction of the first drop; however, the best coagulation usually appears after the addition of about 10 drops of the coagulating agent. The optimum amount of the agar-agar solution is indicated by the flocculation and rapid settling of the precipitate. After settling, the precipitate somewhat resembles coagulated silver chloride.

A study was made of the effects of adding agar-agar to precipitates of barium sulfate in order to determine the limit-

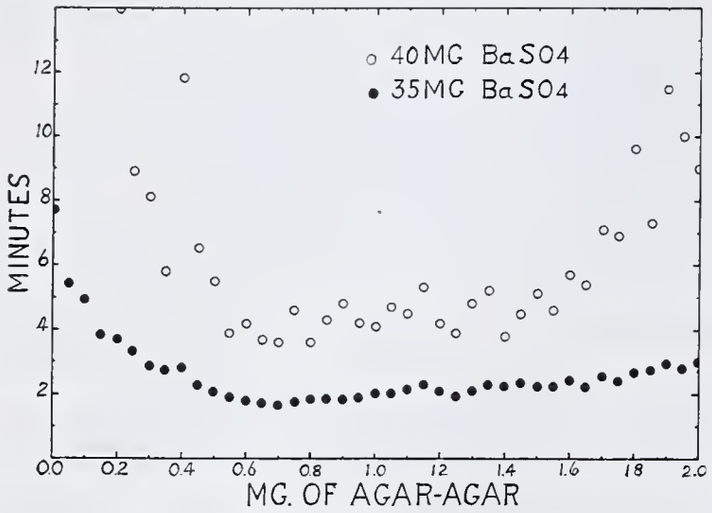

FIGURE 3. TIME OF SETTLING OF SINGLE SUSPENSION OF BARIUM SULFATE
Increased amounts of agar-agar added

ing quantities of agar-agar within which optimum coagulation occurs, the minimum quantity for peptization, and the effect on the accuracy of the determination. The effect of the agar-agar on the electric charge on the particle was also observed by measuring the mobility of the particle in an Abramson electrophoresis cell.

Reagents and Solutions

AGAR-AGAR SOLUTION. Agar-agar, U. S. P., ash 4.2 per cent, was used without further purification. A solution containing 1 mg. per ml. was prepared by suspending 0.5 gram of agar-agar in 50 ml. of cold water and adding this to 450 ml. of near boiling water. The solution was then stirred constantly as it was heated to boiling. Such a solution will retain its effectiveness for several weeks.

BARIUM CHLORIDE SOLUTION. A 5 per cent solution of barium chloride dihydrate was prepared.

SODIUM SULFATE SOLUTION. A sodium sulfate solution of such strength was prepared that 25 ml. of the solution gave approximately 0.1 gram of barium sulfate.

Experimental

The coagulation of the barium sulfate was measured in an apparatus similar to that used by Calbeck and Harner (*2*) in sedimentation measurements. A glass cylinder containing the suspension of barium sulfate was supported over the pan of an analytical balance. A watch glass with a small glass rod through its center was placed in the cylinder and the rod was hooked to the arm of the balance. The rate of settling of the barium sulfate was measured by noting the time required for a given underwater weight of barium sulfate to settle on the watch glass. All precipitations were made in the cylinder from 230 ml. of solution containing 25 ml. of the sodium sulfate solution and 1 ml. of *N* hydrochloric acid. The solution was stirred with an electric stirrer while 20 ml. of the barium chloride solution were added at a constant rate from a buret equipped with a capillary tip.

When precipitation was complete the cylinder was placed over the pan of the balance and the suspension was stirred by pulling the watch glass up and down 20 times in the solution. The watch glass was then hooked to the balance and the time required for 35 mg. of barium sulfate to settle on the watch glass was measured with a stop watch. Each solution was then treated with agar-agar solution and stirred 20 times with the watch glass, and the time-weight measurements were repeated.

Observations were thus made on the effect of adding increased amounts of agar-agar to separate freshly precipitated suspensions of barium sulfate. The results are shown in Figure 1. After the rate of settling was measured for each precipitate, a portion of the suspension was placed in an Abramson (*1*) electrophoresis cell and the mobility of the particles was determined (Figure 2). Less than 0.1 mg. of agar-agar reversed the sign of the charge on the particles. Figure 3 shows the results of a series of measurements on a single suspension. The beginning of peptization is indicated by the rapid increase in the time required for 40 mg. of precipitate to settle after the addition of 1.6 mg. of agar-agar. The time required for 40 mg. of barium sulfate to settle without the addition of the coagulating agent was so long that it was impracticable to measure it.

Several series of analyses were made to determine the effect of the addition of agar-agar on the digestion period and on the accuracy of the method. Five determinations were made without agar-agar in which the precipitates were digested overnight on the steam plate. An average weight of 0.1009 gram of barium sulfate was obtained from 25 ml. of the stock sodium sulfate solution with an average deviation of 0.00018 gram. In ten determinations, agar-agar varying in quantity from 1.2 to 2.0 mg. was added to each one and filtration was accomplished within 30 minutes after precipitation. An average of 0.1008 gram of barium sulfate was obtained from 25 ml. of the sodium sulfate solution with an average deviation of 0.00016 gram. All the determinations indicated that coagulation of the precipitate and 30 minutes digestion gave results equal in accuracy to those obtained without coagulation after overnight digestion. However, the addition of agar-agar did not eliminate high results when barium sulfate was precipitated in the presence of approximately 1 per cent potassium nitrate. No detectable reduction of the sulfate was indicated when the precipitate was moistened and tested with a drop of phenolphthalein after ignition of the barium sulfate which had been coagulated with agar-agar.

The applicability of the procedure to an actual sulfate determination in which difficulty is frequently encountered was tested in a number of coal analyses. The samples of coal were analyzed according to the Eschka method as given by Griffin (*4*). The agar-agar was added dropwise to the hot solution directly after precipitation of the barium sulfate and the solution was filtered immediately without digestion. An average of 1.46 per cent sulfur was found with deviations of ±0.01 per cent in seven determinations. Another analyst found 1.50 per cent sulfur by standard procedure without agar-agar with overnight digestion. In these samples, 10 drops of the above agar-agar solution produced excellent coagulation.

Discussion

Since the optimum concentration of a sensitizing agent varies with the ionic strength of the solution, the most effective quantity of agar-agar will differ slightly in different solutions. Figure 3 shows, however, that the limits are not so critical as to cause difficulty in practical analysis. Slightly less agar-agar is required if it is added dropwise than if it is added all at once.

The measurements of the electrophoretic mobility of the particles show that peptization begins without appreciable change in the negative charge on the particle. The observations also show that less than 0.1 mg. of agar-agar reverses the charge on the particles of barium sulfate.

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Determination of Blood in Packing-House By-Products

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THE determination of the blood content of meat products used in animal feeding was undertaken as part of a study of the various constituents of commercial packing-house by-products. As hemoglobin is the chief constituent of blood, the determination of hemoglobin or its derivatives was used to estimate the quantity of dried blood present. Experiments with fresh blood showed that the quantity of hemoglobin in the dried product depends to some extent upon the method of processing. This must be considered in the interpretation of the results of the analyses.

Development of Method for Dried Blood

The determination of dried blood in tankage and meat scraps was based upon the hemoglobin content of commercial dried bloods. Hemoglobin in fresh blood is usually determined by diluting it with dilute hydrochloric acid and reading the color against a standard colored disk. This method is not applicable to dried blood because hematin dissolved in alkali precipitates when acid is added and the color cannot be read.

Dried blood is readily dissolved by boiling in 1 per cent sodium hydroxide, but the color of the solution is not proportional to the amount of hemoglobin because of the presence of other colored matter. A suitable method was found to be reduction with sodium hydrosulfite in the presence of pyridine, an alkaline pyridine hemochromogen being produced similar to that described by Drabkin and Austin (*2*). Portions of the reduced and unreduced solutions were placed in opposing cups and the difference in color at 550 mμ, the wave length of maximum absorption, was read in terms of density by means of a Bausch & Lomb visual spectrophotometer.

Dried blood did not dissolve well in solutions of sodium phosphates, ammonia, or dilute acids. It was thought that boiling in acid might change the dried blood, so that upon making the boiled mixture slightly alkaline the blood would dissolve, but 0.05 to 1.00 per cent hydrochloric acid and buffered solutions from pH 3 to 10 did not have this effect. The maximum amount of color of a 0.1 per cent of solution of dried blood (density 0.51) was produced with 0.5 and 1.0 per cent sodium hydroxide. A smaller amount of color (density 0.46) was obtained with 0.2 per cent and still smaller (density 0.21) with 0.05 per cent sodium hydroxide.

Approximately 20 minutes were required to dissolve 0.1 gram of dried blood in 100 ml. of boiling 1 per cent sodium hydroxide. Some samples dissolved in less time. When a sample of dried blood was boiled in 1 per cent sodium hydroxide for different periods of time the density of the color was 0.65 when boiled 20 minutes, 0.59 when boiled 40 minutes, and 0.51 when boiled 60 minutes. The hematin is destroyed by continued boiling, so that the time of boiling should be held to the 20 minutes necessary for complete solution.

Drabkin and Austin (*2*) used a 30 per cent pyridine solution for the development of the hemochromogen. Because of the large number of determinations planned for the present work, tests were made to see if less pyridine could be used. Five per cent pyridine was found to be sufficient, since 5, 10, 20, and 30 per cent produced the same quantity of color.

The relation of the concentration of blood to the absorption of light by the color produced was studied with 0.2 gram of a commercial dried blood dissolved in 100 ml. of 1 per cent sodium hydroxide and 5 per cent of pyridine, diluted to various concentrations. The differences in density of color at 550 mμ between the reduced and unreduced solutions are given in Table I, as averages of two determinations. The density is in proportion to the concentration, within the limits of experimental error.

Ten different samples of dried blood were secured from Swift and Company, Armour and Company, and Wilson and Company, whose cooperation is hereby acknowledged. The density of the color derived from hemoglobin was determined by the method described below, with the results given in Table II. The relative dried blood value is the density of each sample divided by the mean density and multiplied by 100. The hemoglobin was calculated by the method described below.

The density of the color of 0.1 per cent solutions of the various samples ranges from 0.39 to 0.71, showing a wide variation in the hematin content. The mean is 0.55, with a standard deviation of 0.10.

The mean density of 0.55 was used for the calculation of dried blood in dried animal by-products. The comparative dried blood content (Table II) ranges from 71 to 129. These differences are probably due partly to the method of drying, since this affects the hemoglobin which remains in the dried blood. The method of preparing the tankage or meat scraps would have some effect upon the quantity of dried blood found by this method.

Development of Method for Hemoglobin

In the work discussed above, comparisons were made with commercial dried bloods and not with hemoglobin. In order to ascertain the relation of the modified hemoglobin of commercial blood to hemoglobin, work was done with fresh cow blood. These samples were furnished by H. Schmidt of the Veterinary Division of the Experiment Station.

TABLE I. RELATION BETWEEN CONCENTRATION OF BLOOD SOLUTION AND DENSITY OF COLOR PRODUCED

Concentration of Blood	Density of Color	Density per 1%
%		
0.02	0.11	5.5
0.04	0.22	5.5
0.06	0.32	5.3
0.05	0.42	5.3
0.10	0.52	5.2
0.12	0.62	5.2
0.14	0.72	5.1
0.16	0.83	5.2
0.18	0.95	5.3
0.20	1.06	5.3
		Mean 5.3

TABLE II. HEMOGLOBIN AND RELATIVE DRIED BLOOD CONTENT OF COMMERCIAL DRIED BLOODS

	Density of Color	Relative Dried Blood Value	Hemoglobin
		%	%
	0.46	84	48
	0.64	116	67
	0.42	76	44
	0.60	109	63
	0.56	102	59
	0.58	105	61
	0.56	102	59
	0.39	71	41
	0.56	102	59
	0.71	129	75
Av.	0.55	100	58

Table III. Effect of Heating and Pressure upon Hemoglobin

(As measured by density of color produced)

Treatment	Time Treated	Wave Length Maximum Absorption	Density at Maximum Wave Length	Density at 550 mμ
	Hours	*mμ*		
None	0	564	1.17	0.78
Autoclaved at 15 pounds' pressure	0.5	550	0.64	0.64
	1	550	0.45	0.45
	1.5	550	0.33	0.33
	2	550	0.26	0.26
	3	550	0.19	0.19
Heated at 200° in dry oven	1	550	0.41	0.41

When fresh blood was dried at 100° C., pulverized and dissolved in alkali and the hemochromogen was developed, the wave length of maximum absorption was near 562 mμ, while that of the hemochromogen of commercial dried blood was 550 mμ. The processing changed the hemochromogen. In order to find the conditions required to convert the hemoglobin of fresh dried blood to the same substance as that of commercial dried blood, so that the two could be compared, 100-mg. samples of fresh dried cow's blood were heated to 200° C. in a dry heat or autoclaved for various lengths of time at 6.8 kg. (15 pounds) pressure and the color of the resulting hemochromogen was measured. The results, given in Table III, show that all these treatments produced a hemochromogen with a wave length of maximum absorption of 550 mμ. Autoclaving longer than 0.5 hour destroyed progressively the hemochromogen-forming powers of the blood, since the density of the color decreased with the time of autoclaving. It is evident that the hemochromogen-forming powers of commercial dried blood must similarly depend upon the temperature of drying or other treatment. This may be the cause of some of the differences in commercial dried blood shown in Table I.

The relation between the hemoglobin of fresh blood and that of dried blood was studied on five samples of fresh cow blood.

Hemoglobin was determined by the acid hematin method of Newcomer (*5*), by means of the Bausch & Lomb spectrophotometer. Water was determined by drying at 100° C. in a vacuum oven. The dried bloods from the water determination were ground in a mortar and amounts containing 200 mg. of hemoglobin autoclaved for 30 minutes at 6.8 kg. (15 pounds) pressure, dissolved in 1 per cent sodium hydroxide, 5 cc. of pyridine were added, and made up to 100 ml. The resulting 0.20 per cent hemoglobin solutions were filtered and diluted to 0.05, 0.10, and 0.15 per cent. Ten milliliters of each dilution were reduced with a few milligrams of sodium hydrosulfite, and, after 10 minutes, the difference in density at 550 mμ between the reduced and unreduced solutions was determined in opposing cups of the spectrophotometer.

The average density for 1 per cent hemoglobin for four of the samples is 9.5 (Table IV). The density is not quite constant at the different dilutions but does not change regularly with concentration, differences at different concentrations apparently being due to experimental error. The extinction coefficients given in Table IV were calculated from the equation $c = \frac{D}{e \div L}$ in which c is the percentage concentration of hemoglobin, D is the difference in density between the reduced and unreduced solutions, e is the extinction coefficient, and L is the depth of the solution in cm. In four of the five samples the mean extinction coefficients were 4.79, 4.61, 4.86, and 4.78. In the fifth blood it was 3.54.

In the preparation of commercial dried blood, the fresh blood is usually coagulated, drained, and dried at a high temperature (*3*).

The five samples of fresh blood contained 70.2 to 77.7 per cent hemoglobin with a mean of 73.6, calculated to the dry basis, but commercial dry blood contained only 58 per cent (Table II). Two of the nine commercial dry bloods contained hemoglobin within the range of the fresh bloods—namely, 67 and 75 per cent, respectively. The low hemoglobin content of the other samples may have been due to the method used in drying the blood.

Another incongruity is the low extinction coefficient of the color developed from one of the fresh bloods. In four samples these values were 4.79, 4.86, 4.61, and 4.78 (Table IV) but in a fifth it was 3.54. This blood had a low hemoglobin content of only 10.8 per cent as compared with 13.6, 13.9, 14.5, and 15.0 per cent for the other samples, yet on the dry basis the hemoglobin content was similar to that of the other samples. It would appear that the hemoglobin of this blood was more easily destroyed by heat than that of normal blood.

Since the hemoglobin in blood is affected both by the time and method of heating, it may be partly destroyed in the manufacture of tankage or meat scraps and the percentage destroyed will depend upon the treatment given. The results secured by the method here described, are more likely to be too low than too high and are not highly accurate.

Determination of Dried Blood or Hemoglobin

Extraction of meat scraps or tankage with ether was necessary to remove substances which cause a cloudy filtrate.

One gram of meat scraps or tankage is washed four times on a filter paper with 10-ml. portions of U. S. P. ether, or is placed in a centrifuge tube, 10 ml. of ether are added, allowed to stand 10 minutes, and centrifuged, and this treatment is repeated twice. It is then ground in a mortar, transferred to a 400-ml. beaker with 100 ml. of 1 per cent sodium hydroxide, and gently boiled for 20 minutes. The solution, now about 75 ml., is transferred to a 100-ml. volumetric flask containing 5 ml. of pyridine, made up to volume, with distilled water and filtered. A few milligrams of sodium hydrosulfite are added to 10 ml. of the filtered solution in a test tube. After standing not less than 10 minutes and not more than 30 minutes, portions of the reduced and unreduced solutions are placed in opposite cups of the spectrophotometer. The density, read at 550 mμ and 2-cm. depth, is the difference in density between the reduced and unreduced hemochromogen, and is read directly from the instrument.

When the quantities and dilutions given above are used, the percentage of dried blood is obtained by dividing the density as read by 5.5, the average density of a 1 per cent solution of com-

Table IV. Relation of Concentration of Hemoglobin to Density and Extinction Coefficient of Color Produced

Laboratory No.	Concentration of Hemoglobin, %	Density 2 Cm.	Extinction Coefficient	Density for 1% Hemoglobin
63,197	0.05	0.50	5.00	10.0
	0.10	0.91	4.55	9.1
	0.15	1.43	4.77	9.5
	0.20	1.91	4.78	9.6
			Mean 4.78	
64,345	0.05	0.34	3.40	6.8
	0.10	0.67	3.85	6.7
	0.15	1.05	3.50	7.0
	0.20	1.57	3.43	6.9
			Mean 3.54	
64,346	0.05	0.44	4.40	8.8
	0.10	0.88	4.40	8.8
	0.15	1.48	4.90	9.9
	0.20	1.89	4.73	9.0
			Mean 4.61	
64,347	0.05	0.46	4.60	9.2
	0.10	0.98	4.90	9.8
	0.15	1.49	4.94	10.0
	0.20	2.04	5.01	10.2
			Mean 4.56	
64,348	0.05	0.43	4.30	8.6
	0.10	0.99	4.95	9.9
	0.15	1.45	4.87	9.8
	0.20	1.03	5.01	10.1
			Mean 4.78	
	Grand mean, 64,345 excluded		4.76	9.5

Table V. Dried Blood and Hemoglobin in Meat By-Products

Description	Dried Blood %	Hemoglobin %
Tankage	30.8	18.1
60% protein digester tankage	30.6	17.8
Tankage	31.0	18.1
60% protein digester tankage	27.0	15.8
50% protein digester tankage with bone	20.2	11.8
60% protein digester tankage	11.9	6.9
	41.9	24.5
Meat and bone scraps	5.2	2.4
	0.9	0.5
	3.1	1.8
	2.3	1.4
	2.3	1.4
	3.2	1.9
	1.1	0.6
	4.1	2.4
50% protein meat and bone scraps	1.4	0.8
Meat and bone scraps	26.5	15.4
	1.3	0.7
	12.8	7.5
Steamed bone meal	0	0
20% protein steamed bone meal	0.5	0.3
65% protein sardine meal	4.7	2.7

mercial dried blood (Table II), and multiplying by 100, or by multiplying directly by 18 (100 divided by 5.5).

The quantity of hemoglobin may be calculated by dividing the density by 9.5 (the average in Table IV) and multiplying by 100, or by multiplying directly by 10.5 (100 divided by 9.5).

With commercial dried blood, 0.10 gram should be used, with corresponding changes in the calculation. Extraction with ether is not necessary. When the color developed from meat scraps or tankage is too deep, the work may be repeated with a smaller quantity of sample.

Dried Blood in Meat Scraps and Tankage

Samples of meat scraps, tankage, and some other feeds were analyzed by the method described, with the results given in Table V. The seven samples of tankage contained from 11.9 to 31.0 per cent of dried blood. Most of the thirteen samples of meat and bone scraps were low in blood, but two contained 12.8 and 26.5 per cent, respectively.

Meat meal and meat scraps, as defined by the Association of American Feed Control Officials (*1*), should not contain blood beyond such traces as might occur unavoidably in good factory practice, while blood is not excluded from tankage. According to Kraybill (*4*) blood is usually present in tankage.

Summary

A method for determination of the approximate quantity of blood in dried meat by-products is based upon the formation of color from hemoglobin. The sample is dissolved in sodium hydroxide, pyridine is added, color is developed by reduction with sodium hydrosulfite, and the difference in the color density with and without reduction is read at 550 mμ in a spectrophotometer. The intensity of the color is in proportion to the concentration. The maximum color is at 562 mμ with fresh blood and 550 mμ with commercial dried blood, showing a change due to heating. Hemoglobin is partially destroyed by autoclaving blood, so that commercial dried blood (though part has been drained off before drying) averages 58 per cent hemoglobin compared with 73.6 per cent for fresh cow blood on a dry basis. The results of the analysis can be expressed either as dried blood or as hemoglobin. Commercial tankage (seven samples) contained 11.4 to 41.9 per cent of dried blood. Three samples of meat and bone scraps contained 6.3, 12.8, and 26.5 per cent of dried blood, while the other ten contained less than 4.1 per cent. The results are probably low.

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Presented before the Division of Agricultural and Food Chemistry at the 103rd Meeting of the American Chemical Society, Memphis, Tenn.

Analysis of Ethylene in Presence of Butane

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THE analysis of cracked gas samples containing the unsaturated hydrocarbons ethylene, propylene, and butylene by specific absorption in sulfuric acid reagents of various concentrations in an Orsat apparatus, was described by Hurd and Spence (*2*). Fuming sulfuric acid was used in the ethylene pipet, and the authors noted that some absorption of butane occurred. In spite of the nonspecific absorption of ethylene in the presence of butane, the reagent has been widely used. The disadvantages of the fuming sulfuric acid and the bromine method for the analysis of ethylene have been discussed by Gooderham (*1*), who suggests the use of concentrated sulfuric acid containing 1 per cent silver sulfate as a catalyst for the analysis of ethylene in fuel gas.

It was found that the reagent described by Gooderham gave rapid absorption of ethylene but was not entirely specific in the presence of butane. About 0.5 cc. of butane was absorbed per passage of 100 cc. of pure butane gas at atmospheric pressure.

In order to eliminate the error due to butane absorption a new reagent was developed. This reagent is prepared by adding 12 grams of silver sulfate to 200 cc. of 72 per cent sulfuric acid. The reagent absorbs ethylene rapidly and gives no error due to butane absorption.

Table I. Absorption Data

Passage	Ethylene–*n*-Butane Mixture Buret	Ethylene–*n*-Butane Mixture Absorption	Pure *n*-Butane Buret	Pure *n*-Butane Absorption
	Cc.	*Cc.*	*Cc.*	*Cc.*
0	100.0	...	94.7	...
1	80.0	20.0	94.7	0.0
2	72.2	7.8	94.7	0.0
3	70.0	2.2	94.7	0.0
4	69.9	0.1	94.7	0.0
5	69.9	0.0	94.7	0.0
		Total 30.1		Total 0.0

A typical set of absorption data for a synthetic mixture containing 30 per cent pure ethylene and 70 per cent pure *n*-butane is given in Table I, with data for the passage of pure *n*-butane. A 2-minute time of passage was used throughout the analysis.

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Simplified Technique in the Use of Liquid Amalgam Reductors

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THE use of liquid amalgams as reducing agents in volumetric analysis has many possible advantages. The saturated bismuth, lead, cadmium, and zinc amalgams range in reduction potential from +0.32 to −0.762 volt, in accordance with the following single electrode reactions:

$$BiO^+ + 2H^+ + 2e^- = Bi + H_2O \quad (+0.32 \text{ volt})$$
$$Pb^{++} + 2e^- = Pb \quad (-0.126 \text{ volt})$$
$$Cd^{++} + 2e^- = Cd \quad (-0.402 \text{ volt})$$
$$Zn^{++} + 2e^- = Zn \quad (-0.762 \text{ volt})$$

Liquid amalgams, among other applications, have been used in the reduction of vanadium, iron, molybdenum, uranium, tungsten, titanium, and chromium in preparation for their subsequent evaluation using standard oxidizing agents. The indirect determination of phosphorus following the formation of the familiar ammonium phosphomolybdate has been carried out. By the choice of suitable liquid amalgams many differential reductions have been accomplished.

The original use of a liquid amalgam reductor was devised by Nakazona (*7*). Many other applications were subsequently described by Someya (*9*). The only other liquid metal reductor used (fused Wood's metal) was described by Smith and Wilcox (*8*).

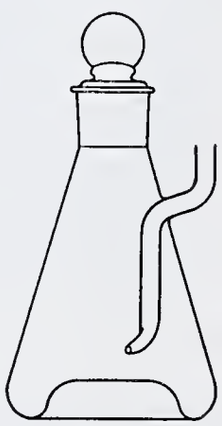

FIGURE 1

The use of liquid amalgams as reductors following the Nakazona procedure has not proved popular as a practical procedure because of the difficulty of removing the excess of reducing agent from the solution containing the element being determined. While the Nakazona procedure is rapid in the reaction of reduction, the apparatus (*3*) employed in the separation of the liquid amalgam and the reduced solution is extremely awkward to manipulate. The use of liquid amalgam reductors has therefore never been generally adopted and the Jones reductor (*2*), the Walden silver reductor (*11*), or other procedures have always been preferred.

The present paper describes a simple and very practical method by which liquid amalgam reducing agents may be rapidly separated from the solutions of metal ions which have been reduced preparatory to their subsequent oxidimetric determination. By the new procedure a reaction time of 2 minutes is generally ample and the removal of the reducing amalgam from the field of reaction is instantaneous. By comparison with the use of any other metallic reductor the new technique is just as accurate, comparably versatile, and much simpler in mechanical manipulations.

Apparatus and Reagents

For reduction of ions which do not require a neutral atmosphere for their final determination, an Erlenmeyer flask of 250- or 500-ml. capacity with a tight-fitting glass stopper is all the apparatus required. Such determinations are those of iron and vanadium after reduction to their ferrous and vanadyl states. For reductions such as titanic salts to titanous ions, which must be subsequently oxidized in an oxygen-free atmosphere, the apparatus shown in Figure 1 is employed.

The only reagents required are saturated bismuth, lead, cadmium, or zinc amalgams, carbon tetrachloride, the standard oxidants such as permanganate, dichromate, or cerate solutions, suitable indicators such as ferroin, methylene blue, or ammonium thiocyanate, and ferric alum and titanic sulfate solutions in dilute sulfuric acid.

The liquid amalgams are prepared according to the procedures described by Someya (*9*). The other reagents are prepared by routine analytical procedures.

Experimental Procedures

The determination of iron and titanium only is dealt with here. The conditions for the determination of the other elements follow the procedures of Someya precisely (*9*), since the only new technique described in this paper deals with the mechanics of the separation of the solutions reduced, from the liquid amalgam reductor.

Iron was determined in an approximately 0.1 *N* ferric alum solution in 0.1 *N* sulfuric acid as follows:

Twenty milliliters of saturated zinc amalgam were placed in a 250-ml. glass-stoppered Erlenmeyer flask, and 100 ml. of sulfuric acid (1 volume of concentrated sulfuric acid to 6 volumes of water) were added, followed by 25.00 ml. of the ferric alum. The flask was stoppered, shaken vigorously during 120 seconds, and then opened, the sides were rinsed, and 30 to 50 ml. of carbon tetrachloride were added.

The amalgam forms the lower layer of the three immiscible liquids in the flask. The carbon tetrachloride (specific gravity approximately 1.6) separates the amalgam layer from the solution being analyzed, which forms the top layer. The top layer is then swirled, using a mechanical stirrer that will stir this layer without causing the carbon tetrachloride to stir materially. The reduced iron is then titrated, using an approximately 0.1 *N* solution of sulfatoceric acid with ferroin as indicator.

Using zinc amalgam, 25.00 ml. of ferric alum = 16.35, 16.30, 16.33, and 16.29 ml. of sulfatoceric acid; average, 16.32 ml. By titration of 25.00 ml. of the same ferric alum following reduction using the Jones reductor, 25.00 ml. of ferric alum = 16.44, 16.39, 16.38, and 16.46 ml. of sulfatoceric acid; average, 16.42 ml.

In the presence of air the liquid amalgam gives slightly low results, disclosed by Someya. The low results can be eliminated by filling the flask containing the reactants with carbon dioxide, either from a cylinder of compressed gas or by adding 0.5 gram of sodium bicarbonate to the acid solution before stoppering the flask and shaking to reduce. In this case 25.00 ml. of ferric alum reduced in a carbon dioxide atmosphere using zinc amalgam required 16.47, 16.45, 16.50, 16.48, 16.42, 16.45, 16.34, 16.51, 16.52, 16.51, and 16.35 ml. of $H_2Ce(SO_4)_3$; average, 16.45 ml. It is thus seen that low results are eliminated through the use of carbon dioxide. The cause of these low results in the absence of carbon dioxide is as yet unknown.

Differential Titration of Iron and Titanium

The same procedure was applied to mixtures of 25.00 ml. of the ferric alum solution plus 25.00 ml. of an approximately 0.1 *N* solution of titanic sulfate. The flask shown in Figure 1 was employed after being filled with carbon dioxide from a cylinder through the side arm. The stopper was inserted, the side arm stoppered, and the flask shaken for 2 minutes. The stoppers were then disengaged, the flask walls rinsed, and 30 to 50 ml. of carbon tetrachloride added. A few drops of 0.1 per cent solution of methylene blue were added as indicator and while a vigorous stream of carbon dioxide gas was passed into the side arm, the titanous ion was titrated, using sulfatoceric acid to the formation of blue by the indicator. A drop or two of ferroin was then added and the titration of ferrous iron completed.

By this procedure as the average of four consecutive determinations the titration of titanium gave 18.78 ml. of sulfatoceric acid. The comparison of the value of the titanic sulfate solution with the Jones reductor was found to be 18.65 ml. of sulfatoceric acid. These results are not believed to be the best obtainable using the method. Determination of iron in the

same set of four results gave, as the average of the closely agreeing results, 16.58 ml. of sulfatoceric acid compared to 16.45 ml. using the Jones reductor for the ferric alum reduction.

The accumulation of reaction products from a large number of determinations is collected in a suitable container and later separated, using a large separatory funnel, and the liquid amalgam as well as the carbon tetrachloride is used repeatedly.

Conclusions

The procedure described above for accomplishing the separation of solution and amalgam, using carbon tetrachloride, makes possible the completion of a reduction in 120 seconds and subsequent immediate titration of the reduced solution. In the oxidimetric determination of iron it is the most rapid procedure at present known.

The present paper is written for those skilled in the art of analysis. For procedures in the extended use of the Nakazona liquid amalgam reductor, the published results of Someya (*9*) should be consulted and the new technique applied. By this procedure the published data may be employed in routine procedures in a really practical manner. Besides the applications of Nakazona (iron, molybdenum, uranium, titanium, and vanadium) using the zinc amalgam (*7*), Takeno (*10*), and Kano (*4*), using the cadmium amalgam, we have the differential determination of titanium and iron, titanium and uranium, and uranium and iron by Kikuchi (*6*) and the differential determination of iron and chromium by Kano (*5*). The indirect determination of phosphorus was described by Hakamori (*1*). The determination of tin by use of the bismuth amalgam should be better than the usual nickel reduction procedure (*3*).

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Elimination of Fluoride Interference in the Molybdenum Blue Reaction

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FLUORIDE ions, even if present in very low concentrations, produce a negative interference in the molybdenum blue reaction (*6*), which is used extensively for determining small amounts of phosphate. The most convenient of the usual methods for removal of fluoride ions is evaporation with perchloric acid. The excess perchloric acid is then neutralized before the determination is made (*3*, *5*). If this neutralization is not carried out with precision, the indicator will cause interference in the photometric procedure. The perchlorate ion, if present in high concentration, may interfere with the development of the molybdenum blue color (*3*). If organic matter is present, varying degrees of hydrolysis and oxidation may occur during the evaporation and thus make the differentiation between organic and inorganic phosphate impossible. Another disadvantage of the usual method is that the evaporation should be carried out in platinum to avoid the larger blank which arises when Pyrex beakers are used (Table I).

Table I. Blank Determinations on Aliquots Containing 0.01 Mole of Ammonium Fluoride

Treatments of Aliquots	Photometer Reading	Apparent Phosphorus, Equivalent P. P. M. in Aliquot
Evaporated with $HClO_4$ in Pyrex beakers	93.4	0.340
Evaporated with $HClO_4$ in platinum dishes	97.6	0.022
Boric acid added, aliquot not evaporated	98.0	0.017

The fluoride removal is unnecessary when boric acid is added to the fluoride-containing aliquot before the phosphate determination is made. The boric acid forms with fluoride the fluoborate ion and thus prevents interference by the fluoride ion. Under these conditions, evaporation with perchloric acid may be omitted and accompanying errors avoided, with a considerable time saving. Boric acid has also been used to remove fluoride interference in iron determination (*1*, *4*).

This scheme for preventing fluoride interference should be generally applicable where phosphate is to be determined by the molybdenum blue reaction. The procedure of Dickman and Bray (*2*) is readily modified to eliminate fluoride interference. Under the conditions of their procedure, neither the excess boric acid nor the fluoborate ions have any appreciable effect on the photometer reading (Table II). The procedure given below is recommended for determining the amount of phosphate extracted from soils by fluoride solutions (*3*).

Table II. Effect of Boric Acid on Fluoride Interference

Concentration of Phosphorus Added	H_3BO_3 Added	NH_4F Added	Concentration of Phosphorus Found
P. p. m.	*Mole/l.*	*Mole/l.*	*P. p. m.*
0.250	None	None	0.254
0.250	None	0.02	0.00
0.250	0.08	0.02	0.245
0.250	0.08	0.20	0.200
0.250	0.24	0.20	0.246
0.250	0.24	0.30	0.248
0.250	0.24	0.60	0.256
0.050	0.24	0.20	0.050
0.500	0.24	0.20	0.492

Analytical Procedure. An aliquot of the unknown solution containing less than 0.015 mole of fluoride ion (equivalent to 0.3 mole per liter in the final volume) is pipetted into a test tube, and 15 ml. of 0.8 molar boric acid are added. The volume is then made up to 35 ml. with distilled water and the reagents are added to give a final volume of 50 ml. The reagents and procedures for development and measurement of the color are those described by Dickman and Bray (*2*). Maximum color, however, develops less rapidly in the fluoborate procedure and photometer readings should be made between 5 and 10 minutes after the addition of the reagents. In the range of concentrations to which this reaction has been applied, the photometer calibration curve for known phosphate solutions may be used without modification. With fluoride concentrations greater than those investigated, it may be necessary to construct a calibration curve, using phosphate solutions which contain the same amount of boric acid as will be added to the unknown solutions.

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Contribution from Agronomy Department, University of Illinois.

A Rapid Kjeldahl Digestion Method Using Perchloric Acid

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A PREVIOUS paper (*4*) suggested a rapid perchloric acid method for microdeterminations of nitrogen. The present paper describes a variation of this method which can be used in the ordinary Kjeldahl digestion. By the proposed procedure a considerable amount of time is saved in digesting, the necessity for using large amounts of salts is eliminated, and as a consequence, the cost of making nitrogen determinations is considerably reduced. An added advantage is that the resulting solution is usually water-clear. If iron is present a yellow tint may persist, but this will disappear upon cooling and dilution.

Previous attempts to use perchloric acid in Kjeldahl determinations have generally resulted in the loss of nitrogen either as the oxide or in the elemental form. Mears and Hussey (*3*) found that such loss could be prevented by decreasing the quantity of perchloric acid used, the increase in rate of digestion obtained was no greater, however, than if they had used mercury or selenium as catalysts. Stubblefield and DeTurk (*5*) refer to a perchloric acid method proposed by Gauduchon-Truchot (*1*) and to a similar procedure of Le Tourneur-Hugon and Chambionnat (*2*) which involved the dropwise addition of perchloric acid, but they say that neither is applicable to general laboratory use.

One reason for the failure of analytical chemists to give more attention to the possibilities in the use of perchloric acid in nitrogen determinations is the tendency of the acid to react violently with easily oxidizable materials. In the method proposed, there has never been any evidence of this explosive property. The cold dilute acid is not dangerous, and the small amount employed is further diluted by 50 times its volume of sulfuric acid before it is used. Furthermore, the easily decomposable materials have already been oxidized in advance of the introduction of the perchloric acid into the system.

Procedure

PLANT MATERIALS AND FERTILIZERS. To 1 gram of dried material add 25 ml. of concentrated sulfuric acid, 10 grams of sodium sulfate, and 1 ml. of a selenium oxychloride–sulfuric acid solution (12 grams of selenium oxychloride per liter of concentrated sulfuric acid). Heat over a vigorous flame for 10 minutes. (During this interval the sample will char, go into solution, and pass to a dark red, light brown, or yellowish solution, depending on its composition: plant materials usually take on a reddish tint; fertilizers become brown. For most materials, a digestion time of 10 minutes is sufficient.) If nitrates are present, as in mixed fertilizer, and zinc is used to reduce the nitro groups following the addition of salicylic acid, start the digestion with a low flame and continue for about 5 minutes in order to expel the hydrogen, after which heat it to vigorous boiling for an additional 10 minutes. Rotate the flasks occasionally to aid the refluxing acid to wash the walls of the flask clean. Turn off the flame and allow the flasks to cool for 10 minutes on the digestion rack and for a few minutes more under the cold water tap until they are cool enough to be grasped with the bare hands.

Add 10 drops (0.5 ml.) of a 35 per cent aqueous solution of perchloric acid (diluted from 70 per cent acid) directly into the digest. Reheat the flasks over a flame so small that the digest does not boil. Continue the digestion at this low temperature until the solution clears, normally between 10 and 15 minutes. After it has become clear, allow the digest to cool, dilute with 200 ml. of water, and distill by the usual procedure.

The digest must be kept from boiling, otherwise a loss in nitrogen will occur, and if the perchloric acid is volatilized, the period of digestion may be prolonged. The end of the digestion is preceded by the evolution of a small quantity of white fumes and a slight effervescence in the digest. The final solution is usually colorless, but certain materials having insoluble components may yield a grayish turbid solution. This has no effect, however, on the accuracy of the nitrogen determination. For moist samples, such as fresh compost, 1 ml. of the 35 per cent perchloric acid (rather than 0.5 ml.) should be used to compensate for the water contained in the sample.

SOILS. Except for peats and other highly organic soils, the sodium sulfate should be omitted. The sample is digested directly with 25 ml. of sulfuric acid and 1 ml. of the selenium oxychloride solution for 10 minutes over a vigorous flame. The subsequent perchloric acid phase of the digestion is as previously described. Since soils usually contain comparatively little organic matter, the solution is normally light colored when the perchloric acid is added. Accordingly, there is no definite color change to indicate the completion of the oxidation, and a standard period of 15 minutes is used for the perchloric acid phase of the digestion.

Experimental

The perchloric acid procedure was tested by comparing the per cent nitrogen obtained on a variety of materials by this method with that found by the official A. O. A. C. method (Table I). The values by the official method were determined by the members of the Soils Department in connection with other investigations. The fact that the results were

TABLE I. NITROGEN OBTAINED BY RAPID PERCHLORIC ACID AND A. O. A. C. METHODS

Material	Perchloric Acid Method	A. O. A. C. Method	Deviation
	%	%	%
Sludge	1.79	1.78	+0.01
Hynite tankage	9.45	9.57	−0.12
Bovung	2.03	2.01	+0.02
Smirow	7.23	7.01	+0.22
Garbage tankage	2.87	2.66	+0.21
Animal tankage	8.77	8.33	+0.44
Cocoa meal	3.13	2.98	+0.15
Peanut hulls	1.22	1.24	−0.02
Dried blood	13.77	13.83	−0.06
Tobacco stems	1.06	1.00	+0.06
Alfalfa	2.73	2.82	−0.09
Horse manure	1.43	1.45	−0.02
Dry fish scrap	9.48	9.28	+0.20
Acid fish scrap	8.75	8.54	+0.21
Chicken manure	2.26	2.25	+0.01
Processed tankage	9.59	9.76	−0.17
Cocoa tankage	2.36	2.52	−0.16
Beetle scrap dust	19.05	19.02	+0.03
Milorganite	5.67	5.66	+0.01
Tankage 1	8.78	8.85	−0.07
Tankage 2	8.42	8.50	−0.08
Tankage 3	8.67	8.65	+0.02
Tankage 9b-2200	6.55	6.60	−0.05
Tankage 8b-220	9.42	9.42	0.00
Mixed fertilizers 1	2.12	2.14	−0.02
Mixed fertilizers 2	4.19	4.20	−0.01
Mixed fertilizers 3	8.13	8.33	−0.20
Mixed fertilizers 4	6.00	5.93	+0.07
Compost 6A	0.37	0.31	+0.06
Compost 7A	0.53	0.48	+0.05
Compost 7B	0.36	0.35	+0.01
Compost 8B	0.29	0.26	+0.03
Compost 8C	0.26	0.29	−0.03
Dutchess loam soil			
Ap Horizon	0.231	0.245	−0.014
A_2 Horizon	0.190	0.197	−0.007
B Horizon	0.082	0.092	−0.010
Sassafras loam soil			
Plot 7A-Ap Horizon	0.057	0.064	−0.007
Plot 7A-B_1 Horizon	0.020	0.020	0.00
Plot 9A-Ap Horizon	0.084	0.089	−0.005
Plot 11A-Ap Horizon	0.100	0.104	−0.004
Plot 18A-Ap Horizon	0.132	0.143	−0.011
Plot 18A-B_1 Horizon	0.047	0.053	−0.006

obtained by independent analysts, one of whom used the A. O. A. C. and the other the suggested method, indicates the reliability and accuracy of the proposed procedure. The average deviation from the official method for the 33 materials, other than the samples of soil, was 0.09 per cent. The average digestion time for materials other than soils by the perchloric acid method was 30 to 35 minutes, whereas that for the official method was between 1 and 3 hours. For soils, the digestion time by the rapid method was 35 minutes, in comparison with 2 hours by the official procedure.

Discussion

The volume of perchloric acid that can be used without losing nitrogen is a direct function of the organic matter which remains after the preliminary sulfuric acid digestion. For this reason, an alternate method can be employed in which the use of sodium sulfate is not involved.

Heat the sample with 25 ml. of concentrated sulfuric acid and 1 ml. of the selenium oxychloride solution for 25 minutes. Allow the digest to cool, add 1 ml. of 35 per cent perchloric acid, and heat the mixture over a small flame until it is clear, as described above.

This variation of the procedure is as accurate as the previously described method. The elapsed time for the complete digestion, however, is between 40 and 45 minutes.

In the absence of an organic substrate with which to react perchloric acid will oxidize the ammonia, with resulting loss of nitrogen. This explains why sodium sulfate cannot be used in soil digestions. The amount of organic matter in soils is comparatively low and most of it is oxidized by the sulfuric acid when sodium sulfate is added to raise the boiling point of the digest.

During the perchloric acid phase of the procedure the temperature of the digest must be kept below the boiling point. If the solution is made to boil the perchloric acid will react at too rapid a rate, with a resulting loss of nitrogen.

The addition of salicylic acid did not materially lengthen the period of digestion. An average period of 32 minutes was necessary for samples containing approximately 2 grams of salicylic acid. Evidently the carbon contained in the salicylic acid is largely oxidized during the preliminary sulfuric acid treatment and therefore does not lengthen the total digestion period. This also explains why 0.5 cc. of 35 per cent perchloric acid is sufficient in spite of the large amount of carbon added in the salicylic acid.

The perchloric acid method is also suitable as a wet-ashing procedure for the determination of plant ash elements, such as phosphorus, potassium, and calcium. The alternate rather than the first mentioned procedure is to be preferred for this purpose.

Summary

A rapid Kjeldahl digestion procedure involving the use of 35 per cent perchloric acid is proposed. An alternate method eliminates the use of sodium sulfate, with only a small increase in the length of period of digestion. The average digestion time for 1-gram samples is approximately 30 minutes.

No explosive hazard, because of the perchloric acid used, is believed to be involved. The results check closely with the values determined by the official A. O. A. C. method. The cost of the determination is reduced by eliminating certain reagents employed in the A. O. A. C. method, such as mercury and potassium sulfide.

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Pressure Wash Bottle for Volatile Solvents

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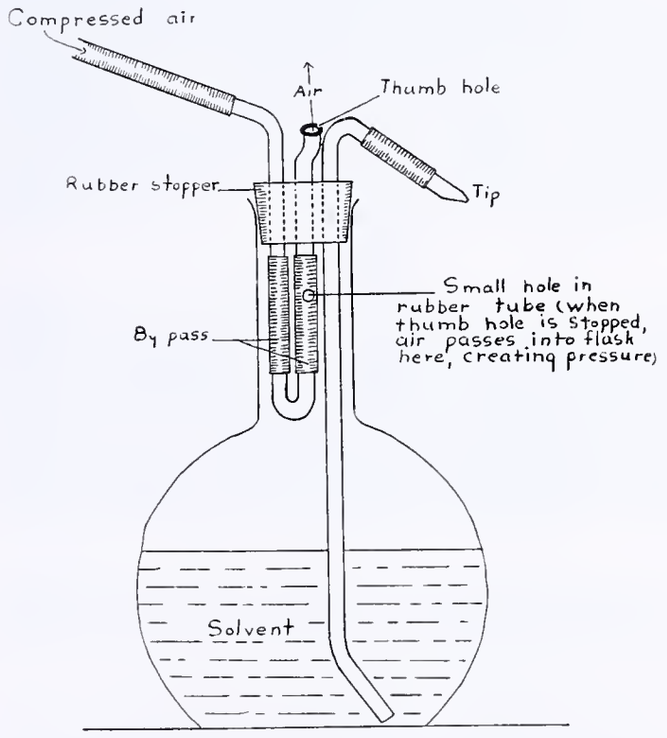
MUCH time and energy can be saved in many laboratory operations by attaching to the mouthpiece of the conventional wash bottle (of the type with the third tube for the thumb) a long piece of light rubber tubing connected to a compressed air line or air pump. In using this device, a slow current of air is turned on and allowed to flow through the wash bottle continuously during the period of use, the air escaping through the thumb-hole tube. Delivery of liquid from the wash bottle is obtained instantly and for any desired period of time down to a small fraction of a second by placing the thumb over the thumb hole, thus creating pressure inside the bottle.



In case the device is used for volatile solvents such as ether, petroleum ether, alcohols, etc., evaporation and consequent loss of solvent which would take place by allowing a current of air to pass promiscuously through the bottle are easily prevented by incorporating into the design a small bypass, best described in the accompanying diagram. This device, the liquid flow from which is entirely under control of the thumb, is the most effective the authors have seen for delivering a small quantity of a liquid in a forcible stream exactly when and where it is wanted.

Colorimetric Determination of Cobalt

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The ammoniacal ferricyanide method for cobalt is modified to control the nature and concentrations of salts present and to permit a precision of 0.06 mg. of cobalt, without careful measurement of the volumes of reagents.

VARIOUS colorimetric methods for the determination of cobalt are described by Snell (*4*). Of these, the one employing ammoniacal ferricyanide appeared to be the most suitable for routine analysis of paint driers. When dealing with concentrations of cobalt usually encountered in these analyses, its working range corresponds to a convenient size of sample, and only common reagents are employed. However, the introduction of various anions required for the separation of interfering metals renders the method unreliable—for example, one sample might also contain lead which would be removed as the sulfate, and another might contain such other metals that a sulfide separation would be needed, in which case the cobalt sulfide would be dissolved in aqua regia. A further complication is that quantities of acids would vary, and would require more or less ammonium hydroxide for neutralization. The resulting variations in salt concentrations also affect the color intensity.

The purpose of the present work was to find such a set of conditions that the color developed would be a function only of the cobalt present, even with rough measurements of the reagents. Since the ferricyanide reagent itself is colored, its volume must be measured accurately. In the method described below, however, all the other reagents can be measured from graduates, and the cobalt determined with a precision of 0.06 mg. The cobalt is first converted to the sulfate, and the time required from the sulfate solution to the final measurement is 10 minutes or less.

Reagents

All reagents should be of the C. P. grade. In the development of the method, Baker's C. P. chemicals were used: Ammonium hydroxide, concentrated (15 *N*), cobalt oxide (Co_3O_4), and potassium ferricyanide, 1 per cent solution in distilled water. The potassium ferricyanide solution should be stored in a brown bottle. On long standing, a precipitate may form, which should be removed by filtration. Sodium hydroxide, 25 per cent solution in distilled water. Sulfuric acid, concentrated (36 *N*) acid diluted 1 to 10 with distilled water, giving approximately 3.6 *N*, and 1 to 4, giving approximately 9 *N*.

Apparatus

A photoelectric colorimeter, measuring in the region of 5200 Å., and, in the development of the method, a Diller colorimeter (*1*) with a Hellige No. 469-40 filter were used.

Procedure

The sample should contain from 0.5 to 4.0 mg. of cobalt, but if the color developed is too dark, it may be diluted in some known ratio with the blank solution, and the color measured. Practically all other cations interfere, by giving precipitates, forming colored ferricyanides, or modifying the color of the cobalt ferricyanide. If a cobalt drier alone is to be analyzed, the extraction method with 6 *N* hydrochloric acid (*3*) yields a solution which may be treated immediately as described below. If the only other metal present is lead, the solution may be treated immediately, and the lead sulfate filtered off. In more complicated cases, cobalt sulfide may be obtained by appropriate means, and dissolved in aqua regia.

To the cobalt solution, free of other cations and nonvolatile anions, are added 6 ml. of 9 *N* sulfuric acid from a graduate. Volatile anions are removed by evaporation to fumes of sulfur trioxide. The solution is cooled, diluted, and transferred to a 100-ml. volumetric flask. The acid is neutralized with 25 per cent sodium hydroxide solution, using litmus paper as indicator. (The formation of cobalt hydroxide also indicates alkalinity.) The solution then is made slightly acid with 3.6 *N* sulfuric acid. These adjustments of acidity do not require great care, for 1-ml. excess reagent does not affect the results.

The volume is brought to 50 to 60 ml. with distilled water to prevent the precipitation of cobalt ferricyanide, and 10.0 ml. of 1 per cent potassium ferricyanide are added from a pipet, followed by 20 ml. of ammonium hydroxide from a graduate. The volume is adjusted to 100.0 ml. with distilled water, the flask shaken, and the color measured. The color is developed immediately, and is stable for at least a day.

A blank solution is prepared in the same fashion, without any cobalt, and the colorimeter is set to 100 per cent transmission with this solution. By thus compensating for the color of the potassium ferricyanide, the 1 per cent solution need not be prepared quantitatively.

To standardize the colorimeter, 1.362 grams of cobalt oxide were dissolved in hydrochloric acid. About 2 ml. of sulfuric acid were added, and the chloride was removed by volatilization. The resulting cobalt sulfate was diluted to 1 liter, giving a solution containing exactly 1 mg. of cobalt per ml. The concentration was checked by precipitation of the cobalt with anthranilic acid (*2*).

To measured volumes of this standard solution were added 6 ml. of 9 *N* sulfuric acid, followed by neutralization, etc., as described above, or by evaporation to fumes of sulfur trioxide, and then as described, or by precipitation of the sulfide, solution in aqua regia, and then as described, with identical results.

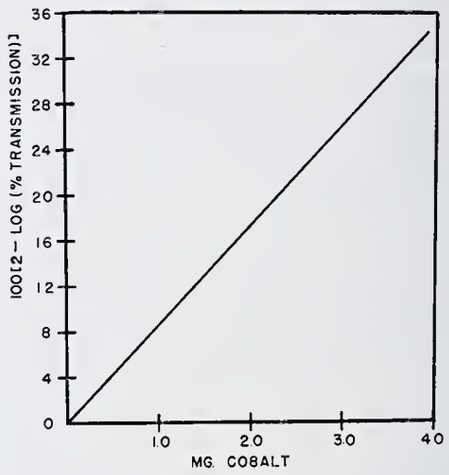

FIGURE 1

The calibration curve obtained is shown in Figure 1. The curve is linear above 4 mg. of cobalt, but as the logarithmic scale becomes compressed above 40, darker colors are preferably aliquoted with the blank solution. A recalibration after 6 months gave an identical curve.

Table I illustrates the negligible effects of reasonable variations in the amounts of reagents used. In developing these data 2.0 ml. of the standard cobalt solution were treated in accordance with the above method, except for the variations noted. Wide variations in the amount of acid present are permissible if sodium hydroxide is used for neutralization. If ammonium hydroxide is used, these variations result in irre-

TABLE I. DETERMINATION OF COBALT

Conditions	Color
Regular method	17.0
5 ml. of 9 *N* sulfuric acid	17.0
10 ml. of 9 *N* sulfuric acid	16.5
1 ml. excess of 25% NaOH in neutralization	17.0
Neutralized with 3 *N* HCl	17.0
Neutralized, and 1-ml. excess of 3 *N* HCl	17.5
Neutralized, and 2-ml. excess of 3.6 *N* sulfuric acid	17.0
15 ml. of ammonium hydroxide (for color development)	17.0
25 ml. of ammonium hydroxide (for color development)	17.0

producibility, because ammonium ions have a pronounced effect on the final color. For the same reason, sulfates were chosen in preference to chlorides.

The colorimeter can be read to $^1/_2$ scale division. Table I indicates that reproducible results can be obtained with only approximate measurement of the volumes of reagents used. Duplicate determinations indicate the same limit of reproducibility which, from the slope of the line in Figure 1, may be seen to correspond to 0.06 mg. of cobalt.

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Use of Phosphate for Separation of Cobalt from Iron

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The well-known tendency of cobalt to be retained by the iron-alumina precipitate produced by ammonia has generally been ascribed to a specific adsorption by the large surface of this gelatinous precipitate. Whatever its cause, it can be overcome by precipitating the iron as phosphate at a pH of 3.5. The precipitate is easily filterable and practically all the cobalt passes into the filtrate.

THE U. S. Geological Survey has been engaged in locating possible new supplies of cobalt ores and the survey laboratory has been called on to determine cobalt in a variety of rocks, minerals, and ores. As the percentages of cobalt are usually small in these materials, it is generally necessary during the course of analysis to concentrate and separate the element from a large excess of iron.

Although cobalt is commonly placed in the group with manganese, zinc, and nickel, not precipitated by ammonia, it has long been known that part of the cobalt will be found in the ammonia precipitate under ordinary conditions, and this feature becomes increasingly important with small percentages of cobalt. Thus, Noyes and Bray (*7*) showed that 2 mg. of cobalt are almost wholly retained by the hydroxides of aluminum, iron, titanium, zirconium, and thorium, and even if the cobalt is added after the hydroxides are already formed it is soon largely adsorbed by the gelatinous precipitates. The basic acetate separation of the iron group from cobalt is better. It has been modified by Funk (*3*). The Ardagh and Broughall method, in which precipitation by ammonia is made in a very concentrated solution, also gives a fair separation of cobalt from iron (*1*).

Fairchild developed a method (*2*) which consists in hydrolyzing the trivalent metals into basic alums at about 100° C. or slightly higher. The basic alums are more pulverulent than the hydroxides, filter more quickly, and most of a large excess of iron or aluminum can be separated from a little cobalt in the presence of ammonium sulfate at a pH of around 3.1 by this method. Correction for the small quantity of iron and aluminum remaining with the cobalt after the removal of the basic alum precipitate is not difficult and was carried out by Fairchild by means of tartrate, hydrogen sulfide at a pH of 5.2, weighing the cobalt as sulfate, and allowing for a trace of iron or aluminum oxide remaining in the cobalt sulfate not soluble in water. Table I shows some of Fairchild's separations of cobalt.

The extraction method of Sandell and Perlich (*8*) is limited by the low solubility of the cobalt dithizone complex in carbon tetrachloride to extremely small percentages of cobalt; it is not applicable to ores of cobalt.

Phenylthiohydantoic acid has been suggested as a reagent for separating cobalt from other elements (*11*), but it does not remove iron completely from the cobalt, and further methods of purification must be used. The marked odor of this compound is objectionable. The use of zinc oxide as an agent to maintain a fixed pH was suggested by Slavik (*9*) as an aid for the removal of iron from cobalt. Hoffman (*4*) showed that this procedure did not make a complete separation with a single precipitation. Hoffman and Lundell (*5*) proposed to remove iron by an ether extraction followed by cupferron. This method seems to give excellent results, although it is rather lengthy.

TABLE I. SEPARATIONS OF COBALT BY FAIRCHILD'S METHOD

Expt.	Fe Taken *Gram*	Al Taken *Gram*	Co Taken *Gram*	Co Recovered *Gram*	Error *Gram*
1	0.2100	....	0.0020	0.0020	0.0000
2	0.2100	0.1536	0.0105	0.0105	0.0000
3	0.3500	....	0.0209	0.0208	−0.0001

Present Method

The method of separation here described is employed after making the usual dehydration of silica and an acid hydrogen sulfide precipitation of such elements as lead, copper, and tin. Iron is then removed as phosphate from an acetic acid solution at a pH of about 3.5. The iron thus precipitated apparently has no retentive or adsorbing power toward cobalt, and the cobalt can be essentially completely separated by a single precipitation of the iron.

Calcium, magnesium, aluminum, alkalies, and other common elements do not interfere. Bismuth, titanium, zirconium, and hafnium are completely precipitated with the iron but do not retain cobalt. Tin and lead would tend to retain some of the cobalt. Copper, nickel, barium, and strontium are not precipitated with the iron, antimony very incompletely.

In the filtrate from the phosphate precipitation cobalt and nickel might perhaps be collected as sulfides at a pH of about 5, but the authors prefer to obtain cobalt at once with nitrosonaphthol (*6*). The cobalt precipitate is ignited and weighed as Co_3O_4 if sufficient in quantity.

For small amounts of cobalt Vogel's thiocyanate method is employed, using the optimum conditions for colorimetric comparisons described by Tomula (*10*). In this colorimetric method with thiocyanate the authors have found that any color caused by traces of iron can be very successfully removed by adding a little of the phosphate reagent described below. The red color caused by rhenium does not develop

TABLE II. DETERMINATION OF COBALT IN SYNTHETIC MIXTURES

Expt.	Added Salts, as Oxides		Cobalt Taken	Cobalt Found
	Grams		*Mg.*	*Mg.*
1	0.1	Fe_2O_3	6.7	6.6
	0.1	Al_2O_3		
2	0.4	Fe_2O_3	6.7	6.7
3	0.3	Fe_2O_3	5.3	5.3
	0.2	Al_2O_3		
4	0.4	Fe_2O_3	16.0	16.1
5	0.3	MnO		
	0.1	Fe_2O_3	5.3	5.3
6	0.4	Fe_2O_3	19.8	19.6[a]
	0.1	MnO		
7	0.4	Fe_2O_3	19.8	19.8[b]
	0.1	MnO		
	5.0	NH_4Cl		
8	0.1	Fe_2O_3	5.3	5.4
	0.1	Cr_2O_3		
9	0.1	Fe_2O_3	0.20	0.20
	0.3	MnO		
10	0.3	MnO	0.20	0.19
11	0.2	Al_2O_3	0.10	0.10
12	0.2	Fe_2O_3	0.10	0.10
13	0.2	CaO	0.10	0.095
14	0.2	MgO	0.10	0.095
15	0.1	Cr_2O_3		
	0.1	Fe_2O_3	0.20	0.18
16	0.1	Cr_2O_3		
	0.1	Fe_2O_3	19.8	19.9

[a] 0.05 mg. of Co found in second treatment of residue.
[b] 0.01 mg. of Co found in second treatment of residue.

TABLE III. DETERMINATIONS OF COBALT IN VARIOUS SAMPLES

No.	Material	Cobalt
		%
1	Manganese ore (Urucum, Brazil), collected by J. van N. Dorr, 2d., Laboratory No. D-1325	0.05
2	Sulfide ore (Calif.), collected by J. S. Vhay, No. 1381	0.56
3	Sulfide ore (Nev.), collected by J. S. Vhay, No. 1275	2.39
4	Manganese ore (Va.), collected by H. S. Ladd, No. 1274, M. Fleischer, analyst	2.25
5	Manganiferous material (Ala.), collected by E. F. Burchard, No. 1347	0.96
6	Soil (Dominican Republic), collected by D. J. Varnes, No. 1375, J. G. Fairchild, analyst	0.10
7	Manganese ore (Tenn.), collected by T. A. Hendricks, No. 1268	2.28
8	Sulfide ore (N. Mex.)	0.01
9	Sulfides (Ariz.), collected by J. S. Vhay, No. 1381	0.01
10	Sulfides (Mass.), collected by R. H. Jahns, No. 1379	0.09
11	Cryptomelane (Cuba), collected by Charles Park, Jr., No. 1175	0.36

under the above conditions (rhenium requires the addition of stannous chloride, but the authors have not encountered rhenium in the minerals so far analyzed).

When molybdenum is present some is retained by the iron and some (0.5 mg. ±) follows cobalt into the nitrosonaphthol precipitate, but there is no interference by molybdenum in the acetone-phosphate-thiocyanate colorimetric test. Any molybdenum color which may develop at first disappears when the solution is nearly neutral, allowing the cobalt color to appear unchanged.

Phosphate has a distinct advantage over stannous chloride in removing the iron color developed with thiocyanate, as it is more permanent in its action and gives no cloudy effect. The quantity of iron likely to escape the phosphate separation varies, usually from 0.04 to 0.08 mg., so that very little phosphate is sufficient to remove the red color. An excess should be avoided.

Chromium is usually incompletely precipitated in the phosphate procedure and a little may separate when the filtrate is concentrated. Aluminum is incompletely precipitated but it does not interfere in the nitrosonaphthol precipitation of cobalt. With high percentages of aluminum more washing is required, but all the cobalt can be washed into the filtrate. Vanadium divides both in the phosphate and in the nitrosonaphthol precipitation and may cause an error, but it has been found in only one cobaltiferous ore. If present, vanadium may be separated from cobalt by cupferron, or cobalt from vanadium by sodium hydroxide and sodium peroxide.

Reagents

PRECIPITATING SOLUTION. Dissolve 10 grams of reagent quality dibasic sodium phosphate dodecahydrate in 250 ml. of distilled water.

WASH SOLUTION. Dissolve 2 grams of reagent quality dibasic sodium phosphate dodecahydrate in 500 ml. of distilled water. Add 2 drops of methyl orange indicator to the cold solution, and then add glacial acetic acid until the solution changes to a faint red color, about 4 ml. of glacial acid being required.

COBALT PRECIPITATING REAGENT. Dissolve 1 gram of purified alpha-nitroso-beta-naphthol in 15 ml. of glacial acetic acid and filter the solution.

Procedure

Dilute the solution, in which iron has been oxidized with nitric acid, to about 400 ml., bring the solution to a boil, and add 3 drops of methyl orange indicator solution. Pour in at least 25 ml. of precipitating solution for each 0.10 gram of iron present. Add ammonium hydroxide while rapidly stirring until the color changes to yellow. Add 1 ml. of glacial acetic acid, when the color of the solution should become slightly pink. Add some paper pulp at this time to facilitate filtering. Place the mixture on the steam bath for about 2 minutes until the precipitate settles, filter while hot through a Whatman No. 40 paper, and wash at least five times with hot wash solution.

Determination of Cobalt

If colorimetric amounts of cobalt are present, concentrate the solution to about 200 ml.; otherwise, adjust the volume to 400 ml. If a few specks of ferric phosphate appear in the solution at this time, filter them off through a small filter. Add 2 ml. of hydrochloric acid for each 100 ml. of solution, heat to boiling, and add about 6 ml. of nitrosonaphthol reagent for every 10 mg. of cobalt present. Allow to stand at least 2 hours. Filter through a Whatman No. 40 paper, wash first with hot 1–4 hydrochloric acid, and finish with hot water. Place paper in a tared porcelain crucible, ignite carefully, at a temperature not exceeding 850° C. and weigh as Co_3O_4.

For small amounts the weight of cobalt oxide has checked sufficiently well with its weight as sulfate.

When the amount of cobalt to be determined is very small, dissolve the oxide in aqua regia while still in the crucible, evaporate to dryness, take up with a little hydrochloric acid, and evaporate again to remove nitric acid. Dissolve the cobalt chloride in 1 to 5 ml. of water, and add the solution to 0.5 gram of ammonium thiocyanate in a 30-ml. beaker. If the solution is colored slightly pink by traces of iron, decolorize it by adding 1 ml. of the precipitating phosphate reagent. Add an amount of acetone equal in volume to the aqueous solution and compare the blue color with that of a solution containing similar amounts of thiocyanate and acetone, to which standard cobalt solution is added until the color matches.

Table II shows the results found for cobalt with various synthetic mixtures. In these mixtures the metal ions were obtained by solution of their respective chlorides and sulfates, all of which were of reagent quality.

Table III gives a few representative results for cobalt on samples from different localities. A very large number of samples have been found to contain no cobalt or less than 0.01 per cent.

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Colorimetric Determination of Trinitrobenzene in Dinitrobenzene

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COLOR reactions of polynitro aromatic compounds with bases have been used for both qualitative and quantitative purposes. An extensive compilation of color tests for nitro compounds was published by Bost and Nicholson (*1*) as an aid in qualitative analysis. Color reactions of various nitration products of benzene and toluene have been reported by Rudolph (*8*), who described the variations produced by acetone and alcohol as solvents and the effects of sodium and ammonium hydroxides.

Benzene in alcohol at dilutions as low as 1 to 1,500,000 can be detected by nitration and reaction with sodium hydroxide (*6*). Methods for determining benzene and toluene in air involve absorption in a suitable nitrating mixture, separation of the nitrated product, and measurement of the color developed in acetone with sodium hydroxide. Compounds determinable by similar reactions include nitrobenzene, dinitrochlorobenzene, and nitrotoluene (*9*). Trinitrotoluene in air has been determined, following absorption in acetone, by developing the color with sodium hydroxide (*5*).

The following spectrophotometric study was undertaken as a basis for developing a routine method for the determination of trinitrobenzene in dinitrobenzene. The only method previously available involves use of melting point data (*3, 4*) and is subject to several limitations.

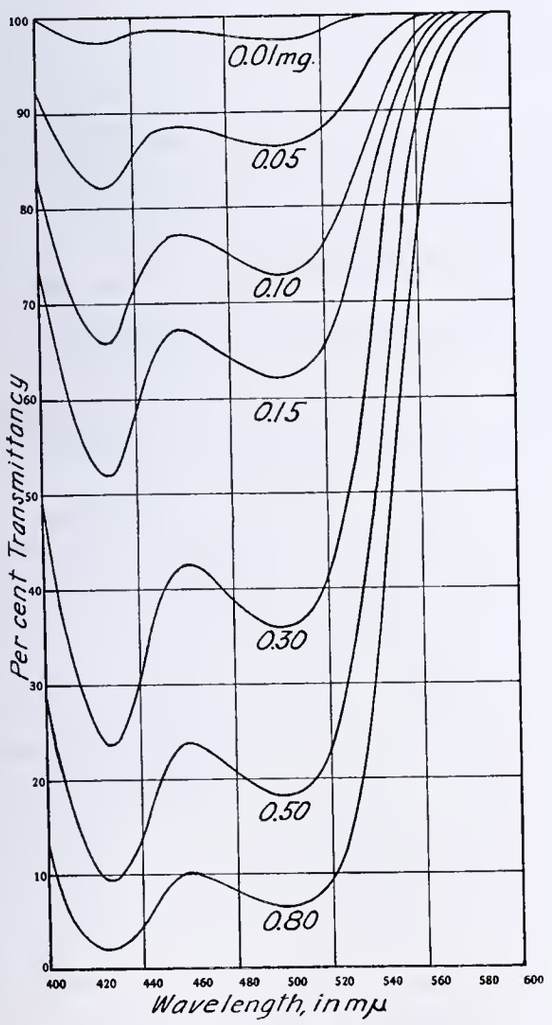

FIGURE 1. EFFECT OF TRINITROBENZENE CONCENTRATION

Mg. of trinitrobenzene per 50 ml. of solution

Apparatus and Solutions

Transmittancy measurements were made with a General Electric recording spectrophotometer set for a spectral band width of 10 mμ. One-centimeter transmission cells were used and the blank cell contained the same quantity of reagents as the sample.

A two-cell filter photometer (*7*) employing the Brice circuit (*2*) and equipped with Corning filters 396 and 428 was calibrated for routine determinations with a 1-cm. cell.

Standard solutions of dinitrobenzene and of trinitrobenzene in acetone and in 95 per cent ethanol contained 0.1 mg. per ml. The nitro compounds were recrystallized material. Aqueous solutions of sodium hydroxide for developing the color were used in concentrations of 1 and 10 per cent.

Color Reaction

EFFECT OF SOLVENT. In acetone, both dinitrobenzene and trinitrobenzene give colors with sodium hydroxide. Although the hues are decidedly different, the direct determination of either in a mixture would be too complicated for routine determinations with a simple instrument. Both colors darken on standing, moreover, and at a rate sufficient to require a separate solution for measurement at each wave length selected.

Trinitrobenzene reacts with ammonia in acetone, giving a reddish purple hue which also becomes more intense on standing. This reaction could be tried as a possible means for determining trinitrobenzene since dinitrobenzene gives no color under the same conditions. A disadvantage of acetone as solvent is that trinitrobenzene solutions begin to darken immediately after preparation.

In ethanol, only trinitrobenzene reacts with sodium hydroxide or with ammonia. This test can be used, therefore, as a means for distinguishing between the two compounds and for determining trinitrobenzene in the presence of dinitrobenzene. The color developed with ammonia fades quickly; but the transmission of a solution containing 0.3 mg. of trinitrobenzene and 10 mg. of dinitrobenzene in 50 ml. of ethanol, basified with sodium hydroxide, increased only 0.7 per cent on standing 10 minutes.

The color reaction was carried out in 95 per cent ethanol, although 2-propanol, and probably other solvents, could be used.

EFFECT OF SODIUM HYDROXIDE CONCENTRATION. The amount of sodium hydroxide added has little effect if a sufficient excess is used. Trinitrobenzene is an acid-base indicator changing from colorless to yellow between pH 12 and 14. For a solution containing up to 2 mg. of trinitrobenzene, 0.5 to 1.0 ml. of 10 per cent sodium hydroxide solution is recommended. No attempt was made to express concentration of base in terms of pH because of the limited working range of the glass electrode pH-meter.

EFFECT OF TRINITROBENZENE CONCENTRATION. Transmission curves are shown in Figure 1 for concentrations from 0.01 to 0.80 mg. per 50 ml. of solution. Each solution contained 0.5 ml. of 10 per cent sodium hydroxide. For the measurements at 502 mμ Beer's law is valid, and the molecular extinction coefficient is 15,800.

The favorable portion of the calibration curve for the filter photometer includes the range 0.1 to 1.0 mg. of trinitrobenzene per 50 ml. with a 1-cm. cell.

EFFECT OF DINITROBENZENE. Dinitrobenzene in ethanol does not react with sodium hydroxide to give a color. It may cause low results for trinitrobenzene, if present in large enough quantities, unless sufficient sodium hydroxide is used. The color produced by 0.3 mg. of trinitrobenzene is not affected by the presence of 100 times that amount of dinitrobenzene if 0.5 ml. of 10 per cent sodium hydroxide is used.

Samples containing from 10 to 75 per cent trinitrobenzene can be analyzed with errors of only 0.5 to 2 per cent of the amount present. This is sufficient accuracy for reporting yields in most organic reactions.

Procedure

Selection and Preparation of Sample. Procure a representative portion of the material to be analyzed, reduce to a fine powder, and dry in a desiccator. Proper care should be exercised, since trinitrobenzene can be detonated.

Measurement. Weigh samples of 50 to 100 mg., dissolve in 95 per cent ethanol, and dilute to 50 ml.

Measurement of Desired Constituent. Withdraw an aliquot containing 0.1 to 1 mg. of trinitrobenzene, add 0.5 ml. of 10 per cent sodium hydroxide solution, and dilute to 50 ml. with ethanol.

Measure or compare the color by any of the usual means within 10 minutes. A blue-green filter such as Corning No. 396 is recommended for photometric measurement. Beer's law is valid for measurements at 502 mμ.

Summary

A rapid colorimetric method for the determination of trinitrobenzene is described. The optimum range for measurements with a 1-cm. transmission cell is from 0.01 to 1.0 mg. per 50 ml. of solution. By dilution samples containing as high as 75 per cent trinitrobenzene can be accommodated satisfactorily. With proper modification, the method should be applicable to samples such as air containing only small traces.

Results were not affected by the presence of dinitrobenzene in an amount 100 times that of the trinitrobenzene.

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Colorimetric Determination of Iron with 2,2′-Bipyridyl and with 2,2′,2″-Terpyridyl

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SINCE the work of Blau (*1*) on complex salts of 1,10-phenanthroline and 2,2′-bipyridyl, various analytical applications of these bases have been found aside from their use as colorimetric reagents for iron (*4*, *12*, *17*, *18*, *19*, *24*). Inasmuch as 2,2′-bipyridyl, 2,2′,2″-terpyridyl, and 1,10-phenanthroline contain the same iron-specific, cyclic N—C—C—N grouping, a basis for comparing the merits of these three compounds as iron reagents is desirable. (Since reactions between iron and possible pyridyl or phenanthroline isomers of 2,2′-bipyridyl, 2,2′,2″-terpyridyl, and 1,10-phenanthroline are not known, the shorter names, bipyridyl, terpyridyl, and phenanthroline, are used in this paper.)

The determination of iron with 1,10-phenanthroline has already been studied (*5*). Various workers have investigated the bipyridyl method, although not with particular attention to the effect of diverse ions (*3*, *6-11*, *13*, *20-23*). Cooper (*2*) used terpyridyl in a study of the distribution of iron in sea water and in marine plankton, and reported that it is capable of detecting 1 mg. of iron in a cubic meter of water or 1 part per billion, a remarkable sensitivity. For low iron concentrations, it was preferred over bipyridyl.

Morgan and Burstall obtained terpyridyl (2,6-di-2′-pyridylpyridine) along with bipyridyl in the dehydrogenation of pyridine (*15*, *16*).

Apparatus and Solutions

Transmittancy measurements were made with a General Electric recording spectrophotometer adjusted for a spectral band width of 10 mμ. A glass electrode pH-meter was used (*14*).

A standard ferric nitrate solution prepared from iron wire of known purity contained 0.01 mg. of iron per ml. and sufficient nitric acid to prevent hydrolysis. Measured quantities of this solution were reduced with 10 per cent solutions of hydroxylamine hydrochloride or sulfate to obtain known concentrations of ferrous iron.

Buffering was accomplished with a 20 per cent solution of ammonium acetate and pH adjustments were made with 6 *N* solutions of hydrochloric acid and ammonium hydroxide. Ten per cent sulfuric acid and 20 per cent phosphoric acid were prepared from the C. P. reagents.

One-tenth per cent solutions of bipyridyl and terpyridyl were used as the color-forming reagents. Bipyridyl dissolves in water, although not readily, while terpyridyl is not sufficiently soluble to permit preparation of a 0.1 per cent solution. Cooper used a 1 per cent solution of the base in 0.2 *N* hydrochloric acid. This is a satisfactory procedure. since a wide latitude in pH is permissible in carrying out the color reaction. Not knowing this in advance, it seemed preferable to use the hydrochloride in order to avoid an excess of acid. The hydrochloride is easily prepared by passing dry hydrogen chloride into an ether solution of the base or by evaporating a small volume of hydrochloric acid containing the desired amount. Since bipyridyl and orthophenanthroline monohydrate do not dissolve readily in cold water, it would be advantageous to use these bases as hydrochlorides also.

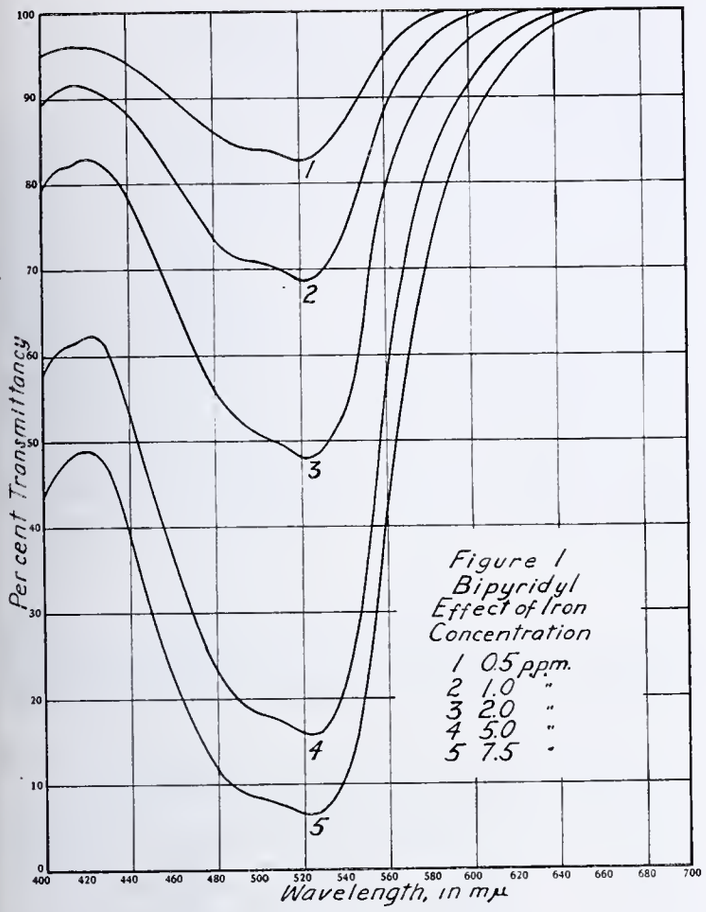
Cobalt nitrate hexahydrate was weighed directly to prepare a solution containing 2.5 mg. of cobalt per ml. Electrolysis of aliquots showed this to be a valid procedure.

Standard solutions of the anions studied were prepared from the alkali metal salts in most cases. Nitrates, chlorides, and sulfates were used for the cation solutions. Each contained 10 mg. of the ion in question per ml. of solution.

Color Reaction. The reddish-purple color formed by bipyridyl with ferrous ion, exhibiting its maximum absorption at about 522 mμ, has been attributed to a complex ion containing 3 molecules of bipyridyl in which the 6 coordination positions of iron are occupied by nitrogen atoms. Similarly, terpyridyl gives a complex ferrous ion. Unlike bipyridyl and phenanthroline, however, only 2 molecules of the base are required, terpyridyl containing 3 nitrogen atoms. The terpyridyl complex resembles permanganate in hue and its absorption maximum occurs at about 552 mμ.

For solutions containing 0.1 mg. of iron, 4 ml. of bipyridyl or 1 ml. of terpyridyl solution develops the maximum color and an excess produces no effect.

Effect of pH. Variations in pH between 3 and 9 do not influence the intensity or hue of the color produced with bipyridyl. Ignatieff (*9*) reports 3.5 to 8.5 as the optimum range. If the pH is less than 2.5, development of the color proceeds very slowly. If less than 2 or greater than 9.5, fading begins immediately and complete color development is not attained. With terpyridyl, pH variations between 3 and 10 do not produce any measurable effect on the intensity or hue



of the color. The workable range for phenanthroline is 3 to 9. Wide tolerances such as these are not always permissible with colorimetric methods.

Effect of Iron Concentration. Beer's law is valid for both reagents at the wave lengths of maximum absorption. The range of iron concentrations amenable to measurement using a 1-cm. transmission cell is indicated in Figures 1 and 2. All solutions contained an excess of hydroxylamine and reagent. Visual comparison in Nessler tubes is best confined between 0.05 and 2 p. p. m. The molecular extinction coefficient for bipyridyl-ferrous ion at 522 mμ is 8650 and for terpyridyl-ferrous ion at 552 mμ is 11,500. The latter value represents only a very small improvement in sensitivity over 11,100 for phenanthroline. A solution containing 0.001 p. p. m. of iron developed a slight color with terpyridyl, the transmittancy at 550 mμ being 98.5 per cent for a 5-cm. thickness of solution.

Reducing Agents. Although the color reactions are with ferrous ion, ferric or total iron may be determined by using a suitable reducing agent. Cooper used a 10 per cent sodium sulfite solution with terpyridyl and allowed 24 hours for development of the color. For bipyridyl, the following reducing agents have been recommended: titanous chloride, hydroquinone, ascorbic acid, sodium dithionite (sodium hyposulfite, sodium hydrosulfite), sodium sulfite, sulfurous acid, and hydrazine sulfate.

Hydroxylamine hydrochloride was found to be very effective with the phenanthroline method (*5*) and works equally well with bipyridyl and with terpyridyl. Its use with bipyridyl has been suggested but not studied (*23*). Good results were also obtained with titanous chloride, hydroquinone, sodium dithionite, and ascorbic acid. Hydrazine and sulfurous acid are not recommended.

Permanency of Standards. The solutions represented in Figure 1 were kept for one year in glass-stoppered Pyrex bottles exposed to daylight. Transmission curves run periodically showed no changes within the limits of visual accuracy in color matching. Bipyridyl-iron standards are valid, therefore, for at least 1 year. Hill (*8*) reported that the colored system was unchanged after standing 3 years in a sealed tube.

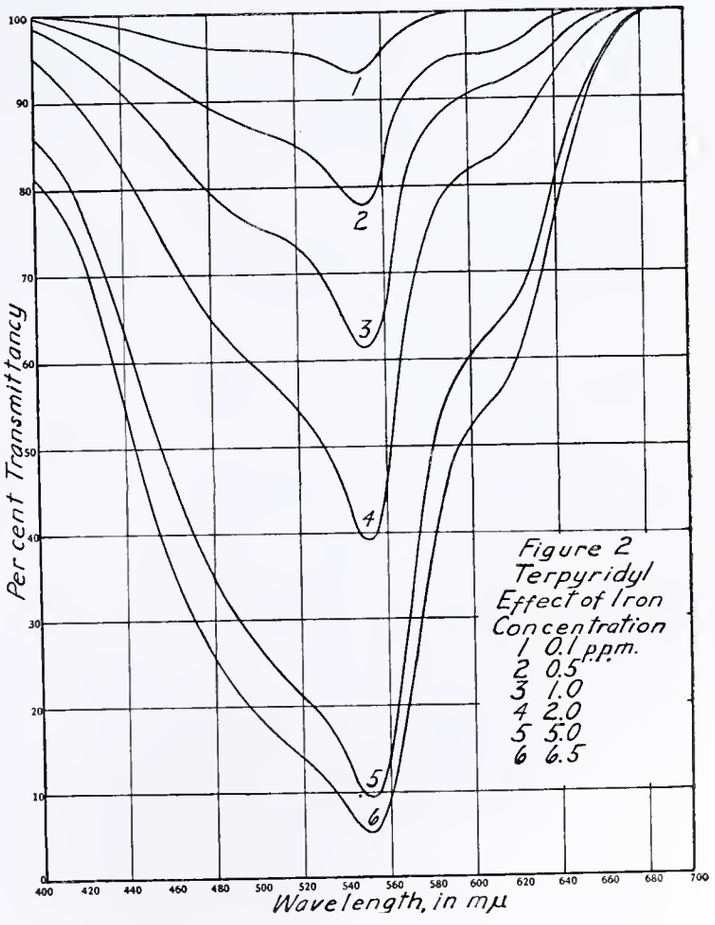
Terpyridyl-iron standards for visual work are reliable for at least 3 months.

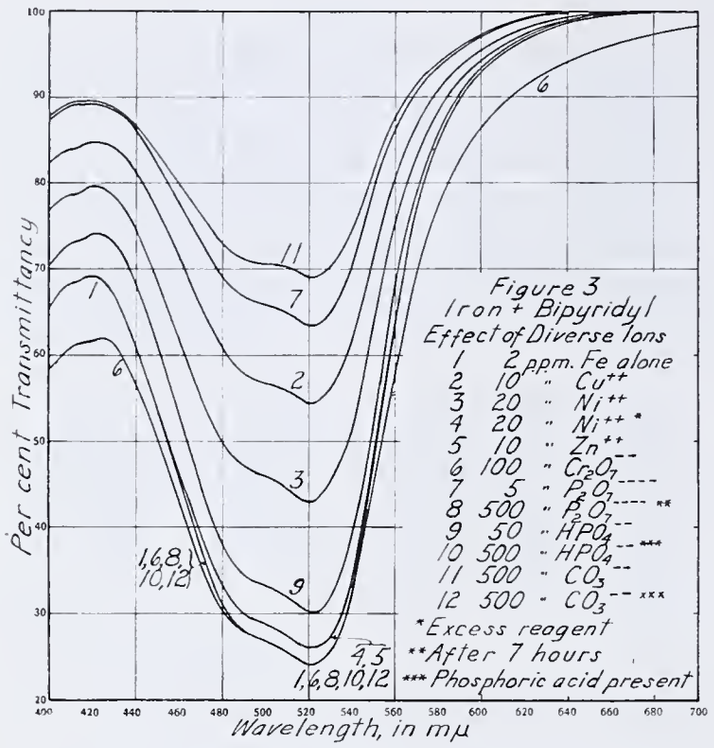
McFarlane recommends cobalt nitrate hexahydrate as a permanent color standard with the bipyridyl method, especially for low iron concentrations (*13*). From the standpoint of hue, the choice of this salt is most appropriate, as will be seen on comparison of the curves in Figure 5. A 2-cm. transmission cell was used. In view of the stability of the iron-bipyridyl complex, the need for artificial color standards is questionable, at least for concentrations exceeding 0.5 p. p. m.

Effect of Diverse Ions. In measuring the effect of diverse ions on the bipyridyl method, a standard solution of the ion in question was added to 0.1 mg. of iron measured as ferric nitrate. One milliliter of 10 per cent hydroxylamine hydrochloride solution and 4 ml. of 0.1 per cent bipyridyl were then added and, after dilution to 50 ml., the apparent iron concentration was determined using 2-cm. transmission cells with distilled water in the blank cell. Two per cent deviation from 2 p. p. m. is negligible for ordinary work and this arbitrary tolerance is used as a basis for defining freedom from interference in the following discussion.

A similar procedure was followed with terpyridyl and 0.5 ml. of reagent solution was used for 0.05 mg. of iron in 25 ml. Hydroxylamine hydrochloride was used as the reducing agent and, in all cases, the ion in question was added to the iron before reducing. This is essential if the over-all effect is desired, because some ions interfere by preventing or retarding the reduction. The color develops immediately with both bipyridyl and terpyridyl (unless ions such as phosphates are present) and was measured within 10 minutes.

In certain cases, the effect of extraneous ions depends on the procedure followed, pH, time of standing, presence of other ions, and the order in which the various reagents are





added, thereby limiting any general statement as to the exact extent of interference.

Five hundred p. p. m. of the following ions may be present without causing more than 2 per cent error in the bipyridyl method: aluminum, ammonium, barium, calcium, cerium, lead, lithium, magnesium, potassium, sodium, strontium, uranyl, acetate, arsenate, arsenite, benzoate, bromide, carbonate, chlorate, chloride, fluoride, formate, iodide, lactate, nitrate, nitrite, orthophosphate, oxalate, perchlorate, pyrophosphate, salicylate, silicate, sulfate, sulfite, tartrate, tetraborate, thiocyanate, and thiosulfate. Uranyl ion does not affect the color in the spectral region 510 to 700 mμ. The slight change in hue below 510 can be excluded readily from the color measurement. Interference of benzoate carbonate, formate, nitrite, orthophosphate, oxalate, pyrophosphate, and silicate was eliminated by using the phosphoric acid procedure (*21*). In the case of fluoride full color development required 45 minutes. Reduction of tetraborate was carried out on the steam bath.

Antimony, bismuth, and tin precipitate. Formation of bipyridyl complexes of cobalt, nickel, beryllium, and certain other metals results in interference which cannot be removed satisfactorily by using an excess of reagent. The extent of interference for various ions is summarized in Table I.

With terpyridyl, 500 p. p. m. of the following ions may be present: aluminum, ammonium, barium, calcium, lead, lithium, magnesium, potassium, sodium, strontium, uranyl, acetate, arsenite, bromide, chlorate, chloride, fluoride, iodide, lactate, nitrate, perchlorate, salicylate, sulfate, sulfite, tetraborate, thiocyanate, and thiosulfate. Certain ions including carbonate may be removed easily. Transmission curves for solutions containing typical interfering ions are shown in Figures 3 and 4. Two-centimeter cells were used.

Phosphoric Acid Procedure. In the determination of ferrous iron in drugs with bipyridyl, Schulek and Floderer (*21*) recommend addition of sulfuric and phosphoric acids before developing the color and use of ammonium acetate as a buffer. This treatment is reported to stabilize the ferrous-ferric ratio. The procedure gives satisfactory results, provided sufficient time is allowed for the color to develop (phosphates retard the color reaction). For most work this is an unnecessary complication, since the color does not develop fully until about 7 hours after adding the bipyridyl.

There may be justification for using phosphoric acid in the analysis of samples containing orthophosphate or a few other interfering anions. The reason for the curious behavior of phosphoric acid in eliminating phosphate interference is not apparent. Conversion of the orthophosphate to pyrophosphate in solution seems out of the question. The effect cannot be explained on the basis of acidity. As shown in Figure 3, 500 p. p. m. of carbonate or orthophosphate can be accommodated satisfactorily. The solutions contained 0.5 ml. of 10 per cent sulfuric acid, 0.5 ml. of 20 per cent phosphoric acid, and 5 ml. of 20 per cent ammonium acetate in 50 ml. Sulfuric acid, and not phosphoric acid, is responsible for the action and the order in which they are added is significant. Phosphoric acid should be introduced before the bipyridyl. Interference by carbonate, formate, nitrite, oxalate, orthophosphate, pyrophosphate, and silicate was completely eliminated by the phosphoric acid procedure, although the method is not satisfactory with metals such as cadmium, cobalt, copper, mercury, or nickel.

Discussion

Inasmuch as the same iron-specific grouping is present in bipyridyl, terpyridyl, and phenanthroline, the reactions of these compounds with iron are similar and there is little basis

Table I. Effect of Diverse Ions with Bipyridyl

Ion	Added as	Present P. p. m.	Error %	Amount Permissible P. p. m.
Ag^+	$AgNO_3$	25	11	5
Be^{++}	$Be(NO_3)_2$	50	0	50
Cd^{++}	$Cd(NO_3)_2$	50	0	50
Co^{++}	$Co(NO_3)_2$	50	5	20
Cr^{+++}	$Cr_2(SO_4)_3$	50	7.5	15
Cu^{++}	$Cu(NO_3)_2$	5	0	5
Hg^+	$HgNO_3$	10	5.5	5
Hg^{++}	$Hg(NO_3)_2$	10	0	10
Mn^{++}	$MnSO_4$	75	2	75
Ni^{++}	$Ni(NO_3)_2$	20	5	10
Th^{++++}	$Th(NO_3)_4$	100	0	100
Ti^{++++}	$Ti(SO_4)_2$	37	5	20
Zn^{++}	$Zn(NO_3)_2$	10	7	5
Zr^{++++}	$Zr(NO_3)_4$	50	0	50
$B_4O_7^{--}$	$Na_2B_4O_7$	100	2	100
CHO_2^-	HCO_2H	100	0	100
$C_2O_4^{--}$	$(NH_4)_2C_2O_4$	5	50	0
$C_7H_5O_2^-$	$C_6H_5CO_2Na$	100	0	100
CN^-	KCN	10	0	10
$Cr_2O_7^{--}$(Cr)	$K_2Cr_2O_7$	100	0	100
MoO_4^{--}	$(NH_4)_2MoO_4$	10	3	7
NO_2^-	KNO_2	50	2	50
VO_3^-	KVO_3	50	2	50
WO_4^{--}	Na_2WO_4	10	3	7

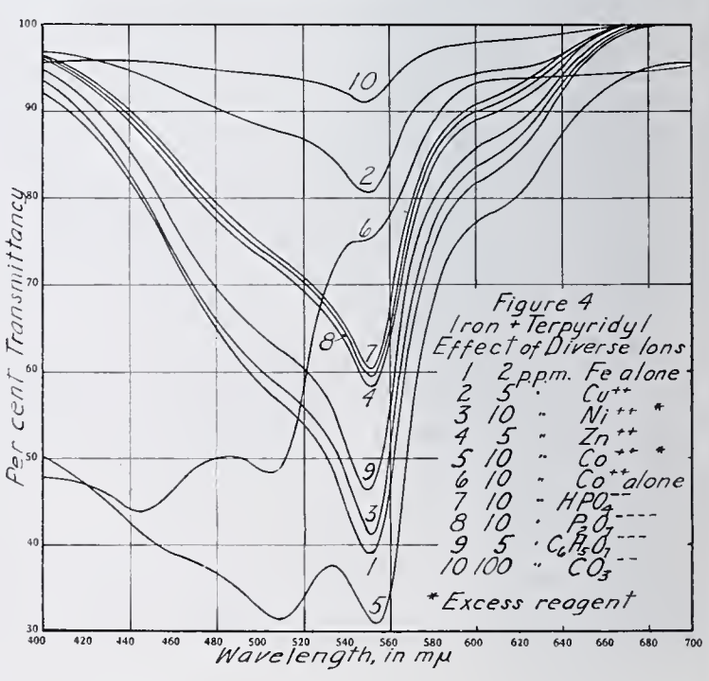


Table II. Effect of Diverse Ions with Terpyridyl

Ion	Added as	Present	Error	Amount Permissible
		P. p. m.	%	*P. p. m.*
Ag^+	$AgNO_3$	150	3	100
Be^{++}	$Be(NO_3)_2$	50	0	50
Cd^{++}	$Cd(NO_3)_2$	100	0	100
Ce^{++++}	$Ce(NH_4)_2(SO_4)_2$	500	3	300
Co^{++}	$Co(NO_3)_2$	5	30	0
Cu^{++}	$Cu(NO_3)_2$	5	77	0
Hg^+	$HgNO_3$	10	2	10
Hg^{++}	$Hg(NO_3)_2$	10	0	10
Mn^{++}	$MnSO_4$	200	0	200
Ni^{++}	$Ni(NO_3)_2$	5	6	0
Th^{++++}	$Th(NO_3)_4$	300	0	300
Zn^{++}	$Zn(NO_3)_2$	10	0	10
Zr^{++++}	$Zr(NO_3)_4$	50	0	50
AsO_3^{---}(As)	Na_3AsO_3	500	2	500
AsO_4^{----}	H_3AsO_4	250	1	250
$B_4O_7^{--}$	$Na_2B_4O_7$	500	2.5	400
CO_3^{--}	Na_2CO_3	10	5	5
CHO_2^-	HCO_2Na	50	0	50
$C_2O_4^{--}$	$(NH_4)_2C_2O_4$	5	0	5
$C_6H_5O_7^{---}$	$(HO)C(CO_2H)(CH_2CO_2H)_2$	5	18	0
CN^-	KCN	5	6.5	0
$Cr_2O_7^{--}$(Cr)	$K_2Cr_2O_7$	50	0	50
MoO_4^{--}	$(NH_4)_2MoO_4$	50	0	50
NO_2^-	KNO_2	100	2	100
$HPO_4^{--}(P_2O_5)$	$(NH_4)_2HPO_4$	10	46	0
$P_2O_7^{----}$	$Na_4P_2O_7$	10	44	0
SiO_3^{--}	Na_2SiO_3	10	4	5
VO_3^-	KVO_3	75	6	25
WO_4^{--}	Na_2WO_4	5	21	0

on which to recommend any one over the others, aside from such considerations as cost and availability. (Terpyridyl is not listed by the chemical supply houses.) Advantages of terpyridyl in sensitivity and freedom from interference by pH variations and presence of diverse ions are hardly significant enough to consider in ordinary work.

There are several outstanding differences, unpredictable in nature, which should be enumerated. Terpyridyl gives a cobalt complex sufficiently colored to be useful in the determination of cobalt. Bipyridyl and phenanthroline give practically no color with cobalt but form highly colored copper and molybdenum compounds. Silver interferes seriously with the bipyridyl and phenanthroline methods for iron, although 100 p. p. m. may be present if terpyridyl is used. Cobalt and copper cause somewhat more interference with terpyridyl than with the other two reagents.

The wave lengths of maximum absorption for phenanthroline, bipyridyl, and terpyridyl are 510, 522, and 552 mμ, respectively, and the hues vary accordingly. Terpyridyl has a more distinct band than the others and its maximum lies in the spectral region most favorable for visual measurements.

A satisfactory method for the determination of cobalt with terpyridyl has been studied. 1,10-Phenanthroline, on the other hand, may be used for determining copper, and its ferrous complex is a valuable oxidation-reduction indicator (*24*). Ferrous complexes of bipyridyl and terpyridyl are not sufficiently stable in hot acid to compare favorably with the "ferroin" indicators. The versatility of these cyclic bases containing the N—C—C—N grouping seems to justify further investigation as new compounds become available.

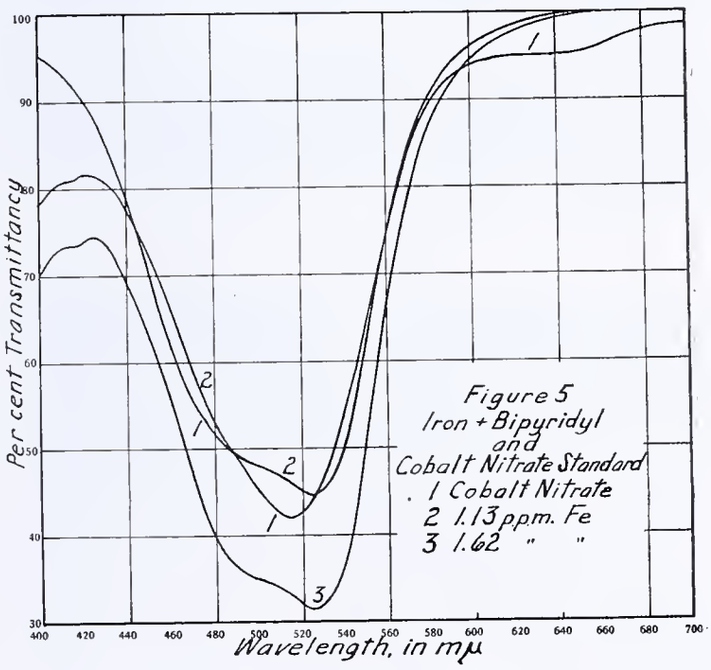
Recommended Procedure

Sample. Procure a representative portion of the material to be analyzed and subject it to the necessary preparative treatment.

Measurement. Weigh or measure by volume a quantity of sample containing 1 mg. of iron or less. This is the maximum iron content advisable for measurements with a 1-cm. transmission cell. For visual work using Nessler tubes, color comparison for solutions containing more than 2 p. p. m. is impracticable.

Treatment. Dissolve the sample by appropriate means. Any of the common mineral acids except phosphoric acid may be used. Remove any interfering ions present, by suitable methods, according to the tolerances listed in Tables I and II. With 2,2′-bipyridyl, interference by 50 mg. of benzoate, formate, nitrite, orthophosphate, oxalate, pyrophosphate, or silicate may be eliminated by adding 2 ml. of 10 per cent sulfuric acid, 2 ml. of 20 per cent phosphoric acid, and 10 ml. of 20 per cent ammonium acetate at this point. Maximum color development requires about 8 hours if phosphoric acid is present.

Desired Constituent. *Measurement.* Transfer the solution to a 100-ml. volumetric flask and add 2 ml. of 10 per cent hydroxylamine hydrochloride solution. Adjust the pH to 3 to 9 (ammonium acetate, 6 *N* ammonium hydroxide, and 6 *N* hydrochloric acid are satisfactory), add 10 ml. of 0.1 per cent bipyridyl solution or 5 ml. of 0.1 per cent terpyridyl solution, dilute to the mark, and mix well. The color develops immediately and may be measured or compared by any of the usual methods. (Iron-bipyridyl standards for visual comparison may be kept in glass-stoppered bottles for at least 1 year. Terpyridyl standards are valid for at least 3 months.) For photometric measurement, a green filter with maximum transmission between 480 and 540 mμ is recommended for bipyridyl. With terpyridyl a filter with maximum transmission in the vicinity of 550 is desirable.



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Determination of Copper with 8-Quinolinecarboxylic Acid

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ANTHRANILIC acid has been widely used as a convenient analytical reagent for the determination of many metallic ions (*1, 2, 3, 7*), but is not adaptable to separations. According to the classification suggested by Haendler and Geyer (*4*), 8-quinolinecarboxylic acid has the same potential chelating group (C, C, C—N, OH) as has anthranilic acid and should therefore exhibit approximately the same behavior towards metallic ions. It was with the intention of investigating this behavior, with its attendant analytical possibilities, that the following study of 8-quinolinecarboxylic acid was made.

8-Quinolinecarboxylic acid gives a copper salt, first reported by Skraup (*9*), which, when dried at 120° C., contains no water and has the composition $(C_{10}H_6NO_2)_2Cu$. Experimental work indicated that when weighed in this form, copper could be quickly and satisfactorily determined when present alone, or with zinc or cadmium.

Solutions

8-Quinolinecarboxylic Acid was prepared according to the method of Skraup (*9*). It was recrystallized at least three times from water, the purified sample melting at 187° C. A saturated water solution of the acid was prepared; 60 ml. of the solution contained about 0.4 gram of acid. The acid was also recovered from the copper precipitates by dissolving these in hydrochloric acid, removing the copper with hydrogen sulfide, and concentrating the filtrate until the 8-carboxyquinolinium chloride crystallized. The acid was obtained upon neutralizing a water solution of the chloride.

Standard Copper Solutions containing approximately 2 grams of copper per liter were made up volumetrically by dissolving electrolytic copper foil (99.97 per cent pure) in a minimum amount of nitric acid and diluting to volume. The concentrations were checked by precipitation of the copper as anthranilate (*6*). The concentrations of these solutions were known to 0.02 mg.

Cadmium Acetate and Zinc Acetate Solutions containing 25 mg. of metal ion in 10 ml. of solution were prepared from the corresponding C. P. chemicals.

Table I. Determination of Copper with 8-Quinolinecarboxylic Acid

Copper Taken	Copper Found	Error
Mg.	*Mg.*	*Mg.*
50.16	50.04	−0.12
50.16	50.20	+0.04
50.16	50.25	+0.09
50.16	50.12	−0.04
50.16	50.25	+0.09
Accuracy, 0.15%		
Precision, 0.14%		

Determination of Copper

Copper was found to be quantitatively precipitated in neutral or weakly acid solution, as shown in Table I. The entire procedure was very simple and required only 30 minutes of actual work.

Procedure. To a solution containing about 50 mg. of copper in 100 ml. of solution add a slight excess of ammonium hydroxide and just remove the blue of the complex ion with 3 *N* acetic acid, following with 5 drops in excess. Heat the solution to boiling and add 60 ml. of acid reagent (0.4 gram for 50 mg. of copper) over a period of 1 minute with stirring. Cool for 2 hours, filter the precipitate on a fine sintered-glass crucible, wash with a little 1 per cent reagent solution and a few milliliters of water, and dry at 110° to 120° C. to constant weight. Factor, 0.15585; log factor, 9.19271 −10.

Table II. Influence of pH on Separation of Copper and Cadmium

Filtrate pH	Copper Taken	Cadmium Taken	Copper Found	Error
	Mg.	*Mg.*	*Mg.*	*Mg.*
4.8	62.30	20	62.45	+0.15
4.7	51.10	20	51.38	+0.28
4.4	49.73	25	49.93	+0.20
4.4	49.77	25	49.98	+0.21
4.1	50.15	25	50.24	+0.09
4.0	49.55	25	49.58	+0.03
3.9	49.05	25	48.97	−0.08
3.7	49.77	25	49.72	−0.05
3.5	49.77	25	49.81	+0.04
2.9	49.77	25	49.56	−0.21
2.8	49.77	25	48.19	−1.58

Determination of Copper in Presence of Cadmium

The usual procedure (*10*) for the separation of copper and cadmium involves the use of a masking agent for the copper, cadmium being removed by precipitation as the sulfide, leaving the copper free to be determined by any standard method. Several organic reagents have been used for determining copper in the presence of cadmium; quinaldinic acid gives satisfactory results in acid solutions of carefully regulated pH (*5–8*). In attempting to determine the conditions under which copper might be quantitatively separated from cadmium by 8-quinolinecarboxylic acid, the influence of changing acid concentration was studied. It was found that complete precipitation depended on pH, as can be seen from Table II, the pH being determined by a color comparator. The best results were obtained in the pH range of 3.5 to 4.0 in the filtrate, and a procedure using these conditions was developed accordingly.

Procedure. For these determinations 25 ml. of the standard copper nitrate solution and the proper amount of cadmium acetate solution were diluted with 150 ml. of water. In ordinary analyses, the solution is prepared as usual and should have a volume of 150 ml. for each 0.05 gram of copper present. To the solution thus prepared, ammonium hydroxide is added to incipient cadmium hydroxide precipitation. The solution is then made just acid to litmus with 0.1 *N* acetic acid and heated to boiling, and 50 ml. of the reagent solution are added. After cooling at least 2 hours, the precipitate is filtered on a No. 4 sintered-glass crucible with the aid of about 30 ml. of 1 per cent reagent, followed by washing a few times with water to remove traces of acid before drying at 110° to 120° C. to constant weight.

When carried out in this manner, copper may be determined with considerable accuracy even in the presence of as much as four times its weight of cadmium, as can be seen from Table III.

Determination of Copper in Presence of Zinc

Copper may also be precipitated quantitatively in the presence of zinc in exactly the same manner and under the same conditions of pH (Table IV).

Interfering Ions

Lead, nickel, mercury, and cobalt ions do not appear to interfere under the above conditions; precipitates form in

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slightly ammoniacal solution. Silver and gold form extremely insoluble precipitates under the given conditions and will interfere if present.

Table III. Determination of Copper in the Presence of Cadmium in Solutions of pH 3.5 to 4.0

Filtrate pH	Copper Taken	Cadmium Taken	Copper Found	Error
	Mg.	*Mg.*	*Mg.*	*Mg.*
3.8	50.16	25	50.28	+0.12
3.7	50.16	25	50.18	+0.02
3.6	50.16	25	50.08	−0.08
4.0	50.16	50	50.28	+0.12
3.6	50.16	75	50.20	+0.04
3.9	50.16	100	50.28	+0.12

Accuracy, 0.16%
Precision, 0.12%

Summary

8-Quinolinecarboxylic acid was found to be suitable as an analytical reagent for the determination of copper in dilute acetic acid solution. The method described has an accuracy of about 0.1 per cent. Copper can also be determined rapidly in the presence of an appreciable amount of cadmium or zinc with an accuracy of about 0.15 per cent. An entire determination involves only about 30 man-minutes.

Table IV. Determination of Copper in Presence of Zinc

Filtrate pH	Copper Taken	Zinc Taken	Copper Found	Error
	Mg.	*Mg.*	*Mg.*	*Mg.*
4.0	50.16	25	50.31	+0.15
3.7	50.16	25	50.22	+0.06
4.0	50.16	25	50.25	+0.09

The results obtained by the above described methods are as satisfactory as the other suggested organic methods, especially those involving quinaldinic acid. 8-Quinolinecarboxylic acid should be less expensive to prepare than is quinaldinic acid.

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Polarographic Determination of Lead in Lead-Bearing Steels

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A GREAT number of rapid methods for the analysis of steels have been brought into use in recent years. Most have such accuracy and simplicity that they have found immediate application not only in steel works laboratories but in research laboratories also. Such methods must be time-saving and capable of being carried out by the average analytical chemist without much previous experience. Colorimetric and potentiometric methods fulfill these conditions; hence their wide application at the present time.

Of the more common elements found in steels, lead is the only one for which no rapid method of analysis has so far been developed. Its usual estimation as lead sulfate or molybdate (*2–5*) is tedious and takes at least 4 hours. Such a procedure is therefore not suitable for checking the composition of steels during manufacture. As lead is not very readily introduced into steels, and burns out quickly, there are good reasons to look for a more suitable method for its rapid estimation.

Tests carried out on the polarograph show the eminent suitability of this instrument for this purpose.

The use of the dropping mercury electrode for estimating lead has already been the subject of many investigations. Terui (*6*) and Abraham and Huffman (*1*) used it in the analysis of lead in zinc and paints.

The steel is dissolved in hydrochloric acid and nitrogen is passed through the solution vessel in order to avoid oxidation of ferrous iron. Gelatin is added as a maximum suppressor and the polarogram is taken. The large concentration of ferrous chloride acts as supporting electrolyte.

Reagents. Concentrated hydrochloric acid and 2 per cent gelatin solution.

Method. Dissolve 1.0 gram of millings in 10 ml. of hydrochloric acid in a 50-ml. measuring flask, in an atmosphere of nitrogen in order to keep iron in the ferrous state. Apply gentle heat to help dissolving. When this is complete add 2 ml. of gelatin solution, cool to room temperature, make up to the mark with boiled water, and polarograph from 0.1 to 0.7 volt. A standard graph can be established by using leaded steels of known lead content. If such lead-bearing steels are not available, artificial standards may be prepared by adding varying measured amounts of lead acetate solution to solutions of mild steels, dissolved as above, before making up to 50 ml. A solution containing 0.001 gram of lead per milliliter is most suitable for this purpose. By addition of quantities from 1 to 3 ml. standards covering the normal range of lead-bearing steels may be obtained.

The lead step occurs at about 0.45 volt.

The time required, including weighing, is from 20 to 25 minutes. By the use of an automatic recording potentiometer, this could be shortened still more.

Tolerance. Tolerance is 0.01 per cent for lead contents between 0.1 and 0.4 per cent. (An attempt was also made to determine lead in lead-bearing 18–8 stainless steels but the method was found to be unsuitable. Some modification would be necessary to make it applicable.)

Table I. Comparative Analyses

Sample	Gravimetric	Polarographic
	%	%
1	0.27	0.275
2	0.17	0.166
3	0.18	0.175
4	0.14	0.166
5	0.20	0.207
6	0.21	0.230
7	0.28	0.290

Acknowledgment

The authors wish to thank the Mond Nickel Company for the loan of its polarograph.

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Measurement of Thickness of Fired-on Gold Coatings

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A STRONG need for a practical method of measuring the local thickness of fired gold coatings on ceramic base materials has been felt for some time both in the laboratory and in commercial operations. The jet-test method here described was developed primarily for this purpose, but with only minor changes it should be applicable to electroplated and other types of gold coatings.

The oldest and best-known test for determining the local thickness of base metal coatings is the Preece test (*5*), which consists in the gradual removal of the coating by repeated immersion in a suitable stripping solution. Later investigators (*1*) showed that the rate of solution of zinc and cadmium coatings was more constant if the solution was applied to the coating in successive drops instead of all at once as it is in immersion. Hull and Strausser (*4*) used a fast dropping rate and found that the rate of solution of electroplated coatings then becomes nearly independent of the rate of dropping, making it possible to express thickness merely in terms of the time of dissolution of coating. In 1937, Clarke (*2*) described the jet test, consisting of a continuous stream of reagent from a small orifice impinging on the surface instead of successive drops. A stripping factor was calculated for each particular solution and the thickness of zinc and other base metal films was computed directly from the time required for penetration of coating and the respective stripping factors.

The major part of this investigation consisted of developing a suitable reagent for the jet test on gold coatings. Since the customary gold decoration is composed of gold metal plus a flux, it was necessary that the reagent independently attack both components of the coating. An acidified ammonium iodide–iodine solution, containing Gardinol as a wetting agent, met this requirement.

Test Apparatus and Reagents

APPARATUS. The jet-test apparatus consists essentially of a 250-ml. separatory funnel, the stem fitted with two glass stopcocks instead of the usual single cock. The lower end of the stem is cut to produce a plane face making an angle of 30° with the long axis of the funnel. A ringstand is used for supporting the separatory funnel and specimen and a large dish serves as a receiver for the dropping reagent. Figure 1 shows the assembled apparatus with specimen in correct test position.

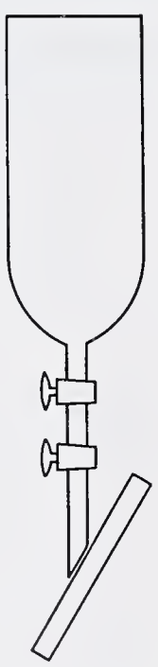

FIGURE 1. JET TEST APPARATUS

REAGENTS. There are two common types of gold decorations used on ceramics—the so-called "liquid bright" type, which is usually below 0.00025 mm. (0.00001 inch) in thickness, and the "burnish" or "coin" gold type, which is usually several times thicker than the liquid bright gold decoration. In order to have the time of dissolution of coating in both cases fall within a practical test period, two solutions designated as Reagent A and Reagent B were used. The latter reagent is much stronger than the former and is therefore recommended for determining the thickness of burnish gold decorations. The hydriodic acid contained in the reagents is relatively unstable, and for most accurate results the reagent should not be used after standing longer than 2 to 3 weeks.

Reagent A. This solution is recommended for thickness measurements of liquid bright gold decorations. Weigh 2.2 grams of iodine crystals (resublimed) and 5.5 grams of ammonium iodide crystals into a 1-liter glass-stoppered bottle, add 100 ml. of distilled water to the iodide-iodine mixture, and shake until the solids are dissolved. Pour 900 ml. of distilled water into a large graduate and add 8 grams of Gardinol (du Pont, WA flakes). Stir until the Gardinol is dissolved and then add the Gardinol solution to the iodide-iodine solution. Add 31 ml. of hydrochloric acid (concentrated C. P., 1.19 sp. gr.), shake, and then filter the reagent. Store the reagent in a glass-stoppered bottle in a darkened cabinet.

Preparation of Reagent B. This solution is recommended for tests on the heavier type gold decorations. It is prepared like Reagent A, using the amounts shown in Table I.

TABLE I. REAGENT B

Iodine (resublimed), grams	11.1
Ammonium iodide, grams	27.8
Distilled water, ml.	1000
Gardinol (WA flakes), grams	8.5
Hydrochloric acid (1.19 sp. gr.), ml.	37

Standardization of Reagents

The ammonium iodide–iodine–hydrochloric acid reagents used in this work were standardized by two independent methods.

1. A jet test was made on a gold decoration of known area, the remaining gold coating was dissolved, and the weight of gold was determined by a standard colorimetric test (*3*). The stripping factor of a particular reagent was calculated from the time of the jet test, the weight of gold, and the area of coating.
2. A large porcelain crucible was coated on the outside with gold. The stripping factor was again calculated from the time of the jet test, the known area covered, and the weight of gold as determined by weighing the crucible on an analytical balance.

STRIPPING FACTORS. The thickness of gold decoration may be calculated directly by taking the product of the time of jet test and the following factors:

Reagent A. One minute equivalent to 4.1×10^{-6} cm.
Reagent B. One minute equivalent to 24×10^{-6} cm.

The weight of gold metal per square centimeter may be calculated from the time of dissolution and the following factors:

Reagent A. One minute equivalent to 7.9×10^{-5} gram per sq. cm.
Reagent B. One minute equivalent to 5×10^{-4} gram per sq. cm.

Recommended Procedure

Select a gold band over 4 mm. in width, which has been given the proper firing cycle, and determine local thickness of gold band by the use of the jet test in the following manner:

Fill the special separatory funnel with the proper stripping reagent at a temperature of about 25° C. Open both stopcocks and adjust lower stopcock so that solution discharges at the rate of 7 ml. (± 0.2 ml.) per minute.

Clamp specimen firmly in position, with the surface of test spot parallel to face of delivery tip and with a slight clearance between the two surfaces. The surface of test area should then make an angle of 60° with the horizontal. In order to produce streamline flow, the reagent should be allowed to flow a distance of about 1 cm. over surface of specimen before reaching the particular spot whose thickness is to be determined.

Degrease the surface of test area by use of organic solvents or by rubbing lightly with a piece of cotton containing a dilute solution of Gardinol.

Open the upper stopcock and note the number of minutes required for removal of decoration in the flow area—i. e., appearance of the base glaze or glass surface.

Calculate the gold thickness from the stripping factors given above and the experimentally determined times of dissolution of coating.

Practical Application

The method has been applied for practical control work with entirely satisfactory results. About 3 minutes is required for each thickness test. It has also been used by certain potteries for estimating the costs of different type of gold decorations. Some tests have been made on the substitution of Reagent B for hydrofluoric acid in removing undesired gold spots from decorated ware.

Summary

A jet-test method for measuring the thickness of liquid bright and burnish gold coatings on ceramics and two independent standardization methods for determining the stripping factors of the ammonium iodide–iodine–hydrochloric acid–Gardinol reagents have been developed. The stripping factors given apply to tests made on gold bands over 4 mm. in width, at a temperature of 25° C., and for gold coatings containing no interfering substances—e. g., platinum and silver. The method would be applicable to thickness measurements of ceramic coatings of gold alloys, but a stripping factor for each particular alloy would have to be ascertained. The method is rapid and with reasonable care should be accurate to ±6 per cent.

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Spectrophotometric Determination of Iron

With *o*-Phenanthroline and with Nitro-*o*-phenanthroline

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IN RECENT years various spectrophotometric methods of analysis have been developed, making use either of a reference curve correlating transmittancy at a given wave length with concentration or of the extinction coefficient of the color system at a given wave length. The senior author has determined manganese as permanganate (*4*) by the former procedure and copper with ammonia (*5*) and iron with salicylic acid (*6*) by the latter.

The orange complex formed by the combination of three molecules of *o*-phenanthroline with one ion of ferrous iron, first reported by Blau (*1*), has been used for a number of years as an internal indicator in oxidimetric titrations. A more recent development has been the use of the cherry-red nitro-*o*-phenanthroline ferrous complex for the same purpose. Saywell and Cunningham (*7*) have applied *o*-phenanthroline to the visual colorimetric determination of small concentrations of iron in various food products and Hummel and Willard (*3*) have used it for the colorimetric determination of iron in biological materials. Fortune and Mellon (*2*) have made a critical spectrophotometric study of the Saywell-Cunningham method with special attention to the effect of diverse ions and hydrogen-ion concentration and to the stability of the color system.

The purpose of the work described in this paper was to apply both *o*-phenanthroline and nitro-*o*-phenanthroline to the spectrophotometric determination of iron in ores, making use of the fundamental Lambert-Beer equation

$$I = I_0 \times 10^{-klc}$$

in which I_0 represents the intensity of the light of a given wave length entering the system, I the intensity of the light transmitted by the system, l the length in centimeters of the solution through which the light passes, c the concentration in grams per liter of the substance absorbing the light, and k the specific extinction coefficient, a constant which is a measure of the absorption due to a single molecule.

Apparatus and Solutions

All spectrophotometric measurements were made with a Cenco-Sheard spectrophotelometer.

o-Phenanthroline. A 0.1 per cent solution of the monohydrate prepared by dissolving in water warmed to 80°.

Nitro-*o*-phenanthroline. A 0.1 per cent solution in 95 per cent ethanol.

Hydroxylamine Hydrochloride. An aqueous solution containing 10 grams per 100 ml.

Stannous Chloride. A 0.25 *M* solution was prepared by dissolving 56.49 grams of the dihydrate in 350 ml. of 6 *M* hydrochloric acid and diluting to 1000 ml. with water.

Standard Iron Solution. A standard solution was prepared by dissolving 0.7029 gram of 99.9 per cent pure ferrous ammonium sulfate hexahydrate in water and diluting to 100 ml. Ten milliliters of this solution were made up to 1000 ml. with distilled water slightly acid with hydrochloric acid. Each milliliter of this final solution contained 0.01 mg. of iron.

Diverse Ion Solutions. Standard solutions, each milliliter containing 10 mg. of the ion in question, were prepared from the chloride or nitrate salt of the cations and from the sodium, potassium, or ammonium salts of the anions.

The Color Reaction

To produce the color system the amount of the standard solution of iron required to give the desired concentration of iron was measured into a 100-ml. volumetric flask and 1 ml. of hydroxylamine hydrochloride solution was added to reduce any iron which might have been oxidized by dissolved oxygen. The solution was diluted to approximately 75 ml., 10 ml. of *o*-phenanthroline or nitro-*o*-phenanthroline solution were added, and the volume was accurately made up to 100 ml. The solution containing nitro-*o*-phenanthroline was allowed to stand 2 hours for the full development of the color. All transmission measurements were made with a Cenco-Sheard spectrophotelometer by setting the wave-length scale, adjusting the incident light by means of a diaphragm to provide the desired displacement of the pointer on the photometer scale, and reading the transmission of the standard solution and of the blank solvent. The transmittancy was calculated by dividing the former by the latter. A 1-cm. cell was used throughout. The spectral transmission curves for the two systems are very similar with the peak of the absorption band in each case at about 508 mμ. Since they closely resemble the curves obtained for the *o*-phenanthroline–iron system by Fortune and Mellon (*2*) using the self-recording Purdue–General Electric spectrophotometer, they are not shown.

That Beer's law is followed by both color systems is proved by the straight line which resulted when the logarithms of the observed transmittancies at 505 mμ for five solutions containing from 0.5 to 4 p. p. m. of iron were plotted against the respective concentrations. This is in accord with the observations of Fortune and Mellon (*2*) for *o*-phenanthroline.

Determination of Specific Extinction Coefficients

Specific extinction coefficients were determined for both color systems at 490 and 505 mμ by obtaining the respective transmittancies at those wave lengths of a series of known iron

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Table I. Results Obtained with o-Phenanthroline

Sample No.	Iron by Dichromate Method	Iron Obtained from Transmittancy at 490 mμ	505 mμ	Average Iron Found	Deviation
	%	%	%	%	%
1	37.62	37.66	37.71	37.69	+0.07
2	36.84	36.90	36.86	36.88	+0.04
3	36.12	36.20	36.19	36.20	+0.08
4	35.11	35.10	35.08	35.09	−0.02
5	54.04	54.00	53.90	53.95	−0.09
6	50.68	50.55	50.51	50.53	−0.15
7	49.59	49.63	49.70	49.67	+0.08
8	52.83	52.79	52.93	52.86	+0.03

solutions as above. The average values calculated for the o-phenanthroline–iron system are: 490 mμ, 10,590; 505 mμ, 11,080; and for the nitro-o-phenanthroline–iron system: 490 mμ, 11,130; 505 mμ, 11,410.

Determination of Iron in Ores

Approximately 0.5 gram of iron ore was accurately weighed and transferred to a 250-ml. beaker. Twenty-five milliliters of concentrated hydrochloric acid were added, the beaker was covered with a watch glass, and the mixture was warmed on a hot plate until solution had been effected or only a white, siliceous residue remained. The solution was transferred to a 1000-ml. volumetric flask, filtering if necessary and thoroughly washing any residue, diluted to the mark, and thoroughly shaken. An aliquot of 10 ml. was measured into a 100-ml. volumetric flask, diluted to the mark, and well mixed. A 10-ml. aliquot of this solution was placed in a second 100-ml. volumetric flask and 1 ml. of hydroxylamine hydrochloride solution was added together with enough water to make the volume about 75 ml. Ten milliliters of the o-phenanthroline or nitro-o-phenanthroline solution were then added and the solution was diluted to the mark. Transmission measurements of this solution in a 1-cm. cell were made at 490 and 505 mμ. The wave length of maximum absorption, 508 mμ, was not used because the scale setting can be more accurately made at wave lengths ending in 0 or 5. Repeated readings on a single setting instead of readings at different settings are recommended for routine work. The transmittancy was obtained by multiplying the transmission by 100 and dividing by the transmission at the same wave length of a blank solution. The percentage of iron was calculated by use of the appropriate value for k, the specific extinction coefficient, as found above for the wave length in question.

The results obtained with o-phenanthroline for eight ores and with nitro-o-phenanthroline for thirteen ores are shown in Tables I and II, respectively, which also include for comparison the values given by the dichromate method.

Reducing Agent

In a search for a less expensive reductant than hydroxylamine hydrochloride Fortune and Mellon (*2*) found that sodium sulfite, sodium formate, and formaldehyde are unsatisfactory because they form complexes with ferric iron. While they did not try stannous chloride, they did determine that as much as 20 p. p. m. of stannous ion and 50 p. p. m. of stannic ion do not interfere with the color.

The authors made a series of determinations on ten iron ores with nitro-o-phenanthroline as above, substituting for the hydroxylamine hydrochloride 1 ml. more than enough 0.25 M stannous chloride solution to reduce all the iron. The deviation from the dichromate results was less than ±0.10 per cent in five cases, but ranged as much as ±0.40 per cent in the others. The average difference was ±0.13 per cent compared to ±0.07 per cent for the hydroxylamine procedure. Stannous chloride is not recommended as the reductant for the spectrophotometric method, but it could be used in the less precise visual colorimetric method.

Effect of Diverse Ions

In a comprehensive study of the effect of 55 diverse ions on the o-phenanthroline–iron color system Fortune and Mellon (*2*) found that very few interfere. To determine the effect on the nitro-o-phenanthroline–iron color system of certain ions commonly encountered, the authors measured the transmissions at 490 and 505 mμ of solutions of the system containing 2 p. p. m. of iron reduced by hydroxylamine hydrochloride and as much as 500 p. p. m. of the ion in question. When the difference between the transmission of the solution and that of the standard iron solution was less than 0.1 scale division, it was assumed that the added ion caused no interference. The following ions gave no interference: acetate, bromide, chloride, nitrate, sulfate, aluminum, ammonium, calcium, lead, magnesium, potassium, and sodium. Chromic and cupric ions produced a change in hue, but their interference is negligible below 10 p. p. m.

Table II. Results Obtained with Nitro-o-Phenanthroline

Sample No.	Iron by Dichromate Method	Iron Obtained from Transmittancy at 490 mμ	505 mμ	Average Iron Found	Deviation
	%	%	%	%	%
1	37.62	37.84	37.60	37.72	+0.10
2	36.84	36.68	36.87	36.78	−0.06
3	36.12	36.07	36.10	36.09	−0.03
4	35.11	35.05	35.03	35.04	−0.07
5	54.04	54.07	54.24	54.16	+0.12
6	50.68	50.71	50.95	50.83	+0.15
7	49.59	49.53	49.66	49.60	+0.01
8	52.83	52.83	52.90	52.87	+0.04
9	51.52	51.50	51.58	51.54	+0.02
10	52.20	52.18	52.13	52.16	−0.04
11	56.00	55.91	56.00	55.96	−0.04
12	34.45	34.56	34.57	34.57	+0.12
13	57.62	57.55	57.51	57.53	−0.09

Discussion

The spectrophotometric method for the determination of iron in ores by use of either o-phenanthroline or nitro-o-phenanthroline gives results which are well within ±0.20 per cent of those given by the dichromate titrimetric method and most of them are within ±0.10 per cent. Results may be duplicated on the same sample with a precision of ±0.10 to ±0.20 per cent. The method is most sensitive for concentrations of about 0.5 to 4 p. p. m. of iron when nitro-o-phenanthroline is used and of about 1 to 3 p. p. m. when o-phenanthroline is used.

The nitro reagent has the disadvantage of requiring about 2 hours for full development of the color. It is also more expensive than o-phenanthroline.

This method has the advantage over the dichromate and similar titrimetric methods of not requiring a standard solution.

Since the method can be used to determine iron in concentrations as low as 0.5 p. p. m. and since very few diverse ions interfere with the color, it can advantageously be adapted to the determination of iron in food and biological materials and in analytical reagents.

Two important advantages of this method over many colorimetric methods for iron are that the pH value need not be regulated closely (*2*) and the color formation occurs in acid solution, eliminating the difficulties usually caused by precipitation of metal hydroxides and hydrated oxides in alkaline solution.

The advantages of this method over the salicylic acid spectrophotometric method for iron (*6*) are its much wider pH range and greater freedom from interference by diverse ions. Both methods give equally satisfactory results.

Summary

A spectrophotometric method has been developed for the determination of iron in ores which depends upon reducing the iron with hydroxylamine and measuring the light transmittancy at 490 and 505 mμ of the colored solution produced by either o-phenanthroline or nitro-o-phenanthroline.

The results agree closely with those obtained by the dichromate titrimetric method.

The method is easily carried out and requires no longer, possibly little less, time than usual titrimetric methods.

Very few diverse ions interfere with the color and a wide range in pH values is possible.

An alternate procedure for reducing the iron with stannous chloride is not recommended.

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Detection and Semiquantitative Estimation of Group I Cations

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IN THE classical procedure for the qualitative analysis of Group I (silver, lead, mercury), the chlorides are precipitated and the lead chloride is extracted with hot water. The residue is then treated with aqueous ammonia to separate the silver from the mercury.

Two valid objections to this procedure are: The extraction of lead chloride is complete only after many washings and hence is necessarily time-consuming. Silver ion in the presence of comparatively large amounts of mercury sometimes escapes detection (in the hands of a beginner) when ammonia is added to separate the silver from the mercury. This is due to the reduction of silver ion to metallic silver by the mercury formed as a result of the reaction between mercurous chloride and ammonia. Small amounts of silver may thus escape detection due to their absence in the ammonia extract. Of course, the silver may be recovered later when the mercury residue is treated with aqua regia and then diluted. (The silver chloride separates out.)

In the authors' procedure these objections are overcome by extracting the lead chloride with an ammonium acetate solution containing acetic acid. Acetic acid is added to the ammonium acetate to reduce to a minimum the hydrolysis of the latter. This must be done to avoid loss of mercury, for the ammonia formed by hydrolysis interacts with the mercurous chloride and the resulting $HgNH_2Cl$ enters into the lead chloride extract. The residue is then treated with aqua regia and after subsequent dilution with water and centrifuging, the silver is separated as chloride from the mercury.

Experimental

In a 15-ml. centrifuge tube take the solution of the unknown (not more than 100 mg. of metal) and dilute to 10 ml. With the aid of a 1-ml. pipet add 1 ml. of concentrated hydrochloric acid. Stir for 2 minutes (to allow lead chloride to crystallize) and centrifuge for 3 minutes. The supernatant liquid now contains Groups II to V. Pour off and set aside.

To the residue in the tube add 5 ml. of L reagent (a solution 2.6 *M* in ammonium acetate and 0.8 *M* in acetic acid). Stir thoroughly for 0.5 minute and centrifuge until clear (not more than 3 minutes). Pour extract into another tube. Repeat with 5 ml. more of L reagent. Test for and estimate lead in the combined extracts by adding 2 ml. of potassium chromate. [A known number of milligrams of lead (say 20) are pipetted into a calibrated centrifuge tube (the blank or control) and the volume is made the same as in the unknown by using water and identical volumes (or weights) of reagents. When the potassium chromate is finally added simultaneously to both, the lead chromate which forms is allowed to settle under the influence of gravity (5 minutes). The number of milligrams of lead in the unknown is proportional to the volume of lead chromate in the blank. The procedure is similar for the silver (estimate as silver chloride) and the mercury (estimate as mercuric sulfide)].

Add 2 ml. of aqua regia to the residue in the centrifuge tube and boil down by inserting the tube in an upright position in a sand bath (to prevent bumping and subsequent loss of solution) until the aqua regia is destroyed (to about 1 ml.).

Dilute the mixture to 10 ml. and centrifuge until the supernatant liquid is clear (an exceedingly faint cloudiness may be ignored). The residue is silver chloride.

Pour off supernatant liquid equally into two tubes. To the first add stannous chloride reagent. A gray to black precipitate indicates the presence of mercury. Estimate it as mercuric sulfide (by passing in hydrogen sulfide) in the second tube only if a test has been received with the stannous chloride reagent.

Add concentrated ammonia with stirring to the final residue (in the second paragraph preceding) until it dissolves. Add dilute nitric acid until the solution is just acid. Dilute to 10 ml. and estimate silver as chloride.

A new method proposed for the detection and semiquantitative estimation of Group I cations is much less time-consuming than the classical one. It allows for the detection of about 100 mg. of metal as maximum with the lower limit of 0.2 mg. for silver and mercury, and 15 mg. of lead, each metal alone or in the presence of each other. This method may be used in a semimicro system of analysis. Time of analysis is less than 30 minutes.

Table I. Results of Detection and Semiquantitative Estimation of Group I Cations

(As performed by beginners in analysis at Brooklyn College)

Unknown[a]	Ions Present			Reported by Student			Reported by Student			Reported by Student		
	Ag^+	Pb^{++}	Hg^+	Ag^+	Pb^{++}	Hg^+	Ag^+	Pb^{++}	Hg^+	Ag^+	Pb^{++}	Hg^+
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
1	20	60	None	30	70	None	20	45	None	5	30	None
2	5	95	None	25	40	None	35	70	None	10	45	None
3	5	40	5	5	35	5	40	35	5	5	85	5
4	None	90	20	None	50	80	None	45	20	None	65	10
5	30	30	50	25	15	40	10	25	75	10	25	25
6	None	30	40	None	15	40	None	45	50	None	20	80
7	50	None	10	30	None	55	40	None	10	50	None	30
8	30	None	40	20	None	50	20	None	40	10	None	20
9	20	20	None	25	15	None	10	20	None	60	30	None
10	None	40	10	None	20	20	None	40	10	None	30	5
11	20	None	10	20	None	25	25	None	80	25	None	30
12	5	20	100	5	10	220	10	15	75	30	25	30
13	25	None	25	50	None	50	20	None	70	10	None	25
14	None	20	None	None	50	None	None	15	None	None	25	None

[a] Three different students for each unknown: 42 students.

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Refractometric Determination of Casein in Skim Milk

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CASEIN is the most important industrial by-product of milk. It is used in coating paper for fine halftone reproduction, on washable wall paper, and in water-soluble paints, glues, plastics, and wool-like fibers.

Skim milk from which casein is precipitated is usually purchased in tank car or tank truck lots from butter and cream plants. It has usually been bought at a flat price irrespective of the casein content, the only information as to the composition of the milk being that obtained from a not-too-sensitive hydrometer, less than one third of which value is attributable to casein. With the increase in the price of skim milk, a simple, accurate method for the quantitative determination of casein became of increasing importance.

Methods previously suggested or tried may be grouped, on the basis of the principles involved, as follows:

Precipitating, washing, drying, and weighing the casein.

Kjeldahl nitrogen determination. The nitrogen content before and after precipitation or in the washed casein precipitate is determined and converted to protein by using the factor 6.38 (*1*, *9*).

Volume of precipitate. The casein is precipitated with acid and the volume of the centrifugally packed precipitate determined (*4*).

Formol titration. Formol titration of milk times a factor is used to indicate the casein content (*3*, *10*, *11*, *13*, *14*, *15*, *21*, *23*).

Alkali-binding power of casein. Since casein possesses appreciable alkali-neutralizing power, titrations made before and after the precipitation and removal of casein are used to indicate casein content (*6*, *8*, *16*, *22*).

Difference in specific gravity before and after removal of casein (*2*, *7*, *12*).

Refractometric method. Casein is precipitated with acid, the precipitate is washed and dissolved in alkali, and the increased refraction of the solution is determined (*17*).

The refractometric method was selected because of the general satisfaction which refractometric methods have given in this type of determination, because of the high degree of accuracy which can be obtained on medium-sized samples, and because neither accurately standardized solutions nor accurate adjustment to a single specific temperature is necessary. This paper describes a technique which yields reliable results.

Experimental

Robertson (*17*) proposed a refractometric method for the determination of casein in milk, but apparently examined only one sample of milk. The authors applied his procedure to 32 samples of skim milk, using a dipping refractometer.

The casein in 50 ml. of milk was precipitated with 0.1 *N* acetic acid, and the casein was washed, filtered, and allowed to drain 30 minutes. The casein and paper were then treated with 100 ml. of 0.1 *N* sodium hydroxide to dissolve the casein. The difference between the refraction of the casein solution and 0.1 *N* sodium hydroxide, divided by a constant, gives the casein content of the milk. Robertson stated that the dilution of the solution by the water retained by the casein and paper caused an error which he did not consider great enough to be of significance.

The results which the authors obtained with Robertson's method were encouraging, but the water held by the casein and paper led to errors larger than should be tolerated in refractometric determinations. The increase in refractometer scale reading per unit of casein varied by 12 per cent in the different samples. Data obtained by the original Robertson method compared with the Association of Official Agricultural Chemists' method (*1*) involving precipitating the casein, washing, and determining the nitrogen in the precipitate are shown by the points marked *X* in Figure 1.

To serve as criteria of reliability in developing a method for milk, highly purified moisture-free casein (ash 0.016 per cent, *20*) was added in varying amounts to 0.1 *N* sodium hydroxide and the refractometer readings were made at 25° C. The values obtained are represented by the circles in Figure 1.

The nitrogen content of the purified casein, as determined by Kjeldahl method, ranged from 15.36 to 15.46 per cent, with an average value of 15.41 for 7 determinations. This value for nitrogen is slightly lower than the 15.67 usually given. Other preparations of purified casein also gave low values. Since the objective was to make the refractometric method agree with the A. O. A. C. method, the A. O. A. C. nitrogen factor of 6.38 was used in calculating casein.

The increase in refractive index resulting from the solution of 1 gram of casein in 100 ml. of 0.1 *N* sodium hydroxide was 0.00181, as shown in Table I. Robertson (*18*) used the value 0.00152, which he determined after extracting and drying some reagent casein (*19*). He gave no analytical data relative to the casein which he used. The value of 0.00152 is in fair agreement with the *X* values in Figure 1. The refraction of a number of samples of casein was determined and the values are presented in Table II. The refractive index of the purer caseins approaches the limiting value of 0.00181.

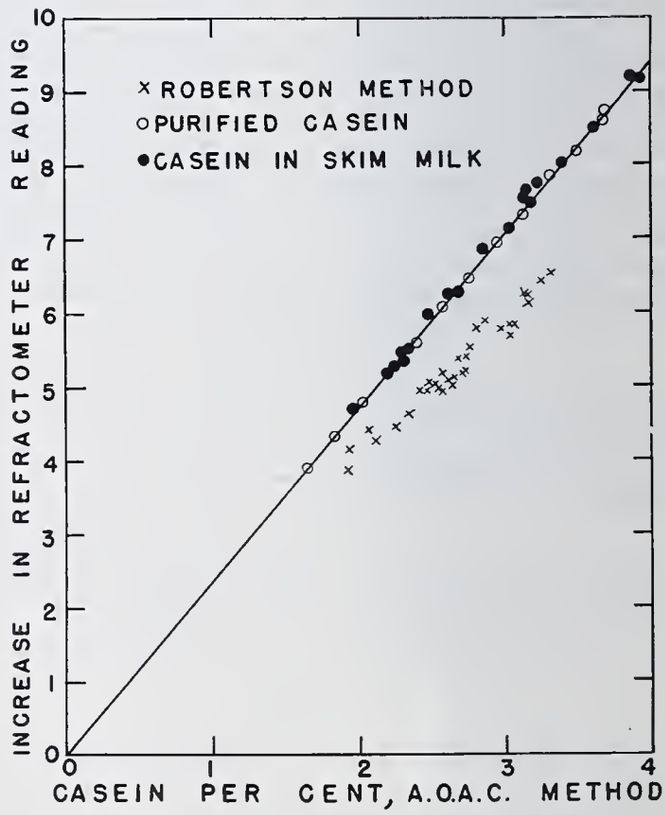

FIGURE 1. DETERMINATION OF CASEIN

Relation between increase in dipping refractometer scale reading of casein dissolved in 0.1 *N* sodium hydroxide and amount of casein as determined by method of Association of Official Agricultural Chemists.

TABLE I. INCREASE IN IMMERSION REFRACTOMETER READING

With varying amounts of purified moisture-free casein in 0.1 *N* sodium hydroxide solution)

Casein Made to 50 Ml. with 0.1 *N* NaOH	Casein Basis of 25-Gram Sample of Milk	Increase in Scale Reading Due to Casein at 25° C.	Increase in Refraction Produced by 1 Gram of Casein in 100 Ml. of NaOH	
			Immersion refractometer scale reading	Refractive index
Gram	%			
0.414	1.66	3.90	4.71	0.00182
0.461	1.84	4.30	4.66	0.00180
0.507	2.03	4.76	4.69	0.00181
0.553	2.21	5.16	4.67	0.00181
0.599	2.40	5.59	4.67	0.00181
0.645	2.58	6.05	4.69	0.00181
0.691	2.76	6.45	4.67	0.00181
0.736	2.94	6.93	4.71	0.00182
0.783	3.13	7.31	4.67	0.00181
0.829	3.32	7.85	4.73	0.00183
0.876	3.50	8.18	4.67	0.00181
0.921	3.68	8.68	4.71	0.00182
0.921	3.68	8.73	4.74	0.00183
			Av. 4.69	0.00181

TABLE II. INCREASE IN REFRACTIVE INDEX AT 25° C. PRODUCED BY DISSOLVING 1 GRAM OF CASEIN in 100 ML. OF 0.1 *N* SODIUM HYDROXIDE

Description of Sample	Moisture Content	Increase in Refractive Index	
		Uncorrected	Corrected for moisture
	%		
Ash-free	6.3	0.00168	0.00179
Grain curd 1	8.8	0.00166	0.00182
Grain curd 2	5.1	0.00170	0.00179
Crude, herd milk	8.5	0.00155	0.00169
Crude, Jersey milk	10.6	0.00153	0.00171
Crude, Guernsey milk	10.6	0.00151	0.00169
Crude, Holstein milk	8.1	0.00152	0.00166
Crude, commercial 1	6.6	0.00167	0.00179
Crude, commercial 3	6.7	0.00167	0.00179
Crude, commercial 5	7.2	0.00161	0.00174
Purified moisture- and ash-free, Table I			0.00181

The approximate increases in refractive index resulting from 1 per cent of the following substances dissolved in water were sodium chloride, 0.00177; potassium chloride, 0.00140; calcium chloride, 0.00203; disodium phosphate, 0.00244; lactose, 0.00148; and sucrose, 0.00149. These results indicate that, depending on the substances present as impurities, the refraction of solutions of crude casein might show a variation through a considerable range.

Method Applied to Skim Milk

The following procedure for obtaining casein from skim milk gave values which agreed well with those obtained with purified casein:

To 25 grams of skim milk (the authors calibrated a pipet to deliver this amount based on a specific gravity of 1.036) at 40° to 42° C. add with stirring 225 ml. of tap water at 40° to 42° C., containing 3.8 ml. of a solution of 10 per cent acetic acid. Let stand for 3 minutes and decant through a 12.5-cm. No. 4 Whatman filter paper. Wash the casein in the beaker twice by decantation with 75-ml. portions of water acidulated to pH 4.6 (0.2 ml. of glacial acetic acid per liter of water). Transfer the casein to the filter and then refilter the entire filtrate. When all the free liquid has passed through, transfer the casein and filter paper to a test tube 25 mm. in diameter and capable of containing a volume of about 70 ml. The tube should be marked accurately to contain 51.2 ml. Add 25 ml. of 0.2 *N* sodium hydroxide and mash the filter paper and casein with a rubber-tipped stirring rod, rinse the rod with distilled water, and make up to the volume of 51.2 ml. with distilled water. The filter paper occupies a volume of about 1.2 ml. and consequently the casein is dissolved and made up to a volume of 50 ml. of 0.1 *N* sodium hydroxide.

Care must be taken to work out all the air bubbles before adjusting to volume. Because the filter paper can be disintegrated and the air bubbles worked out more readily in a test tube than in a volumetric flask, a test tube was used for adjustment to final volume, in spite of the disadvantage of its large cross section at the meniscus.

After making up to volume, continue the disintegration of the filter paper with the glass rod. Stir occasionally during 30 minutes and filter through a No. 3 Whatman filter paper. Determine the refraction of the solution at approximately 25° C. with a dipping refractometer. Perform a control determination by adding only filter paper to the test tube and otherwise follow the procedure described. Subtract the scale reading obtained with the control solution from that obtained with the casein solution and divide the difference by 2.37 to obtain the percentage of casein in the milk.

This method has the very great advantage that if a control is run at the same time, the temperature at which both are determined may vary from 20° to 30° C. and the alkali added need be only approximately 0.2 *N*. Robertson (*18*) and others (*5*) have shown that the difference in refraction is relatively independent of these two variables. The authors have confirmed this observation.

The results obtained on applying this method to a series of different samples of skim milk are represented by the black dots in Figure 1. The method of least squares applied to the "ash-free" casein solutions gave Equation 1.

TABLE III. DETERMINATION OF CASEIN IN SKIM MILK

(Refractometric method applied to skim milk from individual cows, white light, 25° C.)

Sample No.	Breed	Difference in Scale Reading at 25° C.	Casein, A. O. A. C. Method	Casein Calculated from Refraction			
				Equation 1		Equation 2	
				Casein	Error	Casein	Error
			%	%	%	%	%
1	Holstein	4.70[a]	1.98	2.00	+0.02	1.98	0.00
2	Holstein	5.17	2.22	2.20	−0.02	2.18	−0.04
3	Holstein	5.25	2.24	2.23	−0.01	2.21	−0.03
4	Holstein	5.28	2.25	2.25	0.00	2.23	−0.02
5	Holstein	5.37[a]	2.29[a]	2.28	−0.01	2.26	−0.03
6	Holstein	5.48[a]	2.30	2.33	+0.03	2.31	+0.01
7	Holstein	5.55	2.37	2.36	−0.01	2.34	−0.03
8	Holstein	5.99	2.48	2.55	+0.07	2.52	+0.04
9	Holstein	6.30	2.62	2.68	+0.06	2.66	+0.04
10	Guernsey	6.26	2.69	2.66	−0.03	2.64	−0.05
11	Jersey	6.84[a]	2.84	2.91	+0.07	2.88	+0.04
12	Mixed	6.89	2.87	2.93	+0.06	2.90	+0.03
13	Ayrshire	7.12	3.03	3.03	0.00	3.00	−0.03
14	Ayrshire	7.54[a]	3.13[a]	3.21	+0.08	3.18	+0.05
15	Jersey	7.54	3.15	3.21	+0.06	3.19	+0.04
16	Jersey	7.58	3.16	3.23	+0.07	3.20	+0.04
17	Jersey	7.74	3.21	3.29	+0.08	3.26	+0.05
18	Jersey	8.03[a]	3.42[a]	3.42	0.00	3.39	−0.03
19	Jersey	8.63	3.67	3.67	0.00	3.64	−0.03
20	Jersey	9.24[a]	3.84[a]	3.93	+0.09	3.90	+0.06
21	Jersey	9.17	3.92	3.90	−0.02	3.87	−0.05

[a] Single determination.

Table IV. Summary of Deviations in Determination of Casein by Refractometric and A. O. A. C. Methods

Equation Used in Calculations	Deviations from A. O. A. C. Method, %	Number of Samples	% of Samples	Deviation Av. deviation	Deviation Av. algebraic
1	±0.07–0.09	6	29		
	±0.04–0.06	3	14	±0.037	+0.028
	±0.00–0.03	12	57		
2	±0.07–0.09	0	0		
	±0.04–0.06	11	52	±0.035	0.00
	±0.00–0.03	10	48		

$$\text{Casein } \% = \frac{\text{difference in scale reading} - 0.005}{2.348} \quad (1)$$

and to the solutions obtained from the casein precipitated from milk the equation:

$$\text{Casein } \% = \frac{\text{difference in scale reading} - 0.003}{2.371} \quad (2)$$

The intercepts are negligibly small and can be disregarded. The relation is linear. Equation 2 differs from Equation 1 by representing a line slightly steeper in slope. This would be expected because of traces of impurities still remaining in the casein. From the practical analytical standpoint, the extra effort required to lower the impurities further is not justified, since Figure 1 clearly indicates the close agreement between the results obtained with milk and with the pure casein.

Table III presents data from the milk of individual cows. Such milk is more variable than mixed milk and presents greater difficulties in analysis. This table gives the difference in refractometer scale reading, casein determined by the A. O. A. C. method, casein content as calculated from the refractometer reading by Equations 1 and 2, and deviations between the calculated and the casein by the A. O. A. C. method. The differences are small as can be expected, considering that slight errors are involved in both methods. The samples are arranged in order of increasing casein content and the deviations seem to be about equally distributed through the series. The agreement with Equation 2 is slightly better than with Equation 1 and this would be expected since Equation 2 was being applied to the data from which it was derived. The fact that the agreement between the equation derived from the purified casein and the casein from the milk is good indicates that the amount of contaminating material was small. The deviations summarized in Table IV show that with Equation 2 no error greater than 0.06 was encountered.

The warming prior to precipitation and the filtration procedure were developed only after numerous attempts to obtain clear filtrates of uniform nitrogen content and reproducible refraction of the solution on dissolving the precipitate. Experiments were made in which the original first filtrate was refiltered before adding the wash water and casein, and after the first, and after the second wash water had been filtered, as well as after the casein had been transferred to the filter. Results of a typical experiment are presented in Table V. The filtrates were clearer, more uniform, and lower in nitrogen and more of the small particles of casein were retained on the filter if the last-mentioned procedure was used. This type of filtration procedure is also employed in the A. O. A. C. method. Although refiltering earlier in the procedure may save time and may occasionally give fairly good results with some samples of milk, yet milk is encountered frequently where the shortened procedure gives turbid filtrates and leads to error. Therefore, it is recommended that filtration of all samples be carried out as described in the method.

Table V. Effect of Introducing Refiltration Step at Various Stages

(High-protein milk, total protein 4.76%)

Stage of Refiltering Step	Condition of Filtrate	Protein in Filtrate %	Total Protein Less Filtrate %	Difference in Scale Reading of Dissolved Precipitate	Difference ÷ 2.371 Refractometer Casein
After first filtration	Turbid	1.03	3.73	9.08	3.83
After first wash water	Turbid	1.01	3.75	9.04	3.81
After second wash water	Faintly turbid	0.95	3.81	9.25	3.90
After transfer of casein	Faintly turbid	0.96	3.80	9.24	3.89
A. O. A. C.	....	0.92	3.84	..	..

Summary

The casein in 25 grams of skim milk is precipitated with dilute acetic acid at 40° to 42° C., the precipitate is washed and dissolved in 0.1 *N* sodium hydroxide, and the refraction is compared with a 0.1 *N* sodium hydroxide solution containing no casein. The refractive index of the solution is increased by 0.00181 when 1 gram of purified casein is dissolved in 100 ml. of 0.1 *N* sodium hydroxide.

This method has the distinct advantage that if a control solution containing no casein is run for comparison, the concentration of alkali need be known only approximately and the temperature may range from about 20° to 30° C., provided that the unknown and the control solution contain the same amount of alkali and the refraction of both is determined at the same temperature.

The average deviation between results obtained with the refractometer procedure and the A. O. A. C. method was 0.04 per cent of casein, and the maximum error was 0.06 per cent when applied to 21 samples of skim milk of a wide range of composition.

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Analytical Reactions Involving Ignition with Manganous Nitrate

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IN ORDINARY analytical procedures, wet reactions are preferred to dry reactions for both practical and theoretical reasons. When wet reactions are lacking or are unsatisfactory, the analyst may have to resort to dry methods. Two such cases are reported below.

Ignition with Manganous Nitrate

Table I shows the principal products obtained when representative anions are ignited with manganous nitrate. Solutions of alkali salts of these anions were carefully neutralized with sodium carbonate and evaporated to dryness. The residues were moistened with concentrated manganous nitrate solution and heated to incipient red heat. After cooling, the black residues were extracted with warm water and the extracts were analyzed for the anions present. In most cases the effect of this ignition was oxidation. The halates, on the contrary, were catalytically reduced to halides by the manganese dioxide. In several cases the decomposition was not complete—e. g., bromide, cyanide, acetate, etc. The oxidation reaction generally occurs between 250° and 300° C., and is sometimes accompanied by a flash or puff, harmless for moderate amounts of the reactants.

The active oxidizing agent in these reactions seems to be manganese dioxide, formed by the decomposition of manganous nitrate. This is indicated, not only by the fact that pure manganese dioxide will give all the reactions noted, but by the fact that manganous nitrate is broken down into manganese dioxide and nitrogen dioxide below 200°, considerably below the reaction temperature. The same consideration precludes the assumption that the oxidation is due to nitrogen dioxide. The oxidizing action can hardly be attributed to the sodium nitrate formed by a reaction between manganous nitrate and sodium carbonate, since sodium nitrate alone shows no oxidation effects below 340°. Manganous nitrate seems more effective than manganese dioxide, probably because of the finer division of the oxidant and a more intimate mixture of reactants.

The proper condition for the oxidation reaction is a faint acidity. This is realized by adding sodium carbonate solution drop by drop as long as effervescence occurs. The slight excess of alkali is removed by the free acid present in the manganous nitrate solution. Excess of sodium carbonate must be avoided, since the oxidizing action of manganous nitrate is strongly diminished by the presence of alkalies. Besides, a faintly acid mixture is favorable to the oxidation of bromide and iodide.

Detection of Perchlorate

The precipitation of potassium perchlorate is not a sensitive test for the perchlorate ion, owing to the appreciable solubility of the salt. The sensitiveness can be increased by adding alcohol or other organic solvent, but the anions whose potassium salts are insoluble in alcohol are apt to interfere.

In 1909 Rothmund (*4*) proposed a test based on the reduction of perchlorate to chloride by reduced salts of titanium, vanadium, or molybdenum, or by zinc in the presence of salts of these metals. The reliability of this test has been investigated in this laboratory, and the reduction was found to be incomplete in every case. There always remained a small but appreciable amount of unreduced perchlorate.

The following dry test is based on the decomposition of perchlorate into chloride when it is heated in contact with manganese dioxide. Halides and other halates interfere and must be removed previously.

MATERIALS. The materials used in this test must be specially prepared, since the perchlorates and chlorates furnished by manufacturing chemists are not sufficiently pure. Ammonium perchlorate, free of chlorate and chloride, was prepared by neutralizing 70 per cent perchloric acid with ammonium hydroxide and recrystallizing the product. Pure potassium chlorate, free from perchlorate, was made by recrystallizing barium chlorate until free from perchlorate, and then adding a slight excess of potassium carbonate. The precipitated barium carbonate was removed by filtration and the solution was evaporated to crystallization. Owing to the presence of traces of chloride in the manganous nitrate of commerce, a chloride-free product was made by warming an excess of powdered metallic manganese with nitric acid.

PROCEDURE. The solution is acidified with dilute nitric acid, and a slight excess of silver nitrate is added to remove the chloride group of anions. The chlorate and perchlorate ions are left in the solution. A reducing agent is then added to reduce chlorate ions to chloride. A number of reagents were tried for this purpose, including sulfur dioxide, ferrous sulfate, and such metals as zinc, aluminum, etc. Of these, reduction by means of granulated zinc and dilute sulfuric acid on a water bath for half an hour was found most satisfactory. This reduces chlorate ions to chloride without affecting the perchlorate ion. Silver nitrate is again added, to remove the "chlorate chlorine". After removing zinc by sodium carbonate, a few drops of 50 per cent manganous nitrate solution are added, and the solution is evaporated and ignited to incipient redness. The chloride formed by the decomposition of the perchlorate may now be present largely, if not wholly, as silver chloride, and ammonium hydroxide is therefore used as the extractant. The solution is acidified with nitric acid and, if necessary, a little more silver nitrate is added to complete the precipitation. The third silver chloride precipitate represents the perchlorate ions present in the original mixture.

SENSITIVENESS. The decomposition of perchlorate in the presence of manganese dioxide is quantitative, so that the procedure admits of high accuracy (Table II). As little as 0.001 millimole (0.0995 mg.) of perchlorate can be detected, even in the presence of one thousand times that amount of chloride or chlorate, or both. The procedure is simple and fairly rapid. Its most serious defect is the slowness of the

TABLE I. IGNITION OF ANIONS IN THE PRESENCE OF MANGANOUS NITRATE

Anion	Products	Anion	Products
I^-	I_2	$S_2O_3^{--}$	SO_4^{--}
Br^-	Br_2 (incomplete)	NO_2^-	NO_3^-, oxides of N
Cl^-	Cl^-	NO_3^-	NO_3^-, oxides of N
CN^-	OCN^- (incomplete)	ClO_3^-	Cl^-
SCN^-	$SO_4^{--} + OCN^- (+ CN^-)$	ClO_4^-	Cl^-
$Fe(CN)_6^{----}$	$Fe(CN)_6^{---}$	$C_2O_4^{--}$	CO_3^{--}
S^{--}	SO_4^{--}	$C_2H_3O_2^-$	CO_3^{--} (incomplete)
SO_3^{--}	SO_4^{--}	AsO_3^{---}	AsO_4^{---}

TABLE II. DETECTION OF PERCHLORATE IN THE PRESENCE OF CHLORIDE AND CHLORATE

Composition of Material			AgCl Formed
NH_4ClO_4	KCl	$KClO_3$	
Millimoles			
0.001	...	...	Slight precipitate
0.001	0.1	...	Slight precipitate
0.001	1.0	...	Turbidity
0.001	...	0.1	Slight precipitate
0.001	...	1.0	Turbidity
0.001	1.0	1.0	Turbidity

Table III. Solvent Action of Concentrated Nitric Acid on Silver Thiocyanate and Silver Chloride

(Volhard procedure)

Materials Used KCl	Materials Used NH_4SCN	Weight of Residue Calcd.	Weight of Residue Experimental	Weight of Residue Difference
Millimole		*Gram*	*Gram*	*Gram*
0.0116	....	0.0166	0.0154	−0.0012
0.0116	0.01	0.0166	0.0151	−0.0015
0.0116	0.02	0.0166	0.0159	−0.0007
0.0116	0.03	0.0166	0.0165	−0.0001
0.0116	0.04	0.0166	0.0162	−0.0004
0.0116	0.05	0.0166	0.0172	+0.0006
0.0116	0.07	0.0166	0.0169	+0.0003
0.0116	0.10	0.0166	0.0178	+0.0012
....	0.01	0.0000	0.0002	+0.0002

reduction of chlorate to chloride, which may be incomplete in the hands of the impatient analyst.

Detection of Chloride in the Presence of Thiocyanate

Numerous procedures have been proposed for the detection of chloride ions in the presence of thiocyanate ions. A complete bibliography is too extensive for inclusion in this paper. Conflicting opinions have indicated that certain of these procedures should be reinvestigated.

Materials. Since traces of chloride are generally present in the ammonium and potassium thiocyanates furnished by manufacturing chemists, pure materials were prepared as follows:

Ammonium thiocyanate was prepared by the reaction between carbon disulfide and alcoholic ammonium hydroxide, using the method of Millon (*3*). Potassium thiocyanate of "analyzed reagent" grade was recrystallized twice, and the product was shown to be chloride-free by the test below. The trace of chloride in the cupric sulfate used to precipitate cuprous thiocyanate was removed by silver sulfate, and the silver ions thereby introduced were removed by warming the solution with copper turnings. It was surprising to find that the liquefied sulfur dioxide, which was used for reducing cupric compounds, contained considerable amounts of chloride. These were removed by distilling the saturated solution over silver sulfate.

Previous Methods. One of the best known procedures for the detection of chloride in the presence of thiocyanate is that of Volhard (*6*), based upon the assumption that concentrated nitric acid will dissolve silver thiocyanate, but will not affect silver chloride appreciably. Measured amounts of potassium chloride and ammonium thiocyanate were mixed with slight excesses of silver nitrate solution, and the mixtures were heated with 100 ml. of concentrated nitric acid on a hot plate just below the boiling point for 40 minutes. Results for one series of simultaneous runs are shown in Table III. These results indicate that there is always a loss when silver chloride is heated with concentrated nitric acid. If the mixtures are distilled, the distillates are found to contain considerable amounts of chloride. This loss by volatilization is reduced by heating below the boiling point, but still is appreciable. It would be reduced by using a reflux condenser. The presence of silver chloride in the digestion mixture seems to exert a protective action on the silver thiocyanate, as indicated by the gradual increase in the residue with increase in the thiocyanate content. The values are roughly reproducible, varying slightly with the length of digestion, temperature, exposure, etc. Effervescence, due to the escape of the oxides of nitrogen when thiocyanates are present, also favors the loss of chlorine.

Another procedure is based upon the separation of the thiocyanate as cuprous thiocyanate.

In the procedure proposed by Mann (*1*), the solution is mixed with cupric sulfate and the cupric thiocyanate is then reduced to cuprous thiocyanate by hydrogen sulfide. Hall (*5*) improved this procedure by using sulfur dioxide as the reducing agent. To determine the limitations of the method, solutions of thiocyanate and chloride were treated with 10 ml. of a 20 per cent solution of cupric sulfate and 10 ml. of a saturated solution of sulfur dioxide, making a total volume of 30 ml. After boiling for one minute, the mixtures were cooled and the precipitates separated.

Results (Table IV) show that the precipitation of the thiocyanate is not complete, and that enough of this ion is left in the filtrate to give a slight precipitate with silver nitrate solution. The limiting sensitiveness of the precipitation of cuprous thiocyanate is 0.001 millimole (0.058 mg.), in contrast with less than 0.0001 millimole (0.00355 mg.) when precipitated as silver thiocyanate. Most of the chloride is removed as cuprous chloride during the cuprous thiocyanate precipitation, leaving about 0.1 millimole in the filtrate. It follows that the precipitate obtained when the filtrate is treated with silver nitrate solution may consist of either silver thiocyanate or silver chloride, or both.

Another procedure (*2*), sometimes used by manufacturing chemists, is based on the direct oxidation of the thiocyanate by nitric acid. Mixtures of thiocyanate and chloride in a total volume of 100 ml. were mixed with 25 ml. of concentrated nitric acid and heated just below the boiling point for 3 hours. In all cases a loss of chloride occurred. In a blank test containing chloride only, a loss of 0.006 millimole (0.2130 mg.) of chloride was found. The losses are apparently greater with increasing amounts of thiocyanate.

New Procedure. The complete decomposition of the thiocyanate group upon ignition with manganous nitrate affords a rapid and reliable method for separating thiocyanate from chloride. If the medium is kept neutral by the addition of a drop of sodium carbonate solution, no chloride is lost during the ignition.

The solution is made slightly alkaline with sodium carbonate, and any precipitate is filtered out. The filtrate is evaporated almost to dryness in a small crucible, and the residue is moistened with a drop of 50 per cent manganous nitrate solution and heated to incipient red heat. If the residue after ignition is not black, more manganous nitrate is added and the ignition is repeated. The residue is extracted with dilute nitric acid and boiled to expel any hydrogen cyanide. The solution is then tested for chloride with silver nitrate in the regular way.

Table IV. Separation of Thiocyanate from Chloride as Cuprous Thiocyanate

(Mann's procedure)

Materials Used KSCN	Materials Used KCl	Reaction with $CuSO_4 + SO_2$	Reaction of $AgNO_3$ with Filtrate from Cuprous Ppt.
Millimoles			
0.1	...	Precipitate	Turbidity
0.01	...	Precipitate	Turbidity
0.001	...	Slight precipitate	Turbidity
0.0005	...	Slight precipitate	Turbidity
0.0002	...	Precipitate on standing	Turbidity
0.0001	...	No effect	Turbidity
...	1.0	Precipitate	Precipitate
...	0.5	Precipitate	Precipitate
...	0.3	Precipitate	Precipitate
...	0.2	Precipitate on standing	Precipitate
...	0.1	No effect	Precipitate

Several interferences with this test should be noted. Bromides are not completely oxidized upon ignition with manganous nitrate, and some approved procedure must be used to detect chlorine in their presence. When large amounts of thiocyanate are present, or when insufficient manganous nitrate is used, some sulfide will be formed. This will be eliminated during the boiling with dilute nitric acid. Chlorates and perchlorates are changed to chlorides during the ignition; hence they must be separated prior to heating with manganous nitrate. This separation is effected by precipitating the halide group of anions as silver salts, and

Table V. Detection of Chloride in the Presence of Thiocyanate

Composition of Material NH4SCN	KCl	AgCl Formed
Millimole		
0.1	...	No effect
0.1	0.1	Precipitate
0.01	0.01	Precipitate
0.01	0.001	Slight precipitate
0.1	0.001	Turbidity

reforming the halide ions by warming the precipitate with zinc and dilute sulfuric acid in the usual way. Some hydrogen sulfide and hydrocyanic acid may be formed owing to the reduction of thiocyanate by zinc, but this has no effect upon the test for chloride.

Sensitiveness. This test is very sensitive (Table V). As little as 0.001 millimole (0.0355 mg. or 35.5 micrograms) of chloride can be detected in this way, even in the presence of one hundred times that amount of thiocyanates. This sensitivity is superior to that of the older tests, and the procedure is comparable in speed and accuracy.

Summary

Many anions are oxidized when ignited with manganous nitrate in neutral medium. The actual oxidizing agent is manganese dioxide.

A very sensitive test for perchlorate is based upon the formation of chloride when heated in the presence of manganese dioxide. The test is better than the methods based upon the reduction of perchlorates by titanous solutions.

Certain procedures in common use for the detection of chloride in the presence of thiocyanate are not sensitive. A new procedure, based on the removal of the thiocyanate by oxidation with manganese dioxide, has been extended to include chlorate and perchlorate.

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Analysis of Plant Extracts for Chlorophylls *a* and *b*

Using a Commercial Spectrophotometer

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THERE has long been the need of a simple method for the determination of chlorophyll which could evaluate the ratios of the individual components and avoid the difficulties and uncertainties incurred by the use of solid chlorophyll standards (*5*, *7*, *8*). A simple, rapid, and accurate determination for chlorophylls *a* and *b* in green plant tissue has been reported by Comar and Zscheile (*2*, *3*), based upon the fundamental absorption spectra of highly purified chlorophyll *a* and chlorophyll *b* preparations (*8*). The original measurements and analyses were made with a photoelectric spectrophotometer similar to that described by Hogness, Zscheile, and Sidwell (*4*) but employing a large Müller-Hilger Universal double monochromator in the optical system. Errors due to the size of the spectral region isolated or to scattered radiation were negligible.

The utility of the method was limited due to the expensive optical apparatus required; for to the writer's knowledge there are only five installations of this type in the United States. The purpose of the present work, therefore, was to determine whether the reported absorption coefficients could be applied to yield accurate values using a more generally available instrument.

Instrumental

A Cenco-Sheard spectrophotelometer was used in this work. The instrument as described by Sheard and States (*6*) has an absorption cell carriage made to carry two 1-cm. cells of the open top type. It was not found practical to use this type of cell, mainly because of the rapid loss of diethyl ether during the measurements. However, it was possible to obtain from the manufacturer a 5-cm. cell carriage attachment which accommodated 1-, 2-, and 5-cm. cells with ground stoppers. Both the 1- and 2-cm. cells were used in the reported analyses, although the latter have been found more convenient. The light source was an incandescent lamp drawing 70 to 80 amperes at 4 to 5 volts.

In all measurements the 25 Å. exit slit was used and the entrance slit adjusted to 0.4 mm. According to Sheard and States (*6*) the total spectral region isolated would be 41 Å. with the major portion of the energy in the 25 Å. band.

For all measurements above 6500 Å. a red filter, which did not transmit below 6000 Å., was inserted directly before the entrance slit. For the measurements near 6600 Å. it was necessary to use the lamp bulb at its maximum current rating in order to secure satisfactory galvanometer deflections. This should not decrease the life of the lamp unduly, since each measurement usually required less than a minute.

The red absorption peak of the plant extract in diethyl ether solution, prepared as herein described, has always been found at 6600 Å. This is a convenient means of checking the wave length calibration of the instrument in this region; the same correction may usually be applied to the setting for 6425 Å. The calibration in regions of shorter wave length (around 5460 Å.) may be checked with a mercury arc either visually or photoelectrically.

The concentration of the solution to be used for the measurement in the red region should be adjusted to give a $\log_{10} \frac{I_0}{I}$ value of 0.5 to 0.8 at 6600 Å. In the normal plant extract, the same solution is also used for the 6425 Å. reading and yields a $\log_{10} \frac{I_0}{I}$ value which is in the accurate range. At other wave lengths the $\log_{10} \frac{I_0}{I}$ value should be restricted to the customary limits of 0.2 to 0.8 with the optimum value at about 0.4.

Spectroscopic Analysis

Figure 1 shows the absorption spectra of chlorophylls *a* and *b* in ether solution as determined by Zscheile and Comar (*8*). Beer's law is used in the form:

$$\alpha = \frac{\log_{10} \frac{I_0}{I}}{cl} \qquad (1)$$

Table I. Absorption Constants Employed in Analysis

Wave Length Å.	Specific Absorption Coefficients (Ether Solutions) Chlorophyll *a*	Chlorophyll *b*
6600	102	4.50
6425	16.3	57.5
6130	15.6	8.05
6000	9.95	9.95
5810	8.05	8.05
5680	7.11	7.11

where α = specific absorption coefficient (liters per gram centimeter)
c = concentration (grams per liter)
l = thickness of solution layer (centimeters)
I_0 = intensity of light transmitted by the solvent-filled cell
I = intensity of light transmitted by the solution-filled cell

In this system the concentration of each component may be obtained from absorption measurements at any two wave lengths, providing (1) that both wave lengths are not coincident points and (2) that the absorption values for the two components are not in the same proportion at the two wave lengths.

The following equations may be used, since the values of αcl for the individual components are additive:

$$\text{At wave length } \lambda' \ldots\ldots \log_{10}\frac{I_0}{I} = \alpha'_a c_a l + \alpha'_b c_b l \quad (2)$$

$$\text{At wave length } \lambda'' \ldots\ldots \log_{10}\frac{I_0}{I} = \alpha''_a c_a l + \alpha''_b c_b l \quad (3)$$

where $\log_{10}\frac{I_0}{I}$ = absorption measurement at the given wave length
α'_a and α'_b = specific absorption coefficients of chlorophylls *a* and *b*, respectively, at wave length λ'
α''_a and α''_b = specific absorption coefficients of chlorophylls *a* and *b*, respectively, at wave length λ''
c_a and c_b = concentration of chlorophylls *a* and *b*, respectively, in grams per liter

These two equations may be solved simultaneously to obtain values of the unknowns, c_a and c_b.

The total chlorophyll concentration can be obtained from the value of $\log_{10}\frac{I_0}{I}$ at any coincident point by direct substitution in Equation 2 or 3.

Table I presents the absorption coefficients of chlorophylls *a* and *b* in diethyl ether solution used in this work; the reasons for the choice of these particular wave lengths have been previously discussed (*3*).

For routine analysis, measurements need be made only at wave lengths 6600 and 6425 Å. In this case the following equations may be used to simplify the calculation; they are derived from Equations 2 and 3 by substitution of the appropriate absorption coefficients and setting l = 1 cm.

$$\text{Chlorophyll } a \text{ (mg./liter)} = 9.93 \log_{10}\frac{I_0}{I}_{(6600)} - 0.777 \log_{10}\frac{I_0}{I}_{(6425)} \quad (4)$$

$$\text{Chlorophyll } b \text{ (mg./liter)} = 17.6 \log_{10}\frac{I_0}{I}_{(6425)} - 2.81 \log_{10}\frac{I_0}{I}_{(6600)} \quad (5)$$

$$\text{Total chlorophyll (mg./liter)} = 7.12 \log_{10}\frac{I_0}{I}_{(6600)} + 16.8 \log_{10}\frac{I_0}{I}_{(6425)} \quad (6)$$

Although the standard absorption coefficients are expressed in the units liters per gram centimeter it is convenient to express chlorophyll concentration in mg. per liter.

When it is desired to check the plant extract or the instrument, values of total chlorophyll concentration are obtained from measurements at the intersection wave lengths 5680, 5810, and 6000 Å. A check on the percentage composition is obtained from a reading at 6130 Å. used in combination with a value of total concentration as determined from other wave lengths.

Preparation of Plant Extract

The plant extract is prepared essentially as discussed previously (*3*). A weighed sample (usually 2 to 10 grams) of fresh leaf tissue is disintegrated in a Waring Blendor in acetone to which a small amount of calcium carbonate has been added. (Commercial acetone was used without further purification.) If desired, very small samples may be used, satisfactory routine analyses having been made with as little as 0.15 gram of fresh leaf tissue. It was found convenient, especially for smaller samples, to use a Blendor cup made from a 250-ml. (8-ounce) bottle and which required about 100 ml. of solvent to cover the blades. This vessel was made available to the author through the courtesy of Benne (*1*). The macerate is filtered through paper by suction; the residue may be washed with ether if necessary to remove the last traces of pigment. (Merck's ether, labeled "ether purified, for fat extraction", was used without further purification.) The filtrate is made to volume and an aliquot is pipetted into a separatory funnel containing ether. Distilled water is added carefully and the fat-soluble pigments are transferred quantitatively into the ether layer. The ether is then washed exhaustively by scrubbing through distilled water fifteen to twenty times. This may be done conveniently by allowing the solution to flow by gravity from the separatory funnel through a tube, the lower tip of which is submerged below the surface of the water contained in a lower funnel (*5*). The diameter of the tip opening was about the size of a 25-ml. pipet tip. After each scrubbing the water layer is discarded, the funnels are interchanged, and the process is repeated. The solution is made to volume with ether, dried over anhydrous sodium sulfate, and when optically clear is ready for spectroscopic measurement.

Experimental Results

Agreement between analytical values obtained from various wave lengths is possible only if the following conditions are fulfilled: (1) The standard absorption coefficients must be identical with the coefficients of the components as measured in the unknown solution; this implies that the quality of

Table II. Analysis for Total Chlorophyll and Per Cent Chlorophyll *a*

Wave Length, Å.	Moline Elm	European Linden	Crimean Linden	Norway Maple	European Basswood	Lilac	Sunflower	Tomato	Corn Seedling	Barley Seedling	Alfalfa	String Bean
					Total chlorophyll, mg. per gram of fresh leaf							
6600 } 6425 }	2.07	3.12	2.57	4.07	1.77	1.88	1.65	1.57	1.08	1.89	2.45	1.42
6000	2.03	3.07	2.52	4.04	1.80	1.87	1.62	1.53	1.05	1.79	2.37	1.39
5810	2.16	3.21	2.64	4.15	1.85	1.89	1.72	1.63	1.11	1.93	2.51	1.49
5680	2.11	3.16	2.58	4.15	1.83	1.91	1.69	1.58	1.06	1.83	2.47	1.45
					Per cent of chlorophyll *a*							
6600 } 6425 }	73.2	71.9	70.6	69.3	71.4	72.3	73.7	74.3	73.7	70.6	71.4	73.6
6130	72.0	72.8	69.4	67.8	71.3	71.7	71.6	71.6	75.0	69.4	71.9	71.0

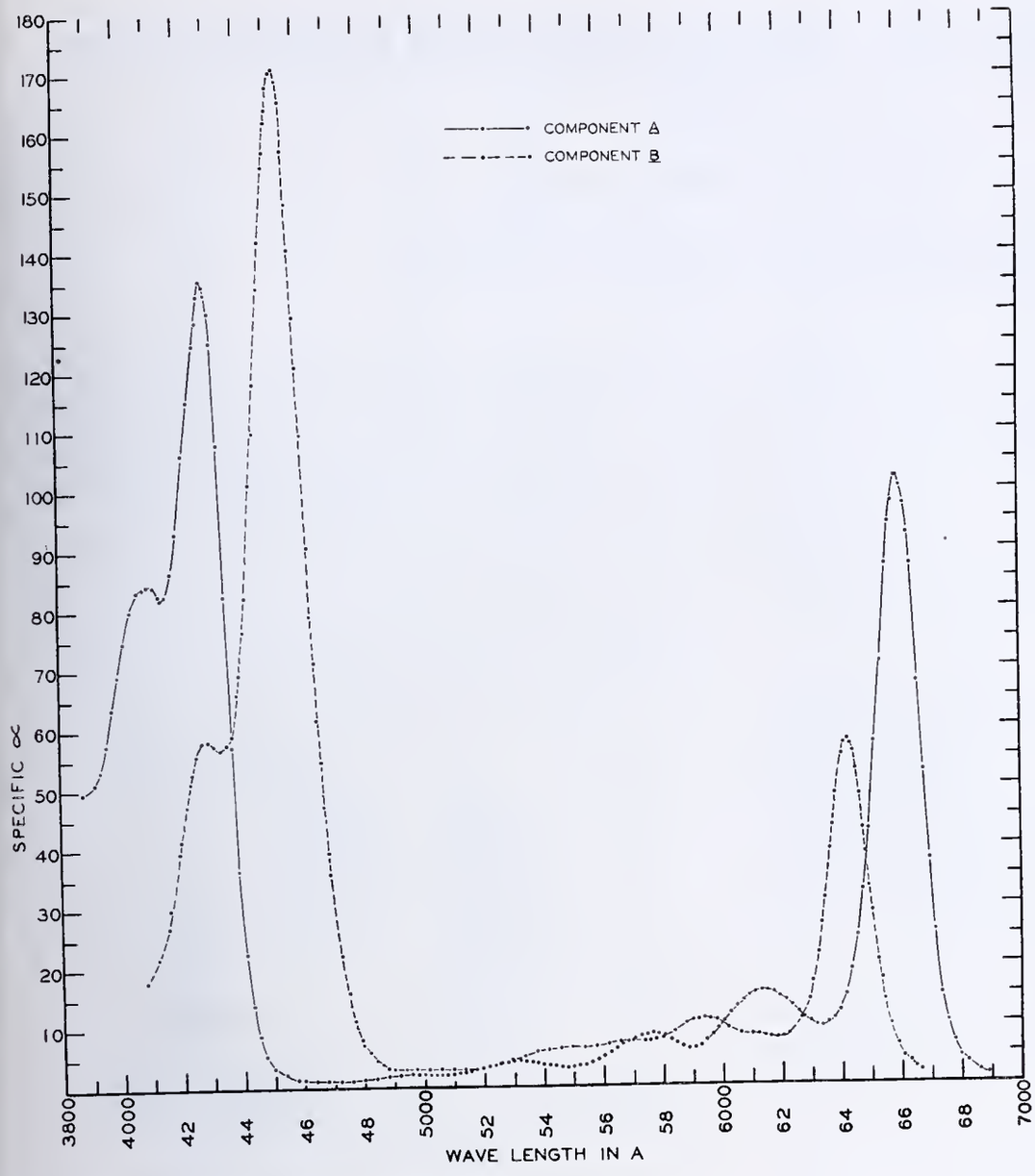

FIGURE 1. ABSORPTION SPECTRA OF CHLOROPHYLLS *a* AND *b* IN ETHER SOLUTION
From Zscheile and Comar (*8*)

It seemed desirable to confirm these results by a direct comparison of results obtained with the spectrophotometer at Purdue University, with which the original measurements were made, and the results obtained with the instrument used in this work. A tomato leaf extract was prepared in the usual manner. Aliquots of the two final solutions (one adjusted to proper dilution for measurements in the region 5680 to 6130 Å. and the other for readings at 6425 and 6600 Å.) were prepared. Measurements were made at this laboratory and 2 days later with the instrument at Purdue. The ether solutions were kept cool and in the dark during the period between the measurements. The results are presented in Table III and the agreement is considered satisfactory.

TABLE III. INTERLABORATORY COMPARISON, CHLOROPHYLL ANALYSIS

Wave Length, Å.	Purdue Instrument	This Laboratory
Total chlorophyll, mg. per liter in measured solution		
6600, 6425	7.84	7.78
6000	58.2	54.8
5810	58.3	59.5
5680	56.7	55.8
Per cent chlorophyll *a*		
6600, 6425	72.5	71.9
6130	69.2	70.3

radiation employed must be comparable to that used in the determination of the standard values. (2) There must be present in the solution no substance other than chlorophyll which absorbs light at the wave lengths employed; this means that satisfactory results would not be obtained if chlorophyll decomposition products were present in the leaf tissue or were formed during the preparation of the extract.

Table II presents analytical values for leaves of various plants as calculated from the different wave lengths. No serious discrepancies are found. For the plants reported, then, one would be justified in using wave lengths 6600 and 6425 Å. for routine analysis of fresh green tissue.

Summary

Absorption coefficients for the chlorophyll components as determined on a photoelectric spectrophotometer of high accuracy and purity of radiation have been successfully applied to the analysis of plant extracts for chlorophylls *a* and *b*, using a commercially available instrument.

Acknowledgment

The writer wishes to express appreciation to F. P. Zscheile for the privilege of using the Purdue spectrophotometer and to L. F. Green for making the measurements on this instrument.

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Determination of Unsaturates in Hydrocarbon Gases

The Hydrogenation Method

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MOST methods currently used to determine the concentration of unsaturated compounds in gaseous hydrocarbon mixtures were introduced years ago. The procedures have been modified occasionally to increase accuracy, but some methods give results which are inherently inaccurate and are retained either because of convenience or because errors have not been recognized or investigated. Recent improvements in older refining processes and the introduction of new processes, such as polymerization, alkylation, and the production of synthetic rubber raw materials, which involve olefinic compounds, have demanded higher accuracy in the analysis for these substances.

A method for determining unsaturates in gaseous hydrocarbons by catalytic hydrogenation has been described by McMillan, Cole, and Ritchie (*5*). It is claimed to give results accurate within a few tenths of 1 per cent and to be as rapid as others in general use at present. The method is apparently satisfactory from the chemical standpoint: the hydrogenation is complete, free of side reactions, and sufficiently rapid for routine use, and the catalyst is easily saturated with gas and produces no interfering effects. However, the method, as originally described by McMillan, Cole and Ritchie, is based on the assumption that the lower hydrocarbons are ideal gases. Since these compounds are known to deviate considerably from the ideal gas laws, the nature of the errors introduced by the assumption was studied.

In order to formulate a convenient procedure for correcting the analytical results for such deviations, it is necessary to consider the nature and sequence of the gas volume measurements made during an analysis. These are:

Volumes of hydrogen and hydrocarbon gas are measured separately, the hydrogen being in excess.

The hydrogen and hydrocarbon sample are mixed and reacted over the catalyst, and the volume of the resulting gas, which consists of saturated hydrocarbons and excess hydrogen, is measured.

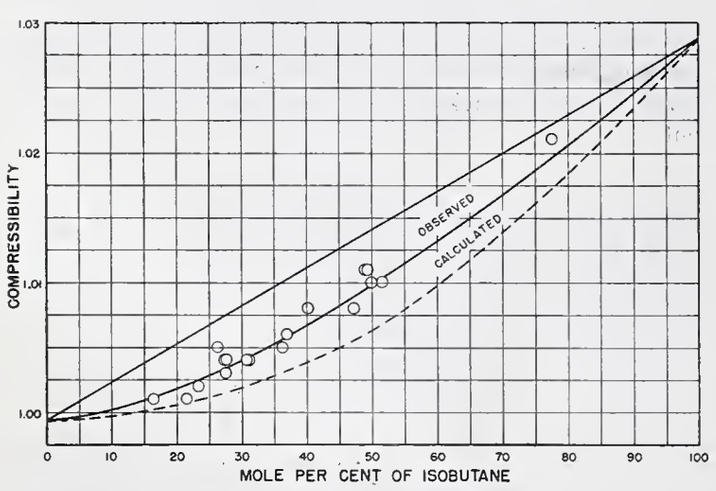

FIGURE 1. COMPRESSIBILITY OF MIXTURES OF ISOBUTANE AND HYDROGEN

Hydrogen conforms to the ideal gas laws within the errors of the analytical measurements and hence requires no correction. However, corrections must be made for deviation of the hydrocarbon sample from ideal gas behavior and deviation of the mixture of saturated hydrocarbons and excess hydrogen resulting from the hydrogenation reaction.

The extent of deviation of the measured volume of a pure gas from the ideal gas volume is conveniently expressed by the compressibility of the gas under the conditions of measurement. The compressibilities of hydrogen and a number of hydrocarbons at 760-mm. pressure are presented in Table I.

TABLE I. COMPRESSIBILITIES OF SOME GASES[a]

Fraction	Gas	Compressibility at 760 Mm. 0° C.	30° C.[b]
	Ideal gas	1.0000	1.0000
	Hydrogen	0.9994 (*2*)	...
	Methane	1.0024 (*2*)	...
C_2	Ethylene	1.0078 (*2*)	1.0081 (*6*)
	Ethane	1.010 (*1*)	...
	Acetylene	1.010 (*2*)	...
C_3	Propylene	1.0204 (*1*)	1.0151 (*6*)
	Propane	1.0207 (*3*)	1.0158 (*3*)
C_4	Isobutane	...	1.0290 (*3*)
	Isobutene	...	1.0288 (*6*)
	1-Butene	...	1.0287 (*6*)
	n-Butane	...	1.0328 (*3*)
	trans-2-butene	...	1.0319 (*6*)
	cis-2-butene	...	1.0324 (*6*)
C_5	Pentane	...	1.0418 (500 mm.) (*3*)

[a] Where compressibility equals: $\frac{pv \text{ extrapolated to zero pressure}}{p_S \text{ at atmospheric pressure}}$.

[b] Data of (*6*) have been obtained from the second virial coefficient, θ, by the approximation

$$\text{Compressibility} = [1 + (2.36 \times 10^{-6}\,\theta)]^{-1}$$

In general, however, the analytical procedure must be applied to hydrocarbon samples which are mixtures of individual hydrocarbons. The compressibility of a hydrocarbon mixture depends upon the compressibilities and relative amounts of the individual components. It is common practice, however, to distill such mixtures into fractions, each containing hydrocarbons of the same number of carbon atoms, prior to the determination of unsaturation. Strictly speaking, it is necessary to know the compositions of such fractions in order to calculate composite compressibilities from the values given in Table I. It has been found, however, that an average value may be chosen which expresses the compressibility of a given fraction with an accuracy sufficient for the purposes of the analysis. Thus, an average value of 1.031 has been chosen for the compressibility of butane-butylene fractions at 30° C. and 760-mm. pressure.

In general, the compressibility of a binary mixture of dissimilar gases such as hydrogen and a hydrocarbon does not vary linearly with the composition of the mixture. Leendertse and Scheffer (*4*) have discussed a method for approximating the deviation of binary gaseous mixtures from the ideal gas laws involving the compressibilities and the a and b values of the van der Waals equation for the individual components. From their discussion, the following approximation has been derived for the compressibility, $C_{1,2}$, of a binary gaseous mixture:

Table II. Experimental Results

Expt. No.	Observed Volumes: Hydrogen	Isobutane	Mixture	Calculated Ideal Volumes: Isobutane[a]	Mixture[b]	Compressibility[c]	Isobutane
	Ml.	*Ml.*	*Ml.*	*Ml.*	*Ml.*		*Mole %*
1	50.00	47.60	97.90	48.90	98.90	1.011	49.4
2	60.10	39.20	99.65	40.35	100.45	1.008	40.2
3	20.95	70.30	91.40	72.40	93.35	1.021	77.6
4	75.85	22.55	98.90	23.22	99.07	1.002	23.4
5	77.00	20.70	98.20	21.30	98.30	1.001	21.7
6	63.50	34.90	98.85	35.90	99.40	1.005	36.1
7	50.00	48.60	99.00	50.00	100.00	1.010	50.0
8	52.00	45.30	97.90	46.65	98.65	1.008	47.3
9	67.90	25.10	93.45	25.85	93.75	1.003	27.6
10	62.40	35.60	98.40	36.65	99.05	1.006	37.0
11	71.30	26.65	98.30	27.40	98.70	1.004	27.8
12	81.55	15.70	97.60	16.16	97.71	1.001	16.5
13	72.60	26.60	99.70	27.40	100.00	1.004	27.4
14	72.10	25.25	97.65	26.00	98.10	1.005	26.5
15	67.70	29.30	97.50	30.15	97.85	1.004	30.8
16	68.40	29.30	98.15	30.15	98.55	1.004	30.8
17	50.40	47.60	98.30	49.00	99.40	1.011	49.3
18	29.40	30.40	60.10	31.30	60.70	1.010	51.6

[a] Observed volume × 1.0290 = ideal volume of isobutane.
[b] Ideal volume of isobutane + volume of hydrogen = ideal volume of mixture.
[c] Calculated by $\frac{\text{ideal volume of mixture}}{\text{observed volume of mixture}}$ = compressibility.

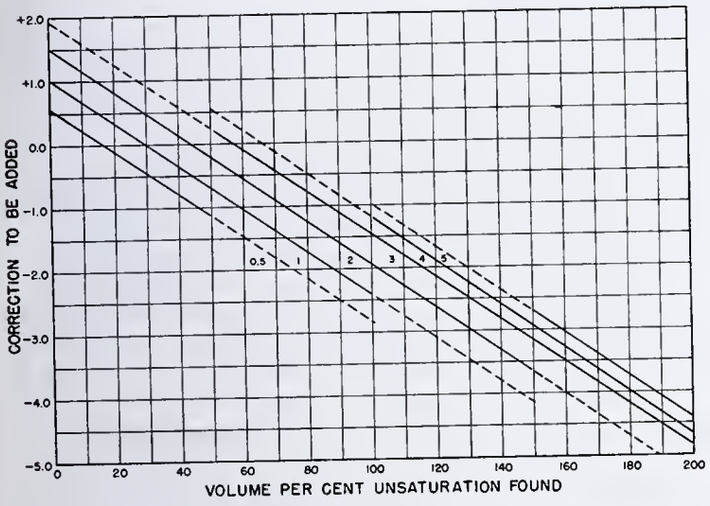

Figure 2. Correction Chart for Hydrogenation of Butane-Butylene-Butadiene Fraction

Various volume ratios of hydrogen to sample

$$C_{1,2} = \frac{\left[\frac{p_1}{1-(1-C_1^{-1})p_1}\right] + \left[\frac{p_2}{1-(1-C_2^{-1})p_2}\right]}{p_1 + p_2 - \Delta} \quad (1)$$

where p_1 and p_2 are the partial pressures of the two gases in the mixture, C_1 and C_2 are their respective compressibilities, and Δ is calculated by means of the following equation:

$$\Delta = p_1 p_2 (RT)^{-2}[2\sqrt{a_1 a_2} - RT(b_1 + b_2)] \quad (2)$$

When p is expressed in atmospheres, $R = 3.66 \times 10^{-3}$ mole volume-atmospheres degree^{-1} mole^{-1}.

For comparison with the calculated values the compressibilities of mixtures of hydrogen and isobutane were determined in the present work.

Experimental

The experimental work consisted of mixing accurately measured volumes of isobutane and hydrogen and measuring the volumes of the resulting mixtures. For these measurements the two 100-ml. burets of the hydrogenation apparatus (*5*) were employed, the catalyst tube being removed and replaced by a straight tube of known volume. The two joints were fitted glass-to-glass with rubber tubing. Although this technique is admittedly less precise than other methods described in the literature, it is sufficiently accurate to determine the compressibility-composition curve within the experimental error of the analytical results to which the data are applied.

Chemically pure isobutane and hydrogen from commercial cylinders were used. Mixing was accomplished by several passes between the burets, all gas volume measurements being made in one calibrated buret.

Discussion

The experimental results are summarized in Table II. The compressibilities calculated from these data are plotted in Figure 1 together with the values calculated from Equation 1. The observed and calculated curves differ somewhat.

The authors prefer to express the unsaturate content of a given sample as the number of moles of hydrogen required to saturate 100 moles of the sample. For convenience, this value will be referred to as the mole per cent unsaturation, M. It is evidently defined by the following equation:

$$M = \frac{100(V_1C_1 + V_2 - V_3C_3)}{V_1C_1} \quad (3)$$

in which V_1, V_2, and V_3 are, respectively, the experimentally measured volumes of hydrocarbon sample, hydrogen, and hydrogen-saturated hydrocarbon mixture resulting from the hydrogenation reaction; and C_1 and C_3 are the corresponding compressibilities.

The degree of unsaturation as calculated by McMillan *et al.* without correcting for the nonideality of the gases will be called the volume per cent unsaturation, U:

$$U = \frac{100(V_1 + V_2 - V_3)}{V_1} \quad (4)$$

U is readily calculated from the analytical measurements. For this reason the authors have chosen to derive the corrections for gas nonideality in terms of an additive quantity, D, such that:

$$M = U + D \quad (5)$$

By combining Equations 3, 4, and 5 the following expression is derived:

$$D = M - U = \frac{100}{C_1}\left[(C_1 - C_3)\left(1 + \frac{V_2}{V_1} - \frac{U}{100}\right) + (1 - C_1)\frac{V_2}{V_1}\right] \quad (6)$$

Accordingly, in Equation 6, D is determined (for a given value of C_1) by the volume per cent unsaturation, U, of the

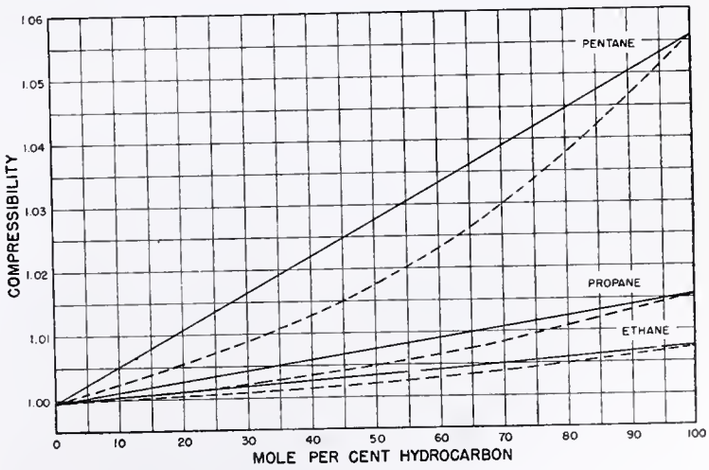

Figure 3. Calculated Compressibility Curves for Mixtures of Saturated Hydrocarbon Gases with Hydrogen

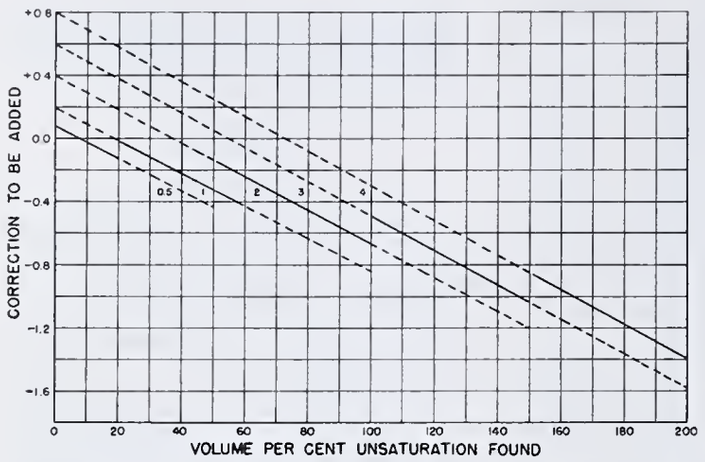

FIGURE 4. CORRECTION CHART FOR HYDROGENATION OF ETHANE-ETHYLENE FRACTION OR ACETYLENE

Various volume ratios of hydrogen to sample

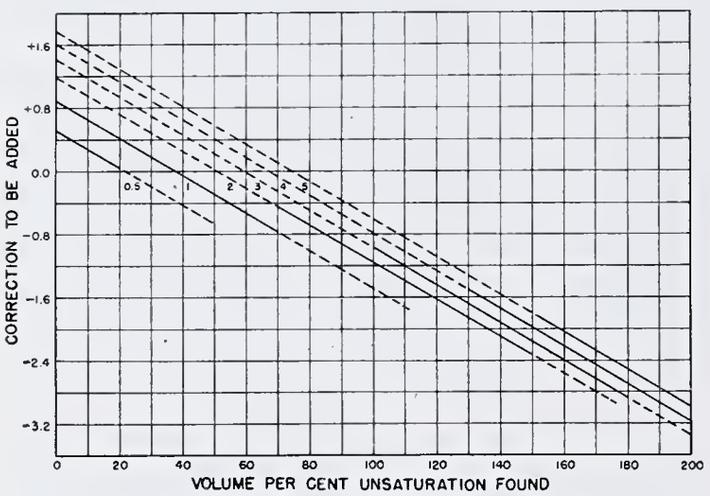

FIGURE 5. CORRECTION CHART FOR HYDROGENATION OF PROPANE-PROPYLENE-PROPADIENE FRACTION

Various volume ratios of hydrogen to sample

sample, the ratio, $\frac{V_2}{V_1}$, of the volumes of hydrogen and hydrocarbon sample taken initially in an analysis, and the compressibility, C_3, of the hydrogen-saturated hydrocarbon mixture from the hydrogenation reaction. Values of C_3 can be easily read from a compressibility-composition curve of the type given in Figure 1 if the composition in terms of the mole percentage, m, of saturated hydrocarbon in the final mixture is known. However, for purposes of reading values of C_3 from the compressibility-composition curve, it is sufficiently accurate to calculate it thus:

$$m = \frac{100\ V_1}{V_1 + V_2 - 0.01\ UV_2}$$

Following the above procedure, it is possible to calculate values of D for a range of values of U and $\frac{V_2}{V_1}$. Figure 2 shows results of such calculations for butane-butylene fractions. A value of 1.031 was assumed for C_1 regardless of the value of U for the original hydrocarbon sample. It has also been assumed that the C_3 values depend only on the per cent of butane in the final mixture, regardless of whether it is n-butane or isobutane.

In order to obtain some experimental confirmation of the corrections calculated in the above manner, a number of pure unsaturated hydrocarbons were analyzed by the hydrogenation method.

TABLE III. ANALYSIS OF PURE ISOBUTYLENE AND BUTADIENE

Gas	Volume of Hydrogen / Volume of Sample	Volume Per Cent Unsaturation Found	Correction from Figure 2	Corrected Value
Isobutylene	1.8	102.3	−2.1	100.2
	3.5	101.2	−1.4	99.8
	9.5	100.0	−0.2 (calcd.)	99.8
Butadiene	4.0	204.7	−4.8	199.9

Samples of highly purified isobutylene and butadiene were analyzed. The isobutylene was prepared by dehydrating redistilled C. P. *tert*-butylalcohol, melting at 24.1° C., by means of C. P. anhydrous oxalic acid, condensing the resulting gas, and redistilling in a 60-cm. (24-inch) vacuum-jacketed column fitted with Stedman-type packing. A middle fraction with a boiling range of 0.1° was taken for use.

The butadiene was obtained from a commercial source and analyzed 99.4 per cent conjugated diolefins by absorption in molten maleic anhydride.

The two purified gases were hydrogenated in the presence of various volume ratios of hydrogen in the prescribed manner. The results are given in Table III. All values are the average of two or more determinations agreeing within 0.2 per cent unsaturation or less.

Since the corrected values are consistent and in good agreement with the probable purity of the gases, it is felt that these data offer confirmation of the validity of the correction curves.

Corrections are also desirable for ethane-ethylene, propane-propylene, and pentane-pentene fractions. The pentane-pentene fraction, although liquid at ordinary conditions, is easily analyzed in the gas phase after vaporization under reduced pressure and blending with a gas such as hydrogen.

In Figure 3 are shown the curves for the compressibilities of mixtures of ethane, propane, and n-pentane, respectively, with hydrogen under ordinary conditions, as calculated by the approximation Equation 1 mentioned above but adjusted by a factor equal to the ratio of the calculated and observed deviations from linearity as found in the isobutane-hydrogen system (Figure 1). Although this method of deriving these curves is admittedly crude, the resulting shift of the ethane and propane curves is within the experimental error of the analytical data to be corrected. The pentane-hydrogen curve is verified by experimental work described below. The hypothetical value for the compressibility of pentane at 30° and 760 mm. was obtained by extrapolating the data of Jessen and Lightfoot (*3*) from lower pressures.

Using Equation 6 corrections for the hydrocarbons containing two and three carbon atoms have been derived and are presented in Figures 4 and 5, respectively.

In the case of the pentane-pentene fraction, the preliminary operations involving vaporization and blending with hydro-

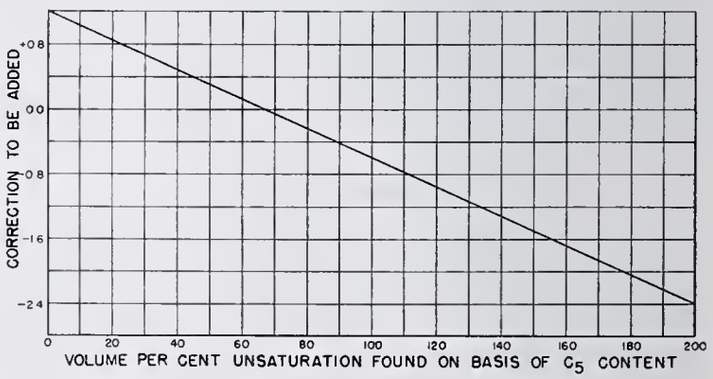

FIGURE 6. CORRECTION CHART FOR HYDROGENATION OF PENTANE-PENTENE-PENTADIENE FRACTION

In 33.8 mole per cent blend with hydrogen, using equal volumes of blend and additional hydrogen

Table IV. Determination of Unsaturation of Five-Carbon-Atom Hydrocarbons and Mixtures Thereof by Hydrogenation

Substance	n_D^{20} [a]	Found	Corrected	Synthesis
		Mole Per Cent Unsaturation [b]		
Isoprene, pure	1.4217	202.0	199.6	200.0
Mixture				
37.0% trimethylethylene	1.3871			
28.0% piperylenes	1.4311			
35.0% isoprene	1.4217	165.0	163.2	163.0
Mixture				
46.4% *n*-pentane	1.3577			
16.5% trimethylethylene	1.3869			
37.1% piperylenes	1.4305	90.7	90.3	90.7

[a] Best literature values: isoprene, 1.4216; mixed piperylenes, 1.4309; trimethylethylene, 1.3869; *n*-pentane, 1.3578.
[b] On basis of hydrocarbon content.

gen are carried out at constant volume, the pressure being measured. The liquid hydrocarbon is vaporized into a previously evacuated vessel until a pressure of 250 mm. is attained. Hydrogen gas is then admitted until the total pressure rises to 750 mm. The partial pressure of the hydrocarbon in the blend is well below the saturation pressure under the conditions of measurement. The blending operation is readily adapted to the ordinary Podbielniak distillation apparatus.

It has been found convenient in this laboratory to prepare all blends of the pentane-pentene fractions at these pressures and use a single correction factor (+0.5 mole per cent) for the composition of the blend. There are obviously many other ways in which the blending can be done, but the additive correction factor applies only to blends made up in the exact manner described.

The corrections for the hydrogenation method when applied to a 33.8 mole per cent blend of pentane-amylene fraction in hydrogen have been calculated and the resulting corrections are plotted in Figure 6. These corrections are based on the use of equal volumes of additional hydrogen and blend in the analysis.

To check the method, a series of pure five-carbon-atom hydrocarbons, and mixtures thereof, were prepared, blended with hydrogen, and the unsaturation determined by hydrogenation. The results, presented in Table IV, offer very good confirmatory evidence for the validity of the method and the corrections.

Acknowledgment

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Contact Sulfuric Acid Manufacture

Evaluation of the Reich Test

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THE Reich test for sulfur dioxide is commonly used in the determination of gas strength in sulfuric acid manufacture. Its flexibility, ease of operation, and simplicity make it a desirable method for the routine testing of gas strengths in different parts of the gas purification or conversion system. Various modifications of the original test proposed by Reich (*11*) both as to apparatus (*2, 3, 4, 7*) and to the nature of the absorbing solution (*5, 10*), have been described, but so far as could be determined, no study of the accuracy and precision of which the test is capable has been reported. The present work evaluates the factors that influence the accuracy and precision of the test as used in brimstone-burning contact sulfuric acid plants. The test as described is applicable to chamber burner gases, but must be modified for use with chamber gases containing oxides of nitrogen.

Apparatus and Materials

Reich test apparatus (Figure 1). Woulff bottle, 4-liter capacity, equipped with thermometer, inlet, and siphon tubes. In operation, this bottle is filled with water to the mark shown.

Reich bottle, 350-ml. capacity, equipped with a 2-mm. bore glass tube with attached stopcock.

Gas pressure bottle, 350-ml. capacity, equipped with T and vent tubes.

Graduate, 125-ml. capacity, graduated in 0.5-ml. units.

Orsat gas analysis apparatus, Bureau of Mines type.

Sulfur dioxide gas mixture, 12 per cent sulfur dioxide, balance nitrogen. This gas mixture was stored in a regulation size cylinder and kept above 21.11° C. (70° F.) to prevent liquefaction of sulfur dioxide.

Barometer, mercurial.

Chromic acid solution, 50 per cent aqueous.

0.1 *N* iodine solution, prepared from C. P. iodine crystals and potassium iodide (20 grams per liter). This solution was standardized against Bureau of Standards arsenic trioxide.

Starch solution, 2 per cent.

Procedure

After flushing the sampling line up to the tip of the inlet tube of the Reich bottle with the gas to be tested, the stopcock was closed and the gas was analyzed according to the following procedure:

Ten milliliters of 0.1 *N* iodine were added to the Reich bottle containing 175 ml. of distilled water, 5 ml. of starch solution, and sufficient iodine to give a light blue color. The Reich bottle was replaced in the Reich assembly, the clamp of the siphon tube removed (the siphon having previously been set), and the water from the Woulff bottle permitted to run to waste. When the flow of water had stopped, the graduate was placed under the siphon tip and the stopcock of the Reich bottle opened. The Reich bottle was shaken during the course of the absorption and the gas permitted to pass until the solution in the Reich bottle again assumed its original light blue color. As the end point was approached, the stopcock was closed, and then momentarily opened to permit the passage of additional small volumes of gas by a rapid turn through a 180° angle. The clamp was again replaced on the siphon tube, after the system had reached its original pressure state as indicated by the cessation of water flow, and the volume of water was read to the nearest 0.5 ml. This volume of water represented the volume of gas analyzed with the

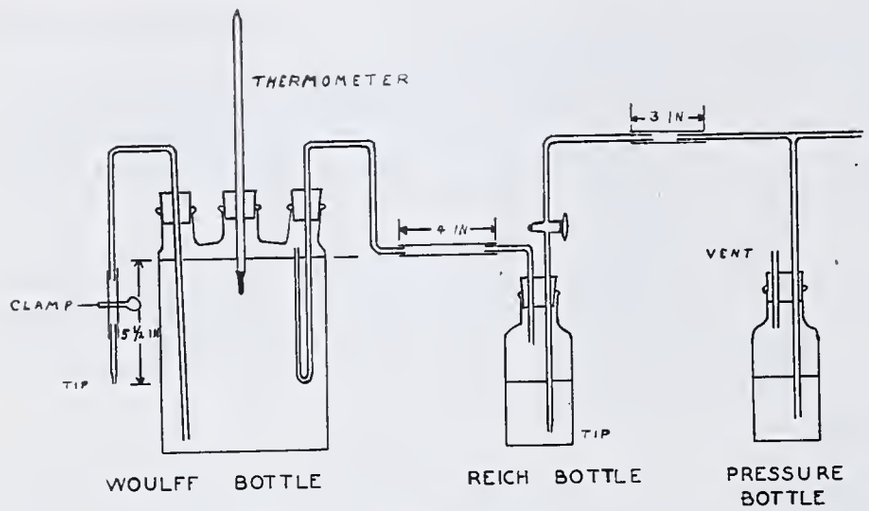

FIGURE 1. REICH TEST APPARATUS

exception of the sulfur dioxide which had been absorbed. The temperature of the water in the Woulff bottle and the barometric pressure were noted and the volume of gas as indicated by the volume of water was corrected to standard conditions of temperature and pressure by the application of the familiar gas laws. The per cent of sulfur dioxide was determined by dividing the volume of sulfur dioxide equivalent to the 10 ml. of 0.1 *N* iodine by the total volume of gas (sum of corrected volume and volume of sulfur dioxide equivalent to 0.1 *N* iodine volume used).

Factors Influencing Accuracy and Precision of Reich Test

A careful study of the method indicates that the following factors have a direct bearing on the accuracy and reproducibility of the test: (1) pressure of the gas within the system, (2) temperature of the gas within the system, (3) accuracy of volume measurement, (4) rate of gas flow, (5) loss of iodine or incomplete absorption of sulfur dioxide in Reich bottle, and (6) value of gram molecular volume used for sulfur dioxide.

PRESSURE OF GAS WITHIN THE SYSTEM. The pressure of the gas within the system after the cessation of water flow, following the decolorization of the iodine solution, is dependent upon the barometric pressure, the water head of the Woulff bottle, and the degree of saturation with regard to moisture content. The first of these factors is obvious. Calculation will show that a difference of 10 mm. in pressure results in a difference of 0.15 per cent in the calculation of the strength of a 12 per cent sulfur dioxide gas. The second pressure factor to be observed is the height of the water head in the Woulff bottle. When a bottle of 4-liter capacity is used, the difference between water head at start and finish of a test is not significant; its absolute value is, however, important, since a 32.5-cm. (13-inch) head, for example, results in a negative pressure of approximately 11 mm. of mercury. This is a large correction and should be taken into account. The third pressure factor is the degree of water saturation of the gas within the Woulff bottle. A study of the sample calculation shown below indicates the water vapor correction and the importance of this factor.

Experimental data to determine a possible state of unsaturation were obtained by placing a water trap between the Reich bottle and the Woulff bottle. No change in result was indicated and the conclusion was reached that this error, if present, was not significant in the present Reich test apparatus. Apparently the gas becomes saturated with water vapor by its passage through the Reich bottle.

TEMPERATURE OF GAS WITHIN THE SYSTEM. Calculations show that a 2° C. difference in temperature of the residual gas collected in the Woulff bottle represents a difference of 0.09 per cent in 12 per cent gas strength. Although a 2° change is a rather large variation, it seems desirable to pass the gas through a U-tube immersed in the water of the Woulff bottle (Figure 1) in order that the gas may assume the temperature of the water. This also permits taking the water temperature as the gas temperature.

ACCURACY OF VOLUME MEASUREMENTS. Calculations indicate that a 1-ml. difference in the volume of aspirated water obtained whenever a 10-ml. portion of 0.1 *N* iodine is decolorized with 12 per cent sulfur dioxide gas results in a 0.14 per cent difference in strength. This indicates the desirability of measuring the water volume to the nearest 0.5 ml. This was accomplished by the use of the special graduated cylinder.

RATE OF GAS FLOW. Experimental tests indicate that it is difficult to obtain satisfactory end points when the rate of gas flow is too rapid. The rate of gas flow is controlled by the size of glass tip in Reich bottle, water head in Woulff bottle, and size of tip from which water is discharged from the Woulff bottle. In the apparatus that was used, these factors were controlled by using a glass tip in the Reich bottle of such size as just to permit the passage of a piece of No. 26 gage wire, cutting the siphon tube to such a length as to provide a 12.5-cm. (5.5-inch) water head and using an exit tip of about 1.5-mm. diameter. This necessarily lengthens the time required for making a Reich test by approximately one minute (over-all time for complete test 4 to 5 minutes); this is not, however, considered excessive.

LOSS OF IODINE OR INCOMPLETE ABSORPTION OF SULFUR DIOXIDE IN REICH BOTTLE. Obviously the loss of iodine or sulfur dioxide from the Reich bottle would result in erroneous results.

TABLE I. LOSS OF IODINE FROM REICH BOTTLE AT ELEVATED TEMPERATURES

(Rate of gas flow, 100 ml. in 70 seconds)

Temp. ° F.	H_2O Aspirated	KI Added *Grams*	Remarks
85	250	0.0	No iodine lost
95	400	0.0	No iodine lost
100	104	0.0	Iodine lost
120	400	5.0	No iodine lost
120	460	3.0	Slight amount of I_2 lost[a]

[a] Color of starch-iodine was dispelled by 1 drop of 0.01 *N* $Na_2S_2O_3$.

The possibility of the loss of sulfur dioxide due to rapid passage and incomplete absorption in the Reich bottle was tested by inserting a trap into the line between the Reich bottle and the Woulff bottle. This consisted of a 20-cm. (8-inch) test tube closed by a two-hole rubber stopper equipped with inlet down through the solution and outlet to Woulff bottle. A light blue starch-iodine solution was placed in the trap, but in spite of frequent runs was not decolorized. Failure to decolorize in spite of rapid passage of high strength gas through the Reich bottle indicated no loss of sulfur dioxide. A similar experiment in which the starch-iodine solution was replaced by a colorless starch solution (previously adjusted to show no capacity for absorbing iodine without change in color) indicated that no loss of iodine was experienced from this dilution—10 ml. of 0.1 *N* iodine per 180 ml. of solution when treated by gas stream as above at room temperature (25° C.).

Additional tests to determine the volatility of the iodine solution were made at higher temperatures, using a 4-liter Woulff bottle, a test tube trap as above, and the Reich bottle with the special glass tip previously described. The solution in the Reich bottle consisted of 10 ml. of 0.1 *N* iodine and 180 ml. of water,

containing 5 ml. of starch solution. The trap contained 1 gram of potassium iodide dissolved in 5 ml. of water plus 3 ml. of starch solution. In these tests, the inlet of the Reich bottle was opened to the air instead of being connected to the gas cylinder. The results indicated that iodine was lost from the Reich bottle at temperatures of 37.78° C. (100° F.) or above. This loss may be corrected by the addition of potassium iodide to the Reich bottle. Under these conditions, the test may be made at 48.89° C. (120° F.) without serious loss of iodine. The results obtained are shown in Table I.

These results indicate that no iodine is lost from the Reich bottle during a normal determination at room temperature. When tests are made at 37.78° C. (100° F.) or above, the addition of potassium iodide is necessary to prevent iodine losses.

Gram-Molecular Volume of Sulfur Dioxide. The gram-molecular volume of the ideal gas has been determined as 22.4 liters. Fairlie (*1*) and Lunge (*6*) have based their Reich test tables on this value for sulfur dioxide. The gram-molecular volume of sulfur dioxide as based on experimentally determined values of density (*8*) is 21.89 liters. Obviously, the use of the one or the other results in a 0.2 per cent discrepancy. Since the 22.4 value rests upon theoretical considerations of the perfect gas and 21.89 rests upon values experimentally determined, it is sound to adopt the latter value. This procedure is also the one followed by Lunge-Berl (*7*), Miles (*9*) and Sullivan (*12*). Using this latter value 10 ml. of 0.1 *N* iodine are equivalent to 10.95 ml. of sulfur dioxide measured at standard conditions of temperature and pressure.

Precision and Accuracy of Test

With the above factors in mind, a series of tests was made on the sulfur dioxide–nitrogen mixture obtained from cylinder source. This particular strength (12 per cent) was chosen because the obtainment of satisfactory results with this gas of high sulfur dioxide concentration would automatically indicate the suitability of the method for gases of lower strength. Apart from sulfur dioxide percentage, the prepared gas mixture differed from normal contact gas produced from brimstone only in oxygen and nitrogen content, since other impurities present in the gas are removed in the purification train and small amounts of sulfur trioxide are removed by inserting a 98 per cent sulfuric acid absorber between the sampling point and the Reich test apparatus. The variation in oxygen and nitrogen content is of no significance, since both are inert with respect to the iodine absorbent. The results that were obtained are shown in Table II.

Table II. Results Obtained by Reich Test

Series 1	Series 2	Series 3
12.19	12.15	12.17
12.23	12.20	12.15
12.17	12.15	12.23
		12.21
Av. 12.19−	12.17−	12.19−

Av. of all runs, 12.19% SO_2

Additional determinations of equal precision were made, but the above are typical and indicate that the reproducibility of the test is within ±0.1 per cent. A sample calculation using proper corrections is shown.

Sample Calculation

Water volume, 89.5 ml.
Temperature of Woulff bottle, 24° C.
Barometer, 757.1 mm. at 26° C.
Normality of I_2, 0.1. (10 ml. of 0.1 *N* I_2 = 10.95 ml. of SO_2 under standard conditions)

Negative pressure	4.0 mm.
Temperature correction on barometer	3.4 mm.
Vapor pressure correction	22.4
	29.8

Corrected barometer 757.1 − 29.8 = 727.3 mm.

$$89.5 \times \frac{727.3}{760} \times \frac{273}{297} = \begin{array}{l} 78.72 \text{ ml. occupied by residual gas} \\ 10.95 \text{ ml. occupied by } SO_2 \\ \hline 89.67 \text{ ml. total volume of gas} \end{array}$$

$$\frac{10.95}{89.67} = 12.21\% \ SO_2$$

The absolute accuracy of the Reich test was shown by comparison with results obtained on an Orsat apparatus (Bureau of Mines model) using mercury as the confining liquid and chromium oxide as the gas absorbent. The results obtained on the same gas mixture as above are shown in Table III.

Results of a confirmatory nature were obtained by absorbing known volumes of the cylinder gas in a 1 per cent solution of sodium hydroxide, oxidizing to sulfate with sodium peroxide, and precipitating as barium sulfate. When analyzed in this manner the values of 12.26 and 12.28 per cent were obtained. These results as determined by the above two methods indicate that the accuracy of the Reich test when properly conducted is within 0.1 per cent. Reich and Orsat techniques both give results on a dry basis.

Table III. Gas Analysis by Orsat Apparatus

(CrO_3 absorbent)

	Run 1	Run 2	Run 3
1st pass	88.30	88.40	88.40
2nd pass	87.90	87.98	88.00
3rd pass	87.90	87.90	87.90
4th pass	87.90	87.88	87.90
% SO_2[a]	12.15	12.17	12.15

Av. 12.16%

[a] Corrected according to gas buret calibration.

Summary

The various factors involved in the accurate analysis of sulfur dioxide gases by Reich test procedure are (1) pressure, (2) temperature, (3) accuracy of volume measurement, (4) rate of gas flow, (5) completeness of sulfur dioxide absorption and loss of iodine, and (6) sulfur dioxide gram-molecular volume value. Experimental data which include comparisons made by accepted Orsat technique, as well as gravimetric sulfate determinations, indicate that the Reich test when properly conducted is capable of an accuracy and precision of ±0.1 per cent on 12 per cent sulfur dioxide gas strength.

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Determination of Pyrophosphate

By Precipitation with Cadmium and Polarographic Measurement of Cadmium in the Precipitate

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IN THE present paper a new method for the determination of pyrophosphate in the presence of varying amounts of orthophosphate is described. The method is of special advantage in the determination of small amounts of pyrophosphate, and may find use in the determination of pyrophosphate in biological material. In the proposed method the molar concentration of orthophosphate may be 16 times as large as the concentration of pyrophosphate, but even in the presence of 40 times the molar concentration of orthophosphate satisfactory results have been obtained. If larger samples are available, as is usually the case in the analysis of commercial phosphates, the proposed method appears not to be superior to existing methods. The effect upon the proposed method of meta- and (or) polyphosphate, which are often present in commercial phosphates, has not been investigated.

Until now no satisfactory method has been described for the determination of small amounts of pyrophosphate in the presence of orthophosphate. Larger amounts of meta-, ortho-, and pyrophosphate present in the same solution can be determined satisfactorily by acidimetric titrations.

Gerber and Miles (*2*, *3*) determined the three phosphoric acids by titrating to different pH values using for the determination of orthophosphate the amount of nitric acid liberated by precipitating the phosphates with silver nitrate. Pyrophosphate is further determined according to Britzke and Dragunov (*1*) by precipitating disodium dihydrogen pyrophosphate with zinc sulfate and titrating the liberated sulfuric acid. In the analysis of mixtures considerable titration error results if the amount of pyrophosphate present corresponds to less than 50 mg. of phosphorus pentoxide. When small amounts of pyro- and orthophosphate are present in a solution a separation has to be made by precipitation.

Several authors have described the separation of pyrophosphate by precipitation with a zinc or cadmium solution. Kiehl and Coats (*6*) found that the precipitate of zinc pyrophosphate was not satisfactorily crystalline but when reprecipitated the pyrophosphate could be determined gravimetrically with an accuracy of 1 per cent. Nylen (*9*) reported an accuracy of 1 to 2 per cent by the zinc method when working in an acetate buffer with a pH of 5. He found no coprecipitation of orthophosphate when 6 to 15 mg. of phosphorus as pyrophosphate were determined in the presence of 15.5 mg. of phosphorus in the form of orthophosphate. An accuracy of about 1 per cent was also found by Madorsky and Clark (*8*), who separated pyrophosphate by precipitation with zinc sulfate at pH 2.7 to 2.8. Gerber and Miles (*3*), precipitating with zinc sulfate at pH 3.0 to 3.3, observed no interference by orthophosphate but a strong interference by ammonium chloride. These authors point out that the conditions for the zinc separation are not yet well enough defined to recommend this method for general application.

Hull (*4*) in qualitative experiments found that precipitation of zinc pyrophosphate is incomplete and that large quantities of orthophosphate are coprecipitated. Precipitation with cadmium in a pH 5 acetate buffer resulted in a much better separation. Wurzschmitt and Schuknecht (*10*), however, stated that cadmium is not suitable for quantitative pyrophosphate determinations. They claim that titration errors in the precipitation of $H_2P_2O_7^{--}$ with zinc are caused by hydrolysis of the ordinary zinc salts, and that they are avoided by precipitating with the complex ammonium zinc iodide.

On the basis of Hull's experiments the authors have investigated the separation of pyrophosphate and orthophosphate by precipitation and determination of pyrophosphate as the cadmium salt. The precipitation was carried out in a buffered solution, and the cadmium in the precipitate was determined polarographically. A few gravimetric determinations have also been made. The results obtained may also be of value in an improvement of the precipitation method of pyrophosphate with zinc.

Reagents Used

SODIUM PYROPHOSPHATE. The salt was prepared by heating dehydrated pure disodium hydrogen phosphate (Merck) over a Méker burner to constant weight. A 0.01 *M* solution was prepared as stock solution. The concentration of this solution was checked by hydrating the pyrophosphate with hydrochloric acid at boiling temperature and titrating the orthophosphate amperometrically with uranyl acetate (*7*). Two titrations gave a deviation of −0.2 and −0.4 per cent, respectively, from the theoretical value, which is within the limit of error of this method.

CADMIUM CHLORIDE. A 0.1 *M* cadmium chloride solution was prepared from dehydrated $CdCl_2.2^1/_2H_2O$ (Mallinckrodt) which had been dried to constant weight at 110° C. The water-free cadmium chloride is hygroscopic but attracts water sufficiently slowly to allow preparation of standard solutions by weighing. The 0.1 *M* solution was used for calibration measurements in the polarographic cadmium determination.

CADMIUM ACETATE. Solutions 0.1 *M*, 0.2 *M*, 0.4 *M*, and 3 *M* were prepared by dissolving Merck's cadmium acetate dihydrate in water. Except for the 0.1 *M* solution the concentrations were not adjusted accurately, since the solutions were used only as precipitating agents.

ACETATE BUFFERS. The following buffer solutions were prepared: 4.7 *M* sodium acetate + 0.14 *M* acetic acid, pH 6.1; 1 *M* sodium acetate + 1 *M* acetic acid, pH 4.7; 1 *M* ammonium acetate + 8 *M* acetic acid, pH 3.6. These buffer solutions are designated below as 6.1, 4.7, and 3.6, respectively.

Solubility of Cadmium Pyrophosphate

Successful results depend in the first place upon the insolubility of cadmium pyrophosphate in the precipitation medium. Therefore, the solubility of cadmium pyrophosphate was determined under various conditions by measuring polarographically the cadmium concentration of saturated cadmium pyrophosphate solutions. It was found that the solubility was negligibly small in water, but was appreciable in 0.1 *M* potassium chloride and in 0.1 *M* potassium chloride plus various concentrations of buffer 6.1. It increased with increasing concentration of buffer 6.1. Since the precipitation of pyrophosphate has to be carried out in a buffer solution in order to prevent the precipitation of orthophosphate, it is necessary to suppress the solubility by using a large excess of cadmium in the precipitation. The precipitate can be washed with water, since the buffer and the excess of cadmium are removed simultaneously.

Gravimetric Determinations

The feasibility of the quantitative determination was checked by a few gravimetric determinations.

Pyrophosphate was precipitated at room temperature from buffer 3.6 with an excess of cadmium acetate. After standing overnight the precipitate was filtered through a Jena 4 G sintered-glass crucible and washed with water. The filtration must be made very carefully, because the precipitate runs through the filter if the suction is too strong. After the precipitate had been washed with water the crucible was heated to constant weight at temperatures between 220° and 290° C. At these temperatures the cadmium pyrophosphate dihydrate obviously loses its water, and the precipitate can be weighed as cadmium pyrophosphate.

Table I. Gravimetric Determination of Pyrophosphate as Cadmium Pyrophosphate

0.01 *M* $Na_4P_2O_7$ Used	Buffer 3.6 Used	$CdAc_2$ Used		$Cd_2P_2O_7$ Calculated	$Cd_2P_2O_7$ Found	Error
Ml.	*Ml.*	*Ml.*	*M*	*Mg.*	*Mg.*	%
25	10	20	0.4	99.7	98.4	−1.3
25	10	20	0.4	99.7	98.6	−1.1
50	10	4	3	199.4	199.7	+0.15
50	10	4	3	199.4	200.7	+0.65
50	10	4	3	199.4	197.5	−0.95

Some results are given in Table I. The precipitation of cadmium pyrophosphate is quantitative under the conditions given in Table I. The deviations are probably due to slight losses in the filtration of this precipitate.

Polarographic Determination of Cadmium in the Precipitate

In preliminary experiments carried out at a pH of 6.1 attempts were made to determine the pyrophosphate in 0.005 *M* solution by adding a known excess of cadmium and measuring the concentration of cadmium in the supernatant liquid. The precipitate plus supernatant liquid was allowed to stand overnight, and the residual cadmium was determined polarographically without filtration. The amount of cadmium added to the pyrophosphate was twice the amount required theoretically for complete precipitation. However, about 20 per cent of the pyrophosphate was not precipitated. The presence of orthophosphate in a molar ratio PO_4 to P_2O_7 of 4 to 1 did not affect the results. This was no longer true when a larger excess of cadmium was used in the precipitation at pH of 6.1 (see Table IV). For complete precipitation of 25 ml. of 0.01 *M* sodium pyrophosphate more than 10 ml. of 0.2 *M* cadmium acetate was required. Under these conditions the determination by subtracting the amount of cadmium left in solution from the total amount used becomes inaccurate. The determination must, therefore, be carried out by measuring the amount of cadmium in the precipitate.

The conditions of precipitation are specified in the tables. In order to improve the settling of the precipitate a few drops of gelatin solution (1 mg. of gelatin per ml.) were added after precipitation and also to the wash water. The precipitate was separated from the supernatant liquid by centrifuging and then washed by shaking and centrifuging with four 50-ml. portions of water. The precipitate was then dissolved in hydrochloric acid (1 to 4), and the solution transferred to a 100-ml. volumetric flask, enough potassium chloride being added to eliminate the migration current in the subsequent polarographic determination of the cadmium.

The diffusion current was measured at 25° C. at an applied e. m. f. of 0.8, 0.9, and 1.0 volt, respectively, with a mercury pool as anode in 0.5 *M* potassium chloride solution. Identical current readings were obtained at these potentials. In order to correct for the residual current, 0.15 microampere was subtracted from the current values measured. The cadmium concentration of the solution was determined by adding successively three known amounts of 0.1 *M* cadmium chloride to the solution and by measuring the increase of the diffusion current. The unknown concentration was determined by linear extrapolation from the three points measured with known concentrations, taking as zero point the diffusion current obtained with the unknown concentration. This procedure is more accurate than using an "internal standard", where only one known amount is added, and it is also better than using a calibration curve, since changes in the properties of the capillary or in the temperature might occur. The error in the concentrations determined in this way is of the same order of magnitude as that in ordinary polarographic work (2 to 3 per cent).

The cadmium pyrophosphate was reprecipitated as follows: The precipitate was dissolved at room temperature in 2 to 3 ml. of hydrochloric acid (1 to 4), and immediately thereafter 6 *M* sodium hydroxide was added dropwise until the color of Tropeolin 00 changed from red to orange yellow. Then 5 to 10 ml. of buffer 3.6 were added. Under these conditions no precipitate of cadmium pyrophosphate appeared. Precipitation was achieved by adding the same amount of cadmium acetate solution as had been used in the first precipitation. The precipitate was centrifuged and washed as described before.

Determination of Pyrophosphate

In Solutions Free from Interfering Substances. Table II contains the results of experiments in which the influence of pH, buffer concentration, reprecipitation, and temperature on the precipitation of pyrophosphate was investigated. Pyrophosphate was precipitated from 25 ml. of 0.01 *N* sodium pyrophosphate solution at room temperature. The precipitate was allowed to stand overnight together with the supernatant liquid. Table II shows that the precipitation is complete in buffer solutions with a pH ranging between 6.1 and 3.6. The error found was of the order of 2 per cent or smaller (occasional errors, not listed in Table II, were presumably due to improper washing of the precipitate or to losses during decantation). Since a low pH is favorable for the prevention of coprecipitation of orthophosphate (Table IV), buffer 3.6 was selected as the medium in most of the experiments. From experiment 15 (Table II) it is obvious that addition of acetic acid instead of the buffer gives quantitative precipitation, since a buffer is formed by addition of the cadmium acetate. Experiments 13 and 14 show that losses by hydration of pyrophosphate do not occur when the cadmium pyrophosphate is dissolved, and when the precipitation is repeated.

Table II. Precipitation of Cadmium Pyrophosphate

(25 ml. of 0.01 *M* $Na_4P_2O_7$ solution taken)

No.	Buffer Used		$CdAc_2$ Used for Precipitation		Cadmium in Precipitate Calculated	Cadmium in Precipitate Found	Error
	Ml.		*Ml.*	*M*	*Millimole*		%
1	5	(6.1)	25	0.2	0.500	0.490	−2.0
2	5	(6.1)	25	0.2	0.500	0.490	−2.0
3	10	(4.7)	25	0.2	0.500	0.494	−1.2
4	10	(3.6)	25	0.2	0.500	0.500	0
5	10	(3.6)	25	0.2	0.500	0.506	+1.2
6	10	(3.6)	20	0.4	0.500	0.490	−2.0
7	10	(3.6)	20	0.4	0.500	0.496	−0.8
8	10	(3.6)	20	0.4	0.500	0.495	−1.0
9	5	(3.6)	2	3	0.500	0.499	−0.2
10	5	(3.6)	2	3	0.500	0.494	−1.2
11	5	(3.6)	2	3	0.500	0.513	+2.6
12	5	(3.6)	2	3	0.500	0.508	+1.6
13	5	(3.6)	2	3	0.500	0.490[a]	−2.0
14	5	(3.6)	2	3	0.500	0.495[a]	−1.0
15	10	1 *M* HAc	15	0.4	0.500	0.513	+2.6
16	10	(3.6)	2	3	0.500	0.480	−4.0
17	10	(3.6)	2	3	0.500	0.478	−4.4
18	20	(3.6)	25	0.2	0.500	0.450	−10.0
19	10	(3.6)	15	0.4	0.500	0.476[b]	−4.8
20	10	(3.6)	15	0.4	0.500	0.470[b]	−6.0

[a] Reprecipitated and again allowed to stand overnight.
[b] Precipitated at boiling temperature and separated immediately.

Experiments 16, 17, and 18 show that too large a concentration of buffer 3.6 causes incomplete precipitation. Low results were also obtained when the precipitation was carried out at boiling temperature (experiments 19 and 20). For this reason the precipitation should not be carried out hot. Precipitation at boiling temperature gives a precipitate which settles more quickly and seems to be coarser.

The concentration of the cadmium acetate in the reagent is of subordinate significance, but in order to prevent coprecipitation of orthophosphate a 0.04 *M* cadmium acetate solution is most advantageous, as shown below.

Table III contains experiments with different pyrophosphate concentrations, in which the time required for complete precipitation was also determined. When the pyrophosphate concentration in the reaction medium was greater than

Table III. Precipitation of Cadmium Pyrophosphate

(25 ml. of pyrophosphate solution taken, 5 ml. of buffer 3.6 used)

No.	Molarity of $Na_4P_2O_7$ Used	$CdAc_2$ Used in Precipitation		Time of Standing after Precipitation	Cadmium in Precipitate		Error
		Ml.	*M*	*Hours*	Calculated	Found	%
					Millimoles		
1	0.01[a]	15	0.4	2	0.500	0.486	− 2.8
2	0.01	3	3	2	0.500	0.498	− 0.4
3	0.01	4	3	1	0.500	5.06	+ 1.2
4	0.02	4	3	0.5	1.000	0.986	− 1.4
5	0.01	4	3	0.25	0.500	0.501	+ 0.2
6	0.0025	15	0.4	Overnight	0.125	0.122	− 2.5
7	0.0025	4	3	Overnight	0.125	0.125	0
8	0.0025	4	3	Overnight	0.125	0.1225	− 2.0
9	0.0025[b]	15	0.4	Overnight	0.125	0.126	+ 0.8
10	0.0025	4	3	2	0.125	0.1225	− 2.0
11	0.0025	4	3	1	0.125	0.1194	− 4.5
12	0.0025	4	3	0.5	0.125	0.116	− 7.2
13	0.0025	4	3	0.25	0.125	0.107	−14.4
14	0.001	4	3	Overnight	0.0500	0.0480	− 4.0
15	0.001	4	3	Overnight	0.0500	0.0495	− 1.0
16	0.001	15	0.4	Overnight	0.0500	0.0468	− 6.4
17	0.001	15	0.4	Overnight	0.0500	0.0460	− 8.0
18	0.001	4	3	3	0.0500	0.0503	+ 0.6
19	0.001	4	3	1	0.0500	0.0460	− 8.0
20	0.001	4	3	1	0.0500	0.0482	− 3.6
21	0.001	4	3	35 minutes	0.0500	0.0390	−22
22	0.001	4	3	0.25	0.0500	0.0145	−71

[a] 10 ml. of buffer 3.6 used.
[b] 10 ml. of 1 *M* acetic acid added instead of buffer.

0.007 *M* the precipitation was complete within 15 minutes when the concentration of cadmium was about 0.3 *M* (experiment 5).

The precipitation of a pyrophosphate solution of the initial concentration of 0.0025 *M* likewise yielded satisfactory results. The time required for complete precipitation was about 2 hours (experiments 10 and 11).

The lowest concentration of pyrophosphate at which precipitation is still complete is about 0.001 *M*. A visible precipitate did not appear until several minutes after the addition of cadmium acetate, and it seemed to be of smaller particle size than the precipitates obtained from more concentrated solutions. When the precipitation from the 0.001 *M* pyrophosphate solution was carried out by using more dilute reagent (experiments 16 and 17) larger errors resulted. However, greater errors were also found in other cases with this dilute pyrophosphate solution, which may be due to the difficulty of centrifuging the precipitate. Three hours seem to be sufficient for complete precipitation. It is recommended that the final concentration of pyrophosphate be at least 0.0012 *M* in order to get quantitative precipitation. Especially in the presence of orthophosphate the concentration of pyrophosphate in the reaction mixture should not be smaller than this value.

In Presence of Orthophosphate and of Calcium. The precipitation of cadmium pyrophosphate is much affected by the presence of orthophosphate and of calcium.

Cadmium orthophosphate has a limited solubility at a pH of 3.6. An incomplete precipitation is obtained, when a 0.1 *M* potassium dihydrogen phosphate solution is made about 0.1 *M* in cadmium. The appearance of the precipitate at the same final concentration of cadmium and orthophosphate is dependent on the concentration of the cadmium acetate solution added. With more concentrated cadmium acetate solution a marked precipitation occurs during the addition, due to local excess of cadmium. With more dilute cadmium acetate solution the precipitate appears slowly. Part of the precipitate formed upon addition of the more concentrated cadmium acetate solution dissolves again afterwards. The final precipitate is coarser than that which is obtained by precipitating with a more dilute cadmium acetate solution. Hence the concentration of cadmium acetate used for precipitation is of subordinate significance regarding the amount of orthophosphate finally precipitated. In the presence of pyrophosphate, however, this is no longer true. When 3 *M* cadmium acetate was added to a mixture of 25 ml. of 0.01 *M* pyrophosphate, 5 ml. of 1 *M* orthophosphate, and 5 ml. of buffer 3.6, much more orthophosphate was coprecipitated than upon addition of 0.4 *M* cadmium acetate to a mixture of 25 ml. of 0.01 *M* pyrophosphate, 10 ml. of 1 *M* orthophosphate, and 10 ml. of buffer 3.6. In the first case one reprecipitation did not suffice to obtain an orthophosphate-free cadmium pyrophosphate (Table IV, experiment 6), but it was achieved with one reprecipitation in the latter case (Table IV, experiment 5). It is, therefore, better to use 0.4 *M* instead of 3 *M* cadmium acetate for precipitation in the presence of orthophosphate.

When the orthophosphate is present in a sufficiently low concentration (about 0.03 *M*), the coprecipitated orthophosphate is eliminated from the precipitate on aging in the supernatant liquid. Simultaneously the coprecipitated orthophosphate promotes the rate of recrystallization of the cadmium pyrophosphate. The latter has a slimy appearance when precipitated in the absence of orthophosphate, and does

Table IV. Precipitation of Cadmium Pyrophosphate in Presence of Orthophosphate

(25 ml. of $Na_4P_2O_7$ solution taken, 5 ml. of buffer 3.6 used)

No.	Molarity of $Na_4P_2O_7$ Used	KH_2PO_4 Present		Molar Ratio $PO_4^{---}/P_2O_7^{----}$	$CdAc_2$ Used for Precipitation		Treatment after Precipitation	Cadmium in Precipitate		Error
		Ml.	*M*		*Ml.*	*M*		Calculated	Found	%
								Millimoles		
1	0.01[a]	10	0.1	4:1	25	0.2	Standing overnight	0.500	1.85	+280
2	0.01[b]	10	0.1	4:1	25	0.2	Standing overnight	0.500	0.518	+3.6
3	0.01[b]	10	1	40:1	20	0.4	Standing overnight	0.500	3.60	+620
4	0.01[b]	5	1	20:1	25	0.2	Standing overnight	0.500	0.83	+66
5	0.01[b]	10	1	40:1	20	0.4	Standing overnight	0.500	0.485[c]	− 3.0
6	0.01	5	1	20:1	4	3	Standing overnight	0.500	0.490[d]	− 2.0
7	0.01	5	0.2	4:1	2	3	Standing overnight	0.500	0.573	+14.6
8	0.01	5	0.2	4:1	15	0.4	Standing overnight	0.500	0.545	+ 9.0
9	0.01	5	0.1	2:1	4	3	Standing for 2 hours	0.500	0.525	+ 5.0
10	0.01[b]	5	0.2	4:1	15	0.4	Standing overnight	0.500	0.505	+ 1.0
11	0.01	5	0.2	4:1	20	0.4	Shaken for 6 hours	0.500	0.548	+ 9.6
12	0.01	5	0.2	4:1	20	0.4	Shaken for 7 hours	0.500	0.528	+ 5.6
13	0.005	5	0.2	8:1	20	0.4	Shaken for 7 hours	0.250	0.242	− 3.2
14	0.005	5	0.2	8:1	20	0.4	Shaken for 10 hours	0.250	0.2525	+ 1.0
15	0.0025	5	0.2	16:1	2	3	Standing overnight	0.125	0.134	+ 7.2
16	0.0025	10	0.2	32:1	2	3	Standing overnight	0.125	0.180	+44.0
17	0.0025	5	0.2	16:1	15	0.4	Standing overnight	0.125	0.1275	+ 2.0
18	0.0025[b]	10	0.1	16:1	25	0.2	Standing overnight	0.125	0.113	− 4.8
19	0.0025	5	0.2	16:1	20	0.4	Standing for 14 hours	0.125	0.095	−24.0
20	0.0025	5	0.2	16:1	20	0.4	Shaken for 14 hours	0.125	0.127	+ 1.6
21	0.0025	5	0.2	16:1	20	0.4	Shaken for 6 hours	0.125	0.1235	− 1.2
22	0.001	5	0.2	40:1	15	0.4	Standing overnight	0.0500	0.0373	−24.6
23	0.001	5	0.2	40:1	15	0.4	Standing overnight	0.0500	0.023	−54.0

[a] 5 ml. of buffer 6.1 used.
[b] 10 ml. of buffer 3.6 instead of 5 ml. used.
[c] Reprecipitated and again allowed to stand overnight.
[d] Twice reprecipitated, second time in greater dilution.

not change its appearance beyond the first 2 or 3 hours of aging. On the other hand, the originally slimy and voluminous precipitate containing coprecipitated orthophosphate was transformed into well-developed crystals after about a day of aging. The mechanism of this aging effect presumably involves a recrystallization of cadmium pyrophosphate, during which process most of the coprecipitated orthophosphate is returned to the solution. This is indicated by the following observations:

1. Addition of the same amount of orthophosphate 30 or 60 minutes after the precipitation of cadmium pyrophosphate also improves the crystalline nature of the precipitate, but not so much as when the orthophosphate was coprecipitated.
2. When the precipitate obtained in the presence of orthophosphate from 0.0025 or 0.001 *M* pyrophosphate solutions was analyzed before the crystal perfection was visible, low values were obtained.

Table V. Precipitation of Cadmium Pyrophosphate in Presence of Calcium

(25 ml. of $Na_4P_2O_7$ solution taken, 5 ml. of buffer 3.6 used)

No.	Molarity of $Na_4P_2O_7$ Used	$CaCl_2$ Present		Molar Ratio $Ca^{++}/P_2O_7^{----}$	$CdAc_2$ Used for Precipitation		Cadmium in Precipitate Calculated	Found	Error
		Ml.	*M*		*Ml.*	*M*	*Millimole*		%
1	0.01	1	5	20:1	4	3	0.500	0.463[a]	− 7.4
2	0.01	5	5[b]	100:1	4	3	0.500	0.163[a]	−67.5
3	0.01	2.5	1	10:1	4	3	0.500	0.480[c]	− 4.0
4	0.01	0.5	1	2:1	2	3	0.500	0.480[c]	− 4.0
5	0.01	1	1	4:1	15	0.4	0.500	0.480[c]	− 4.0
6	0.01	2	1	8:1	20	0.4	0.500	0.470[d]	− 6.0
7	0.01	1	1	4:1	20	0.4	0.500	0.455[d]	− 9.0
8	0.0025	1	1	16:1	15	0.4	0.125	0.114[c]	− 8.8
9	0.0025[e]	1	1	16:1	15	0.4	0.125	0.111[c]	−11.2
10	0.0025[f]	1	1	16:1	15	0.4	0.125	0.116	− 6.7

[a] Precipitate allowed to stand for 2 hours.
[b] Mixture slightly turbid before addition of cadmium acetate.
[c] Precipitate allowed to stand overnight.
[d] Precipitate allowed to stand for 2 hours, then reprecipitated and allowed to stand overnight.
[e] 10 ml. of buffer 3.6 used.
[f] 10 ml. of 1 *M* acetic acid used instead of buffer.

These results might explain the different views expressed in the literature concerning the possibility of quantitatively determining pyrophosphate in the presence of orthophosphate by precipitation with cadmium. Depending upon the time of standing before filtration, too much, too little, or the correct amount of precipitate may be found. The time of standing necessary before the filtration can be considerably shortened by shaking the suspension.

Quantitative Determinations. Table IV contains the results of determinations of pyrophosphate in presence of orthophosphate. "Standing overnight" refers to a time of standing of 18 to 30 hours after precipitation. (The importance of the time of standing is more evident from experiments given in Table VI.)

The amount of coprecipitated orthophosphate increases with increasing pH of the buffer added. With a molar ratio of orthophosphate to pyrophosphate (0.01 *M* in the original solution) of four the error was reduced from 280 to 3.6 per cent, when instead of buffer 6.1 buffer 3.6 was used (experiments 1 and 2, Table IV). The mixture of 25 ml. of 0.01 *M* pyrophosphate solution and 5 ml. of 0.2 *M* orthophosphate solution represents approximately the upper concentration limit at which the coprecipitated orthophosphate is eliminated again on standing. For satisfactory results with this mixture addition of 10 ml. of buffer 3.6 is required (compare experiments 2 and 10 with 8, 11, and 12). Shaking the suspension did not improve the final result. When the molar ratio of orthophosphate to pyrophosphate becomes greater than four at the initial pyrophosphate concentration of 0.01 *M* a reprecipitation is necessary in order to get quantitative results (experiment 5). When 3 *M* cadmium acetate was used for the precipitation, the interference by coprecipitation of orthophosphate was more pronounced (experiments 6 and 15) than with more dilute cadmium reagent (experiments 8 and 17), and the reprecipitation in presence of larger amounts of orthophosphate had to be carried out twice (experiment 6).

From experiments 22 and 23 it is evident that the precipitation of pyrophosphate from an originally 0.001 *M* pyrophosphate solution was incomplete in the presence of a large excess of orthophosphate.

For a quantitative determination in the presence of orthophosphate an initial concentration range of 0.002 to 0.01 *M* pyrophosphate is recommended. Since a molar ratio of orthophosphate to pyrophosphate of 16 to 1 does not cause interference, a reprecipitation is necessary only in extreme cases.

The reduction of the period of time before filtration by shaking is evident from experiments 19, 20, and 21. After 14 hours of standing the cadmium content of the precipitate was 24 per cent too low, while after 6 hours of shaking the correct composition was found.

When pyrophosphate was determined in presence of calcium, a deficiency of cadmium in the precipitate was found in all experiments. Examples are given in Table V. This deficiency was presumably caused by a coprecipitation of calcium pyrophosphate only in case of a large excess of calcium and at higher pyrophosphate concentrations (experiments 1 and 2). With an excess of calcium smaller than tenfold at an original pyrophosphate concentration of 0.01 *M* no complete precipitation was achieved. This is shown by reprecipitation experiments 6 and 7, in which even somewhat greater errors were found. The time of standing of the precipitates before reprecipitating (2 hours) was sufficient for complete precipitation under the conditions of reaction in the absence of calcium. Hence, if the negative errors had been due to a slight coprecipitation of calcium pyrophosphate in the first precipitation, results closer to the theoretical value should have been found after the second precipitation. The appearance of cadmium pyrophosphate precipitated in presence of calcium is as slimy as in precipitation from pure pyrophosphate solution.

Fortunately, the results become much better when calcium and orthophosphate are present together (Table VI). In a mixture of 25 ml. of 0.01 *M* pyrophosphate, 5 ml. of 0.2 *M* orthophosphate, and 1 ml. of 1 *M* calcium chloride (experiments 1 to 6 in Table VI) no longer a deficiency but a slight excess of cadmium was found, as in the absence of calcium. The bulk of the coprecipitated orthophosphate was eliminated on aging, but a slight amount remained in the precipitate, even when the precipitate was allowed to stand for 40.5 hours or when the suspension was shaken for 5.5 hours. When, however, 2 ml. of calcium chloride were added instead of 1 ml., the precipitate showed the correct cadmium content after standing overnight and almost the correct cadmium content after shaking for 5.5 hours. This beneficial effect of the presence of a higher concentration of calcium apparently does not result from a compensation of errors, by which the amount of unprecipitated pyrophosphate would just be balanced by the amount of coprecipitated cadmium orthophosphate, for the following reasons:

1. The experiments in presence of calcium only show that the amount of unprecipitated pyrophosphate is practically the same

Table VI. Precipitation of Cadmium Pyrophosphate in Presence of Orthophosphate and Calcium

(5 ml. of 0.2 *M* KH_2PO_4 solution and 5 ml. of buffer 3.6. Precipitated with 20 ml. of 0.4 *M* $CdAc_2$)

No.	$Na_4P_2O_7$ Used		$CaCl_2$ Present		Molar Ratio $PO_4^{---}/P_2O_7^{----}$	Molar Ratio $Ca^{++}/P_2O_7^{----}$	Treatment after Precipitation	Cadmium in Precipitate		
								Calculated	Found	Error
	Ml.	*M*	*Ml.*	*M*				*Millimole*		%
1	25	0.01	1	1	4:1	4:1	Standing overnight	0.500	0.5225	+ 4.5
2	25	0.01	1	1	4:1	4:1	Standing overnight	0.500	0.570	+14.0
3	25	0.01	1	1	4:1	4:1	Standing for 20.5 hours	0.500	0.540	+ 8.0
4	20	0.01	1	1	5:1	5:1	Standing for 20.5 hours	0.400	0.417	+ 4.3
5	25	0.01	1	1	4:1	4:1	Standing for 42.5 hours	0.500	0.530	+ 6.0
6	25	0.01	1	1	4:1	4:1	Shaken for 5.5 hours	0.500	0.540	+ 8.0
7	25	0.01	2	1	4:1	8:1	Standing overnight	0.500	0.504	+ 0.8
8	25	0.01	2	1	4:1	8:1	Standing overnight	0.500	0.497	− 0.6
9	25	0.01	2	1	4:1	8:1	Shaken for 5.5 hours	0.500	0.522	+ 4.4
10	25	0.005	1	1	8:1	8:1	Shaken for 10 hours	0.250	0.245	− 2.0
11	25	0.0025	1	1	16:1	16:1	Standing for 17 hours	0.125	0.094	−24.8
12	25	0.0025	1	1	16:1	16:1	Standing for 24 hours	0.125	0.100	−20.0
13	25	0.0025	1	1	16:1	16:1	Shaken for 14 hours	0.125	0.127	+ 1.6
14	25	0.0025	1	1	16:1	16:1	Shaken for 5.5 hours	0.125	0.1225	− 2.0
15	20	0.0025	2	1	20:1	40:1	Standing for 14 hours	0.100	0.062	−38.0
16	25	0.0025	2	1	16:1	32:1	Standing for 37.5 hours	0.125	0.122	− 2.4
17	25	0.0025	2	1	16:1	32:1	Shaken for 14 hours	0.125	0.123	− 1.6
18	25	0.0025	2	1	16:1	32:1	Shaken for 5.5 hours	0.125	0.122	− 2.4

with 1 ml. or with 2 ml. of calcium solution (experiments 3 and 5 in Table V).

2. At lower pyrophosphate concentrations the coprecipitated orthophosphate is eliminated on aging in the absence of calcium, but the cadmium content of the precipitate remains the correct one in presence of calcium also.

3. The crystals of cadmium pyrophosphate precipitated from different pyrophosphate concentrations were always better developed when both calcium and orthophosphate were present than in the presence of orthophosphate alone, and the degree of perfection was greater with 2 ml. than with 1 ml. of the calcium chloride solution.

A corresponding behavior was found when the pyrophosphate was present in lower concentrations. In experiments 11 and 12 (Table VI), for example, where the precipitate was allowed to stand for 17 and 24 hours, respectively, the precipitation was very incomplete. It was complete, however, after 5.5 hours of shaking. Experiments 15 to 18 in Table VI demonstrate further the considerable gain of time by shaking the precipitate.

The minimum time of shaking required for obtaining quantitative results appeared to be dependent on the intensity of shaking. In experiments 6, 9, 14, and 18 (Table VI), which were run simultaneously, the precipitate after 4 hours of shaking was still voluminous and fluffy, but became nicely crystalline after 5 hours of shaking. In general, the precipitation is complete after the slimy precipitate has changed completely into well-developed crystals.

Procedure

A procedure for the determination of pyrophosphate in the presence of orthophosphate and of calcium is described below. In the absence of orthophosphate the simplest way to determine pyrophosphate obviously is by hydration. In many practical cases orthophosphate and calcium are present in larger amounts than is the pyrophosphate. In such instances, especially when the amount of pyrophosphate is small, the cadmium procedure seems to be superior to other methods described in the literature.

To 25 ml. of the neutral or weakly alkaline solution, which must be 0.002 to 0.01 *M* in pyrophosphate (corresponding to 7 to 35 mg. of phosphorus pentoxide), sufficient 0.2 *M* orthophosphate solution is added, if necessary, to make the final orthophosphate concentration approximately 0.03 *M*. Five milliliters of acetate buffer of pH 3.6 (8 *M* acetic acid, 1 *M* ammonium acetate) are added and 2 ml. of 1 *M* calcium chloride (or less, if calcium is present). Then 15 to 20 ml. of 0.4 *M* cadmium acetate solution are added, and the mixture is gently shaken for about 5 to 6 hours. When the originally voluminous and slimy precipitate has changed into well-developed crystals, the precipitate is filtered or centrifuged and washed with water. The cadmium in the precipitate is determined polarographically after dissolving in a few milliliters of hydrochloric acid (1 to 4), adding 25 ml. of 2 *M* potassium chloride solution, and diluting to 100 ml. in a volumetric flask.

For the determination of smaller amounts of pyrophosphate correspondingly reduced volumes are used.

The precipitate may also be weighed after drying at 250° C., but in general the polarographic method is more convenient and preferable.

If the orthophosphate content is larger than stated above, the precipitate, after shaking for 5 hours and separating from the supernatant liquid, is dissolved in a few milliliters of hydrochloric acid (1 to 4) at room temperature. The solution is neutralized immediately with sodium hydroxide (6 *M*), using Tropeolin 00 as indicator. After neutralization the volume is made about the same as in the first precipitation and the same amounts of buffer solution and of cadmium acetate are added as before.

Orthophosphate in the mixture is determined by hydrating a a part of the sample with acid at boiling temperature, determining the total phosphorus content and subtracting the pyrophosphate content[1].

Summary

A method is described for the quantitative precipitation of pyrophosphate as cadmium pyrophosphate. After filtering and washing, the precipitate can be weighed in the anhydrous form when dried to constant weight at 250° C. In general it is simpler and more practical to dissolve the precipitate in dilute hydrochloric acid and determine the cadmium polarographically. A procedure is given for the determination of 0.002 to 0.01 *M* pyrophosphate solutions in the presence of from 4 to 16 times the molar concentration of orthophosphate and from 8 to 32 times the molar concentration of calcium.

Acknowledgment

Acknowledgment is made to the Carnegie Corporation of New York for a grant which enabled the authors to carry out the present investigation.

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[1] After completion of this study Jones (5) described work in which he precipitated small amounts of pyrophosphate in the presence of a large excess of orthophosphate with manganous manganese.

Determination of Low Concentrations of Oxygen in Gas

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A method is described for determining in gases concentrations of oxygen as low as several thousandths of 1 per cent. A special type of flask is used. The other equipment and chemicals are likely to be found in most chemical laboratories. About 2 hours are required to obtain results, but during most of this period no personal attention is needed. As compared with previously published methods, this procedure is an improvement with respect to accuracy, delicacy, and facility of manipulation. It is especially adapted to analysis of gases containing highly unsaturated or easily condensable hydrocarbons.

OF THE various procedures proposed for the determination of low concentrations of oxygen in gas, the methods receiving the most attention seem to fall in three classes—namely, absorption, electrical, and oxidation-reduction. There are serious objections to all these methods. Moreover, certain industrial developments in recent months have greatly increased the need for reliable determinations of low concentrations of oxygen in gas and therefore work has been done to evolve a procedure of adequate accuracy and simplicity.

The first group of methods, absorption, represents a more or less routine procedure to the gas chemist, but for low concentrations of oxygen, a few tenths per cent or less, the results obtainable are of very doubtful value, especially for gases containing a large percentage of easily condensable hydrocarbons, such as gases produced in the petroleum and synthetic rubber industries. The electrical methods usually involve a considerable amount of expensive apparatus and at present are better adapted to recording devices than to general analytical work. The chemical procedures may be exemplified by the Lubberger-Wunsch method (*1*) and the method of Steinkampf (*3*), which is a modification of the former and appears in the "Gas Chemists' Handbook". The Lubberger-Wunsch method is almost prohibitively cumbersome and Steinkampf has remedied this difficulty at the expense of delicacy. Furthermore, both methods depend upon the production of a measurable amount of free iodine in the gas phase and obviously certain sorts of hydrocarbon gases now frequently met with industrially could not be analyzed by either procedure.

The writer recently described, in connection with hydrogen sulfide analysis, a special type of flask (*2*) that since that time has proved adaptable to the determination of oxygen in gas. This flask lends itself to a procedure that substantially eliminates difficulties encountered with other methods.

Determination of Low Concentration of Oxygen in Gas

Briefly, the method consists in shaking a sample of gas in the special flask with ferrous hydroxide freshly precipitated by sodium hydroxide from an acidified solution of ferrous ammonium sulfate previously thoroughly purged of its dissolved oxygen. The solution is then acidified and when the ferric salts are dissolved the solution is diluted to a known volume and the ferric iron formed is determined colorimetrically against potassium thiocyanate by a specified procedure. The ferric iron formed is a measure of the oxygen in the gas.

APPARATUS REQUIRED. Two test-tube gas scrubbers, 20 × 2.5 cm. (8 × 1 inch), 1 Shaw sulfur flask (*3*), 1 mechanical shaking device, two 50-ml. tall-form Nessler tubes, three 10-ml. graduated pipets, three 5-ml. graduated pipets, one 50-ml. volumetric flask, one 100-ml. volumetric flask, and one 250-ml. volumetric flask.

CHEMICAL SOLUTIONS REQUIRED. Sodium hydroxide solution, 50 per cent by weight; concentrated C. P. hydrochloric acid; approximately *N* hydrochloric acid (8.7 ml. of hydrochloric acid solution diluted to 100 ml.).

Ferrous ammonium sulfate reagent solution (15 grams of ferrous ammonium sulfate hexahydrate and 8.7 ml. of concentrated hydrochloric acid diluted to 1 liter with water).

Potassium thiocyanate solution (10 grams of potassium thiocyanate diluted to 1 liter).

Standard solution of ferric iron [0.86 gram of ferric ammonium alum dissolved in water and diluted to 100 ml.; 10 ml. of this solution and 1.0 ml. of concentrated hydrochloric acid are diluted to 1 liter. This solution contains 0.01 gram of ferric iron per liter and 1 ml. of the solution is equivalent to 0.001 ml. of dry oxygen at normal temperature and pressure or 0.00105 ml. of oxygen at 60° F. and 76.2 cm. (30 inches) of mercury.]

Sulfuric acid solution, 20 per cent by volume, and potassium hydroxide solution, 30 per cent by weight.

PROCEDURE. Place the two test-tube scrubbers on the sampling line, charging the first one with 25 ml. of 20 per cent sulfuric acid and the second with 30 ml. of 30 per cent potassium hydroxide solution. This amount of alkali is sufficient to purify at least 140 liters (5 cubic feet) of coke-oven gas having a carbon dioxide concentration of 3 per cent. Pass a stream of gas through this train at a rate of from 28.3 to 56.6 liters (1 to 2 cubic feet) per hour, and purge with at least 5.66 liters (0.2 cubic feet) of gas. At the end of this time, with the gas still flowing, attach to the outlet of the train a Shaw sulfur flask (the volume of which has been determined by water displacement) charged with 15 ml. of the ferrous ammonium sulfate reagent solution and purge the flask with at least 14 liters (0.5 cubic feet) of gas. At the end of this period close the outlet valve of the flask and then the inlet valve and remove from the sampling line. Adjust the pressure by opening the outlet valve momentarily and take temperature and barometer readings. With a graduated pipet place 25 drops of a 50 per cent sodium hydroxide solution in the funnel neck of the flask, cool the gas space slightly in water, allow the sodium hydroxide to flow slowly into the flask through the split port in the stopper, and wash the residual caustic into the flask with two portions of water of 1 to 2 ml. each. It is important that no air shall be sucked in with these reagent solutions.

Now place the flask in a mechanical shaker and agitate vigorously for one hour. At the end of this time chill the flask in cold water or evacuate thoroughly if unsaturated hydrocarbons are suspected, place exactly 10 ml. of concentrated hydrochloric acid in the funnel top, and draw it into the flask, wash the residual acid in with two washings of water, and shake the whole thoroughly to mix. If the ferric hydroxide does not go into solution more or less immediately, place the flask in hot water for a while, with the vent in the stopper open, provided the flask has not been previously well evacuated.

When all the iron salt is in solution, cool the contents of the flask, wash into a volumetric flask of suitable size, and dilute to the mark, after adding enough hydrochloric acid to produce a solution having a free acid concentration of approximately normal strength. For this purpose it is sufficient to consider that the

25 drops of 50 per cent sodium hydroxide neutralized 2.0 ml. of the 10 ml. of hydrochloric acid added and that 8.7 ml. of concentrated hydrochloric acid diluted to 100 ml. will yield an acid solution of normal strength. Then, if the solution of ferric iron mentioned is to be diluted to 100 ml., first add 0.7 ml. more of concentrated hydrochloric acid, or 13.7 ml. of acid if it is to be diluted to 250-ml. volume. A normal concentration of acid in this solution has no inherent virtue, but it is a concentration of the desired order of magnitude and a convenient means of keeping a reasonably accurate knowledge of the amount of acid present in the solution. This fact is of importance in the subsequent color comparison, as in the standard and the unknown tubes the acid concentrations must be substantially equal. This solution is now ready for the colorimetric ferric iron determination.

Colorimetric Comparison. In each of two Nessler tubes place 10 ml. of the potassium thiocyanate solution. Into one tube put an aliquot of the sample sufficient to develop a color equivalent to that produced by not more than 5 ml. of the standard solution. If necessary, add sufficient *N* hydrochloric acid solution to produce at least 2.5 ml. of normal acid in the tube and dilute to 20 ml. with water. To the other tube add sufficient *N* hydrochloric acid to equal the total amount of acid in the first tube and follow with a measured amount of iron standard to nearly equal the color in the unknown tube. Dilute to 20 ml. with water and adjust the colors to match with additional standard solution as required. The milliliters of standard used times the dilution factor equals the total milliliters of standard required for the sample. From this figure it is necessary to deduct a solution blank, which is determined as described below.

Solution Blank. Place 25 drops of 50 per cent sodium hydroxide and 15 to 20 ml. of water in a 50-ml. volumetric flask, add exactly 10 ml. of concentrated hydrochloric acid, and cool the solution. Add 15 ml. of the ferrous ammonium sulfate reagent solution and dilute to the mark. To each of two Nessler tubes add 10 ml. of the potassium thiocyanate solution. To one of these tubes add 5 ml. of the blank solution and dilute to 25 ml. with water. To the other tube add 9.2 ml. of the *N* hydrochloric acid and enough standard iron solution nearly to match the color in the first tube. Dilute to 25 ml. with water and add sufficient additional standard to make the colors match. Then ten times the milliliters of standard solution equal the milliliters of total solution blank.

In the writer's experience the total solution blank, using a fresh ferrous ammonium sulfate reagent solution, is in the order of magnitude of 15 ml. of standard solution. Under the specified procedure for oxygen determination the reagent solution is diluted finally to known volume and an aliquot placed in the comparison tubes. It therefore follows that this blank of 15 ml. is to be deducted from the milliliters of standard solution calculated to be required for the known volume to which the reagent solution has been diluted. For a gas containing 0.1 per cent oxygen this is approximately 100 ml. The blank is therefore about one seventh of the volume of standard solution required for the comparison. This ratio will, of course, vary with change in oxygen content of the gas.

Calculations.

$$\frac{(\text{Total ml. of standard} - \text{ml. for solution blank}) \times 0.1}{\text{ml. of gas sample (N. T. P.)}}$$

If gas is corrected to standard conditions, substitute 0.105 for 0.1 in the numerator.

Table I. Summary of Test Data Used as Basis for Method

(Data in table involved the deduction of two blanks—the customary solution blank and a blank on the nitrogen purified by passing through several units charged with sodium pyrogallate and chromous chloride)

Date	Oxygen (N. T. P.) Added	Oxygen (N. T. P.) Found	Oxygen Added to Gas	Added Oxygen Found	Total Oxygen in Gas
	Ml.	*Ml.*	%	%	%
12/10	...	0.011	0.008 purified N_2	...	...
	0.820	0.820	0.595	100.0	0.158
12/11	...	0.0238	0.017 purified N_2	...	...
	0.175	0.176	0.143	100.2	0.126
12/14	...	0.0222	0.0158	...	...
	...	0.0222	0.0158	...	...
	0.0401	0.0415	0.045	103.0	0.030
	0.0401	0.0415	0.045	103.0	0.030
12/16	...	0.0225	0.0159	...	...
	0.078	0.077	0.071	98.6	0.055
12/27	...	0.065	... (N_2 not purified)	...	0.054

Table II. Determination of Oxygen in Compressed Clean Coke-Oven Gas

(Ammonia and hydrogen sulfide added during sampling)

Date	Oxygen Added 1	Oxygen Found 2	Oxygen Found in Unknown Sample (Column 2 Minus Column 1)
	%	%	%
12/20	None	0.38	0.38
	0.13	0.53	0.40
12/21	0.13	0.49	0.36

Table III. Determination of Oxygen in Pyrolysis Gas

(Containing 50% of easily condensable unsaturated hydrocarbons)

New Method	Absorption Method[a]
%	%
0.074	0.4
0.161	0.4

[a] 100-ml. gas buret and chromous chloride absorption solution.

Discussion of Method

Volatile bases, hydrogen sulfide, hydrogen cyanide, and certain unsaturated organic compounds will interfere with the procedure by reducing the ferric iron if they are present when the solution is rendered acid. All these substances except the unsaturates can be readily removed from the gas by scrubbing it with acid and alkali. The unsaturated hydrocarbons can be taken out by evacuation of the flask just before the addition of the acid. The acid and alkali scrubbing solutions have the further advantage that only neutral oxidizing agents can get into the reaction flask. This fact would seem to eliminate substantially all oxidizing agents other than oxygen and possibly peroxides.

A half-hour shaking period did not seem sufficient to complete the removal of oxygen from the sample.

The method is theoretically capable of estimating about 0.001 per cent of oxygen, but at such a low concentration the blank becomes relatively very high. Actually check tests were obtained showing 0.008 per cent of oxygen in specially purified nitrogen. Where the concentration of oxygen in the gas is greater than 1 per cent, some difficulty may be experienced in getting the ferric hydroxide in solution. Such trouble may be minimized by adding greater known volumes of acid, followed by a higher final dilution.

Where the ferrous ammonium sulfate reagent solution is not stored in an inert atmosphere it is preferable to make up a fresh solution every 48 hours. When working with a gas having an oxygen concentration of 0.1 per cent or more, one blank determination in an 8-hour period is satisfactory for most purposes. The author's experience has indicated that an error of not greater than 2 per cent is introduced by this procedure on such a gas. In determining very low concentrations of oxygen or where exceptional accuracy is required it is desirable to determine an over-all blank on the procedure, but this step is somewhat cumbersome and the author's work has indicated that for most purposes it is unnecessary.

Satisfactory agitation is produced by clamping the flask to a 0.95-cm. (0.375-inch) rod with laboratory fittings, the rod being reciprocally operated at a 45° angle by an eccentric having a 10-cm. (4-inch) stroke and traveling at 120 r. p. m.

Special care must be taken to make sure that all connections are vacuum-tight. While the flask is under vacuum it is well to maintain a few milliliters of water in the funnel top to prevent air seepage into the flask. After the solution in the flask has been acidified small air leakages are unimportant.

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Photometric Determination of Silica in the Presence of Phosphates

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THE determination of silica in the presence of phosphates is of importance in phosphate-treated boiler waters and in hot-process phosphate softeners where special attention is being given to silica removal.

The most widely used colorimetric method for silica depends upon the formation of a colored silicomolybdic acid (*5, 7–9, 11–13, 17, 18, 21–24, 27, 29, 32, 36–39;* for a comprehensive survey of the literature up to 1938 see reference *29*). Since phosphate ions react similarly, special attention must be given to the determination of silica in the presence of phosphates. The interference of phosphates has been removed by precipitation prior to colorimetric determination: King (*14*) used magnesia mixture; Thayer (*33*) used calcium chloride; Liebknecht, Gerb, and Bauer (*20*) used calcium chloride and calcium carbonate; King and Stantial (*16*) used calcium chloride and ammonium hydroxide; Schwartz (*28*) used calcium chloride and a sodium borate–sodium hydroxide buffer solution.

Table I. Effect of Oxalic Acid

PO_4	Oxalic Acid[a]	Transmittance[b]
P. p. m.	*Ml.*	%
5	0	69
	1	70
	2	88
	3	94
	4	94
25	0	28
	1	33
	2	79
	3	94
	4	94
50	0	15
	1	21
	2	79
	3	94
	4	94

[a] 100 grams of $(COOH)_2.2H_2O$ per liter.
[b] Filter photometer, filter No. 511, 3 mm.; 50-mm. liquid depth.

Precipitation methods for removal of interfering phosphate are, at best, time-consuming. Another method for avoiding phosphate interference depends upon the effect of pH upon the reduction of the phospho- and silicomolybdic acid complexes; with proper pH adjustment the silicomolybdic acid complex alone is reduced. A recent paper illustrating this method is by Kahler (*12*).

However, the interference of phosphates is most simply avoided by destroying the phosphomolybdic acid complex with various acids and some of their salts—e. g., oxalic, tartaric, citric, and phosphoric (*1, 4, 6, 19, 25, 26, 34, 35, 39*). On the basis of this fundamental information and further experimental work a procedure has been developed for determining silica in the presence of phosphates, using oxalic acid for destroying the interfering colored phosphomolybdic acid. Although it is known that oxalic acid reduces any interfering effect from the presence of tannins, consideration of interference from tannin compounds has been omitted from this paper because of the varying nature of commercial tannin compounds.

Apparatus and Reagents

A filter photometer (*30*) and a Coleman double monochromator spectrophotometer were used for colorimetric analyses of silicate and phosphate concentrations.

Ammonium molybdate solution, 10.0 grams of ammonium molybdate tetrahydrate per 100 ml. of distilled water.

Hydrochloric acid solution, one volume of concentrated acid to one volume of distilled water, 1 to 1.

Oxalic acid solution, 10.0 grams of oxalic acid dihydrate per 100 ml. of distilled water.

Sodium silicate solution. Fuse 3.0 grams of sodium carbonate with 0.200 gram of pure, dry silica. Dissolve in 200 ml. of distilled water. Dilute tenfold if desirable.

Potassium dihydrogen phosphate standard solution, 1.4328 grams of potassium dihydrogen phosphate per liter of distilled water.

The ammonium molybdate, hydrochloric acid, and oxalic acid solutions are kept in Pyrex bottles. The sodium silicate solution is stored in a hard-rubber bottle.

Procedure

Based partly on previous work (*3, 28*) and the existing literature and the remainder on material developed in this paper, the following procedure is recommended.

Filter the sample if necessary, and dilute with distilled water to suit the range of the measuring instrument (filter photometer, spectrophotometer, etc.). Add and mix 4 ml. of ammonium molybdate solution and 2 ml. of 1 to 1 hydrochloric acid solution in rapid succession to a 100-ml. sample. Wait 5 to 10 minutes for full color development, then add and mix 3 ml. of oxalic acid solution. Determine silica color intensity with suitable instrument.

The reagents may be suitably proportioned to the size of sample used and their strengths may be changed to give equivalent values when using different volumes of reagents other than those given. A blank correction should be made to correct for silica in the reagents and water, if necessary.

Experimental

The color developed by various concentrations of phosphate ion after adding ammonium molybdate and hydrochloric acid solutions was destroyed by addition of oxalic acid. The data of Table I show that 300 mg. of oxalic acid dihydrate are sufficient to destroy the phosphomolybdic acid complex formed. Tschopp and Tschopp (*34*) indicate the reaction to be in the proportion of 1 MoO_3 to 1 $(COOH)_2$. Since the phosphomolybdic acid complex is formed in the proportion of 12 MoO_3 to 1 PO_4, it will be seen that 284 mg. of oxalic acid are sufficient to destroy all the molybdate added. This amount of molybdate is equivalent to approximately 180 p. p. m. of PO_4. The residual absorption of 6 per cent shown in the table is the blank correction with the filter combination used.

Table II. Effect of Oxalic Acid

Original SiO_2	PO_4 Added	Transmittance[a] Before adding oxalic acid	Transmittance[a] After adding oxalic acid
P. p. m.	*P. p. m.*	%	%
5	0	80.5	80.5
5	5	75	80.5
5	25	58	80.5
5	50	46.5	80.5
10	0	67	67
10	5	63	67
10	50	42	67
20	0	52.5	52.5
20	5	50	52.5
20	50	37	52.5
50	0	32	32
50	5	31	32
50	50	27	33

[a] Filter photometer, filter No. 511, 1 mm., and No. 038, 2 mm.; 50-mm. liquid depth, silica as sodium silicate.

The data of Table II serve to show that, for the range of silicate- and phosphate-ion concentrations employed, oxalic acid completely destroys the interference of phosphate without affecting the color due to the silicomolybdic acid. If appreciably higher concentrations are encountered, the amount of the oxalic acid may have to be increased proportionally.

The method, as described above, was tried out and compared with the author's earlier method (*28*) on phosphate-conditioned boiler water at the Louisiana Station power plant of the Gulf States Utilities Co. The tests were carried out on a routine basis, by two different men and using a different photoelectric photometer from the one described in this article. The data of Table III indicate satisfactory agreement under the conditions described.

Table III. Boiler Water Silica[a]

(P. p. m. of silica)

$CaCl_2$–Borate Buffer Method	Oxalic Acid Method
25.8	25.7
33.0	33.9
31.2	30.8
24.0	24.7
27.0	26.8
31.2	31.9

[a] Phosphate-conditioned boiler water, Gulf States Utilities Co.

Silica Standards

The preparation of silica standards from silicon compounds has been troublesome because of the apparent lack of stability of such solutions. Previous unpublished work, which has been substantiated during the course of this research, has shown that sodium silicate solutions, as prepared in this paper and also from sodium silicate nonahydrate, are surprisingly stable in hard rubber bottles. The data are presented in Table IV.

The use of liquid standards—e. g., potassium chromate and picric acid—as silica standards is in a somewhat confused state.

In 1914 Winkler (*40*) proposed, for the first time, the use of potassium chromate. He stated that 1 ml. of a solution containing 0.530 gram per 100 ml. was equivalent to 1 mg. of silica. His procedure was to add to a 100-ml. sample 1 gram of solid ammonium molybdate and 5 ml. of 10 per cent hydrochloric acid. He then took 105 ml. of distilled water and added potassium chromate solution until a color match was obtained. The number of milliliters used times 10 equaled p. p. m. of silica.

In 1923 Dienert and Wandenbulcke (*10*) proposed the use of picric acid, stating that a solution containing 36.9 mg. per liter was equivalent to 50 p. p. m. of silica. Their procedure was to add to a 50-ml. sample 2 ml. of 10 per cent ammonium molybdate solution and 4 drops (less than 0.5 ml.) of 50 per cent ("au 1/2") sulfuric acid. Comparison was made with picric acid solutions.

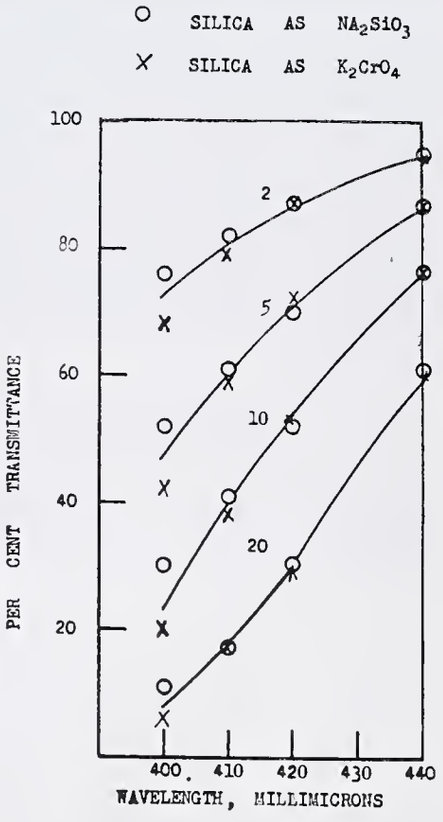

Figure 1

Table IV. Stability of Sodium Silicate Solution[a]

Time *Days*	SiO_2[b] *Gram*
0	0.1057
8	0.1061
35	0.1047
65	0.1054

[a] Stored in hard-rubber bottle.
[b] Silica gravimetrically determined by J. L. Matherne.

In 1928 King and Lucas (*15*), using the same procedure as Dienert and Wandenbulcke, found that purification and vacuum-drying of picric acid changed its relationship as a standard, so that a solution containing 25.6 mg. per liter was now equivalent to 50 p. p. m. of silica.

In 1933 the American Public Health Association (*2*) adopted the Winkler potassium chromate standard but used a different procedure, calling for the use of a 50-ml. sample to which was added 5 ml. of a solution containing 200 ml. of 1 to 1 by volume hydrochloric acid, 30 grams of ammonium molybdate, and 400 ml. of distilled water. One milliliter of potassium chromate solution, containing 0.530 gram per 100 ml. diluted to 55 ml., was considered equivalent to 20 p. p. m. of silica when a 50-ml. sample was tested as described.

In 1934 Swank and Mellon (*31*), comparing picric acid and potassium chromate spectrophotometrically with sodium silicate solutions, proposed the use of buffered potassium chromate solutions as a standard. Using 5 ml. of the A. P. H. A. reagent, described above, and a 50-ml. sample, they proposed a standard containing 0.630 gram of potassium chromate per 100 ml. of water. One milliliter of this solution diluted to 55 ml. is considered equivalent to 20 p. p. m. of silica. In diluting the chromate solution to a final volume of 55 ml., one uses 25 ml. of a 1 per cent solution of borax plus distilled water. As Swank and Mellon pointed out, variations in the volume of sample and reagents, or a change in the concentration of the reagents, may require a different concentration of potassium chromate standard.

As a result the author deemed it advisable to check the Swank and Mellon buffered chromate standards (*31*) against silica standards whose color is developed with the reagents given in this paper. The buffered potassium chromate solutions were prepared from a solution containing 0.63 gram of potassium chromate per liter. One milliliter of solution, diluted with 25 ml. of a 1 per cent solution of sodium tetraborate, made up to 50 ml. with distilled water, plus 3 ml. of water to make a total of 53 ml., is assumed equivalent to 2 p. p. m. of silica.

The data in Figure 1 and Table V, obtained with the Coleman spectrophotometer show, that under the conditions described, the potassium chromate standards are good color matches for silicomolybdic solutions at wave lengths at or above 410 millimicrons, in which range of the spectrum Beer's law is followed. Using the filter combinations Corning No. 511, 4-mm., No. 038, 2-mm., with the filter photometer, the chromate and silicate standards check well.

Table V. Liquid Standards

Concentration *P. p. m. SiO_2*	Per Cent Transmittance[a] *Wave Length, Millimicrons*				
	400	410	420	440	470
2	68	79	87	94	98.5
5	42	59	72	87	98
10	20	38	53	77	95.5
20	6	17	29	59	91.5

	Per Cent Transmittance[b]	
	As SiO_2	As K_2CrO_4 (buffered)
2	83.5	83
5	64.5	64.5
10	44	44
15	32	31
20	24	24
25	19	18

[a] Coleman double monochromator spectrophotometer, 19-mm. liquid depth, SiO_2 as buffered K_2CrO_4.
[b] Filter photometer, filters No. 511, 4 mm., and No. 038, 2 mm.; 50-mm. liquid depth.

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Spectrophotometric Determination of Magnesium by Titan Yellow

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FOLLOWING Kolthoff's (*6*) introduction of Titan yellow as a reagent for magnesium, his method has been adapted to various determinations of this element (*7*). A limited amount of spectrophotometric data is available (*10*). The method has been recommended for the colorimetric determination of magnesium in water (*6*, *8*, *9*, *10*), but there has been no attempt to develop a standard spectrophotometric procedure and to compare it with the official gravimetric method. The present research was undertaken for this purpose, since it seemed likely that there could be worked out a rapid spectrophotometric procedure, comparing favorably in accuracy with the official gravimetric method, and less subject to uncertainties than standard volumetric methods. The increasing availability of fine yet comparatively inexpensive spectrophotometers also made this work seem worth while.

Principle of the Method

When magnesium hydroxide is precipitated in the presence of Titan yellow by sodium hydroxide the yellow color of the reagent changes to red or orange-red. With dilute magnesium solutions the lake which is formed remains dispersed for rather long periods, particularly in the presence of protective colloids such as starch, agar, or dextrin, and the suspension appears clear to the eye. In the presence of calcium ion the red color is deeper, and the suspension has a minimum transmittance at a wave length of 525 millimicrons when compared spectrophotometrically with a suitable blank. Transmittance readings are highly reproducible and sufficiently constant to allow ample time for precise observation. The lake is apparently kept in suspension by electrostatic forces created by the calcium salt and the protective colloid, since suspensions prepared without either calcium sulfate or starch, or similar materials, flocculate readily or give transmittance readings which are not reproducible. Statements made in this connection by Hirschfelder and Serles (*5*) in criticizing Becka's (*2*) procedure should not be interpreted as meaning that accurate analyses cannot be made in the presence of excess calcium.

Apparatus and Reagents

A Coleman 10-S-30 spectrophotometer was used to measure transmittances. Matched square cuvettes 1.308 cm. in depth were used to hold the blanks and test solutions. Measurements were made at temperatures from 25° to 29° C.

Several sets of reagents were prepared during the work. The chromogenic agent was a 0.05 per cent solution of Eastman's Titan yellow. Calcium sulfate solution was made by stirring 5 grams of reagent grade calcium sulfate in contact with 1 liter of distilled water for about 4 hours and filtering the liquid. One per cent starch solution was prepared every few days from soluble starch. Normal sulfuric acid and 2 *N* sodium hydroxide were prepared in the usual manner.

Table I. Concentration-Transmittance Data for Magnesium by Titan Yellow

Magnesium *Mg./100 ml.*	Transmittance %	Magnesium *Mg./100 ml.*	Transmittance %
0.1000	83.9	0.700	31.8
0.200	70.6	0.800	28.8
0.300	59.5	0.900	26.8
0.400	50.1	1.000	25.3
0.500	42.4	1.100	23.8
0.600	36.3		

Calibration Experiments

Calibrations were made by the procedure described below for analyses. In some cases the test solutions contained quantities of magnesium accurately measured by dilution of primary standard solutions of magnesium sulfate whose concentrations were established by several gravimetric analyses. Additional calibration points were also calculated from the transmittances of the test solutions prepared with different-sized portions of all the waters analyzed, the corresponding magnesium concentrations being established by the official gravimetric analyses. The calibration data in Table I are values read at even concentrations from the smooth curve drawn through the fifty-odd calibration points obtained by these two methods, to minimize the accidental errors graphically. It is convenient to construct the final calibration curve with these data on Coleman C-202 concentration-transmittance semilogarithmic paper.

Procedure

After any colloidal sulfur or hydrous ferric oxide in the water to be analyzed has settled out, two or three different-sized samples are measured accurately into 100-ml. volumetric flasks. The samples should contain between 0.1 and 1.1 mg. of magnesium.

Table II. Summary of Magnesium Analyses

Water	Spectrophotometric Analyses Sample *Ml.*	Trans-mittance %	Mg found *P. p. m.*	Gravimetric Analyses, Mg Found *P. p. m.*
Tyler city	100.0	73.0	1.8	2.0
	150.0	62.6	1.8	
	200.0	58.7	1.6	
Waco city	40.0	79.7	3.3	4.4
	46.0	73.2	3.9	
Dallas city	40.0	72.9	4.5	4.9
	46.0	70.0	4.5	
Austin city	20.0	62.4	13.6	11.6
	30.0	53.1	12.3	
	40.0	44.5	11.8	
Corpus Christi city	20.0	66.2	11.9	11.6
	40.0	45.6	11.4	
San Antonio city	10.0	76.6	15.4	15.8
	20.0	58.9	15.3	
	25.0	51.5	15.4	
	30.0	46.6	14.8	
Austin, Capitol fountain (artesian)	20.0	35.7	30.7	29.6
	25.0	29.6	30.8	
Austin, Scout pool (artesian)[a]	10.0	50.5	39.6	39.1
	20.0	28.8	40.0	
	25.0	24.6	41.7	
Synthetic Arkansas city Rosborough Springs[b]	10.0	43.6	48.4	49.0
	1.00	69.0	213	211
	2.00	48.2	212	
	3.00	34.6	212	
	5.00	24.3	213	
San Antonio, Terrell wells[c]	0.500	81.4	232	231
	1.00	67.0	231	
	2.00	45.4	230	
	5.00	23.2	227	
Sea at Galveston	0.250	64.0	1032	1027
	0.500	42.1	1010	
	0.750	29.4	1038	
	1.00	24.7	1037	

[a] Precipitated hydrous ferric oxide and sulfur on standing.
[b] Heavy hydrous ferric oxide precipitate on standing.
[c] Heavy sulfur precipitate on standing.

For waters requiring samples less than 10 ml. a microburet readable to 0.001 ml. may be used. The largest sample that can be used is about 49 ml. and if the water contains less than 2 p. p. m. of magnesium the samples should be acidified slightly with hydrochloric acid and concentrated by evaporation, preferably in platinum, since inconsistencies are sometimes observed when ordinary glassware or porcelain is used.

To each volumetric flask are then added in order 1 ml. of 1 *N* sulfuric acid, 10 ml. of 1 per cent starch solution, 20 ml. of saturated calcium sulfate solution, 10.0 ml. of 0.05 per cent Titan yellow solution, and 10 ml. of 2 *N* sodium hydroxide. The solution is made to exactly 100 ml. with distilled water, poured into a 250-ml. glass-stoppered Pyrex Erlenmeyer flask, and shaken vigorously for 5 minutes. A portion of the suspension is then transferred to a cuvette of the spectrophotometer and as soon as air bubbles have disappeared the transmittance relative to a blank is determined at a wave length of 525 millimicrons. The blank is prepared in exactly the same manner as the test solution, except that the unknown water sample is not added. The blank is good only for any one day's determinations.

The milligrams of magnesium for the samples taken are read from the calibration graph, converted to p. p. m., and averaged. The complete analysis requires about 20 minutes.

Table III. Analyses of Concentrated Magnesium Chloride Solution

Spectrophotometric Analyses Aliquot *Ml.*	Transmittance %	$MgCl_2$ found *Wt. %*	Gravimetric Analyses, $MgCl_2$ Found *Wt. %*
0.500	64.1	31.4	32.1
0.750	51.4	31.6	31.6
1.50	29.4	31.8	..

Table IV. Effect of Iron on Magnesium Analyses

Water	Iron Added *P. p. m.*	Mg Found (Spectroscopic) *P. p. m.*	Mg Found (Gravimetric) *P. p. m.*
San Antonio city (10-ml. samples)	0	15.4	15.8
	1	15.0	
	5	14.4	
Austin, Scout pool (10-ml. samples)	0	39.6	39.1
	1	39.7	
	5	41.1	

Summary of Results

The above procedure has been compared with the official gravimetric method for various Texas waters covering a wide range of magnesium, calcium, and iron concentrations. No analyses were made for the latter two elements, since in most cases sufficient data are available elsewhere (*3*). The spectrophotometric analyses were made without removal of iron and calcium. In the gravimetric analyses iron and calcium were removed in the usual manner with ammonium hydroxide and ammonium oxalate, and magnesium was determined by the official method (*1*). Results of the analyses are summarized in Table II.

In Table III similar data are given for analyses of a highly concentrated magnesium chloride solution, such as is obtained in the commercial preparation of magnesium metal. This solution contained 0.12 per cent of calcium, 0.11 per cent of sulfate, and 90 p. p. m. of manganese, and had a density of 1.281 at 29° C. A 5.00-ml. sample was diluted to a liter and aliquots were taken for analysis as indicated in the table.

Over the wide range of concentrations represented by the data in Tables II and III the agreement between the results obtained by the official gravimetric and spectrophotometric methods is excellent, particularly after they are rounded off according to standard procedure. It thus appears that for most practical purposes the Titan yellow method is sufficiently specific and accurate for magnesium in the presence of potentially interfering substances normally found in municipal water supplies. Moreover, interferences have been

studied thoroughly (*4*, *7–10*) and may be taken into account if necessary.

The effect of varying the iron concentration was studied for two water samples containing relatively small and large amounts of magnesium, respectively, and the results are summarized in Table IV. Iron in natural waters rarely reaches concentrations which would interfere seriously in the spectrophotometric procedure.

Although the amount of sulfide in the water samples was not determined, some of the ground waters were known to contain considerable amounts, since they yielded colloidal sulfur on standing exposed to air. The resulting error is negligible if the sulfur is allowed to settle out.

Summary

Various natural and treated waters and concentrated magnesium solutions may be analyzed rapidly and accurately for magnesium by a spectrophotometric adaptation of Kolthoff's Titan yellow method. For most practical purposes the interferences likely to be encountered do not appreciably affect the results. The procedure is recommended for control use.

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Determination of Rotenone

Improvements in the Gravimetric Method

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FOR the determination of rotenone in plant materials the method adopted as official by the Association of Official Agricultural Chemists (*1*) is most widely used. The procedure involves room-temperature extraction with chloroform, crystallization as a solvate from carbon tetrachloride solution, and purification of the solvate by allowing it to stand in alcohol. A variation developed by Jones (*2*), and until recently extensively used by the writer of this paper, depends upon the isolation of the rotenone as a dichloroacetic acid solvate. This variation has been found satisfactory in this laboratory, but has met with some objection from others because of the difficulty of obtaining the solvate free from interfering gummy material.

In order to get a shorter and simpler procedure the official method has been varied with respect to the mode of extraction and purification of the solvate.

Procedure for Powdered Roots

Although parts of the official method are duplicated more or less, for the sake of continuity and ease of following the method through, the procedure will be completely outlined.

Weigh a sample of finely powdered root of such size that the quantity of extract finally obtained and used for analysis will contain at least 1 gram of rotenone. This will not be possible in the case of roots of exceptionally low rotenone content, and the method is modified accordingly, as noted later. Transfer the powder to a 1-liter round-bottomed flask, preferably one having a standard glass joint. Add 10 grams of decolorizing carbon and then an accurately measured volume of chloroform at some definite (near room) temperature in the ratio of 10 ml. to each gram of root used. Connect the flask to an efficient reflux condenser—that is, one shown to be able to conserve all the chloroform—and boil the contents for 1 hour. If there is any question as to the efficiency of the condenser, the flask and contents should be weighed before heating and any loss in weight made up by the addition of fresh chloroform.

As soon as the condenser has drained, remove the flask, immediately stopper it, and cool it first under the tap and then in the refrigerator for 30 minutes. Filter the contents rapidly through a fluted filter, keeping the funnel covered with a watch glass to avoid evaporation of the solvent.

Bring the filtrate back to the original temperature by immersing the flask in warm water and shaking until the chosen temperature is reached. Measure the volume of the filtrate in a graduate to the nearest milliliter, and calculate the proportion of the sample that it represents. Transfer the chloroform extract to a round-bottomed flask, distill until about 25 ml. remain, and then transfer this remainder to a 125-ml. Erlenmeyer flask with a little chloroform and distill almost to dryness; in this way most of the solvent is recovered. Remove the last traces of solvent by warming slightly under diminished pressure and evaporating twice more after adding two small portions of carbon tetrachloride.

Dissolve the residue by warming under a reflux condenser with exactly 25 ml. of carbon tetrachloride, cool, and seed if necessary to induce crystallization. If at this point crystallization does not take place readily or occurs only in small amount because of low rotenone content, add an accurately weighed quantity of pure rotenone to bring the amount up to at least 1 gram, and keep the flask at 0° C. overnight.

Pour the contents of the flask quickly through a small fritted- or sintered-glass funnel of medium porosity, allowing the flask to drain as completely as possible. (These glass funnels and also crucibles have been found very useful by the author and can be cleaned easily with acetone and used repeatedly.) Continue the suction for about 5 minutes after the solvent has drained off. Place the flask in which the solvate was crystallized, and which still contains some crystals adhering to the sides, under the funnel containing the filtered solvate. Dissolve the solvate by pouring small portions of acetone through the funnel, about 25 ml. usually being sufficient. Evaporate the acetone solution of the solvate to dryness on the steam bath, and then place it under gentle suction for a short time to remove all traces of acetone.

Treat the residue from the acetone with 25 ml. of ethyl alcohol saturated with rotenone at room temperature, and boil the contents, still in the flask originally used for crystallization of the solvate, gently under reflux until completely dissolved. If necessary, add more alcohol to effect complete solution. Allow the alcohol solution to come to room temperature. Usually crystallization begins readily, but it may be induced, if necessary, by adding a crystal of pure rotenone; it then usually proceeds very rapidly. Shake the flask vigorously for 2 full minutes, and allow to stand at room temperature for 30 minutes.

Filter the rotenone crystals through a weighed sintered-glass or Gooch crucible, using about 10 to 15 ml. of alcohol saturated with rotenone at room temperature to transfer and wash them. After aspiration for 3 to 5 minutes dry to constant weight at 105° C., which takes about 30 minutes.

Weigh the final product, which consists of purified rotenone, add 0.07 gram to correct for the solubility in 25 ml. of carbon tetrachloride, and subtract any amount of pure rotenone that may have been previously added. Multiply the net weight by 100 and

Table I. Comparison of Boiling Method with Complete Extraction of Rotenone from Derris and Cube Roots

Sample No.	Root	Total Extractives	
		Boiling method	Complete extraction
		%	%
1	Cube	22.1	22.0
2	Derris	14.2	14.1
3	Derris	13.3	13.3
4	Cube	15.1	14.9
5	Derris	19.7	19.9

Table II. Determination of Rotenone in Root Samples

(Proposed Method as Compared with the A. O. A. C. Method)

Sample No.	Root	Rotenone	
		By proposed gravimetric method	By A. O. A. C. method
		%	%
1	Derris	3.8	3.9[a]
2	Derris	4.8	4.8[a]
3	Derris	4.6	4.5
4	Cube	4.8	4.8
5	Cube	5.0	5.1
6	Cube	4.8	4.7
7	Derris	5.9	5.7
8	Derris	3.6	3.7
9	Derris	8.9	8.6
10	Derris	4.4	4.2

[a] Analyzed by titrimetric variation instead of complete A. O. A. C. method.

divide by the weight of sample represented by the aliquant taken, to obtain the percentage of pure rotenone.

Discussion of the Method

It is agreed that chloroform is one of the best and most convenient solvents for the extraction of the powdered root, and the official method recommends shaking a weighed quantity of powdered root and decolorizing carbon with a definite volume of chloroform for not less than 4 hours, preferably interrupting the process with an overnight rest, or else shaking continuously overnight. The author accomplishes the same result by refluxing for only 1 hour.

To show that 1 hour of refluxing will give sufficient extraction of the rotenone, five root samples were extracted in two ways. First, they were completely extracted in a Butt-type extractor with chloroform, the solvent was distilled off, and the residue dried in an oven at 105° C. overnight and weighed. Other portions of the same samples were then boiled with chloroform as in the proposed method, except that the charcoal was omitted, since it absorbs part of the nonrotenone resins and would cause low results for total extract. An aliquant was then taken, the solvent distilled off, and the residue dried and weighed as before. It can be assumed that under these conditions the weight of total extractives would be proportional to the weight of rotenone, and would give a measure of the completeness of extraction. Table I gives the results obtained.

The results show that, for all practical purposes, one method of extraction is as good as the other. The boiling method, however, has some advantages over any other extraction method. It greatly reduces the extraction time, and it does not require a shaking machine or any special extraction apparatus. It also enables much larger samples (up to 200 grams with a reduction in proportion of solvent) to be conveniently handled in one container.

By using the entire filtrate instead of a fixed aliquant it is possible to recover all the rotenone and solvent present in one step, in the course of the method; hence, small portions of solution do not have to be accumulated for subsequent recovery.

In the proposed method the contents of the flask containing the rotenone solvate, after crystallization, are poured quickly through a small fritted- or sintered-glass funnel of medium porosity. No weighing is necessary, and the transfer is generally complete within a few seconds. The flask need not be put back into an ice bath, but it should be allowed to drain as completely as possible. Suction is continued for about 5 minutes. Usually at this point a fairly light colored crystalline product is obtained. Since the crystals are not washed at all, a possible source of loss, especially in warm weather, is eliminated. The drying to constant weight for about an hour is also avoided, since weighing as crude solvate is unnecessary.

Instead of decomposing the solvate by allowing it to stand from 4 hours to overnight at room temperature, the same result was obtained by complete solution in hot alcohol saturated with rotenone and allowing to crystallize for about 30 minutes. A test sample of pure rotenone under these conditions gave practically complete recovery of the rotenone. Thus a considerable saving of time is effected.

Experimental Results

Ten samples of derris and cube roots were analyzed by this method. Eight of the samples were also analyzed by the regular A. O. A. C. method, and the other two by the titrimetric variation. The results are listed in Table II.

These results indicate fairly good agreement between the method as proposed and both the official and titrimetric methods.

Adaptation to Other Types of Samples

Occasionally a root sample is encountered which contains an unusually large proportion of nonrotenone resins. In this case the crystallization will not proceed correctly and there is no choice except to add enough pure rotenone to the extract to bring the ratio of rotenone to resin up to about 1 to 5. Otherwise the procedure is the same.

Dusting powders consisting of ground roots and an inert diluent such as talc, kaolin, etc., which is insoluble in the solvents used, may be treated in the same way except that the sample should be large enough (up to 200 grams) to give a minimum of 1 gram of rotenone, and the proportion of chloroform adjusted to the ratio of 5 ml. of 1 gram of sample. If the amount of material available is limited, a smaller sample can be used and pure rotenone added to make up the amount.

Samples containing sulfur have to be treated somewhat differently.

The boiling chloroform takes up a large quantity of sulfur, most of which crystallizes out and is filtered off with the marc. The whole solution may be taken down to dryness and the residual chloroform removed under vacuum. The residue is then digested with several portions of warm acetone. Each portion of the solution is then cooled and filtered into a 125-ml. Erlenmeyer flask. The acetone dissolves all rotenone, but only a small amount of sulfur. The acetone is then completely removed by evaporation, the residue dissolved in carbon tetrachloride in the usual way, and the normal procedure resumed. What would normally have been the final product consists of rotenone still contaminated with a little sulfur, and should be weighed. A saturated solution of sulfur in acetone is poured through the crucible until all the rotenone is dissolved out, and the crucible containing the small amount of sulfur is dried and weighed. The loss in weight represents the rotenone content.

Summary

A new procedure for the gravimetric determination of rotenone has been developed from the official A. O. A. C. method, shortened and simplified in several respects. Extraction time was shortened by refluxing the sample with the solvent for a short time. This procedure also permits the use of samples up to 200 grams as well as mixtures of powdered roots with sulfur and most inert ingredients. The purification of the crude rotenone was also modified and several unnecessary steps were omitted.

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A Laboratory Bellows Pump

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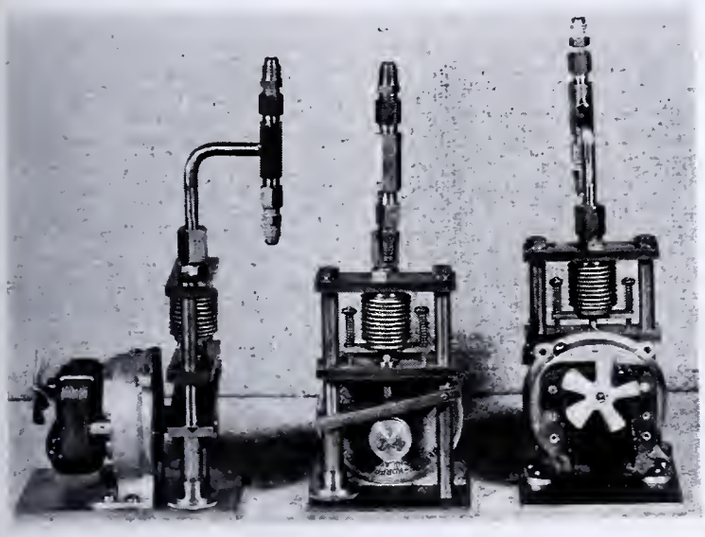

FIGURE 1. PUMP

THIS article describes a laboratory pump capable of delivering 5 to 2500 cc. of liquid per hour at essentially atmospheric pressure. It is a modification of the device described by Tropsch and Mattox (*1*).

Design of the Pump

The design of the pump (Figures 1 and 2) is simple; it can be built in 3 to 5 days. The working parts are exposed, facilitating the detection of leaks. It can be broken down for inspection, or reassembled, in 5 minutes.

The motor-speed reducer assembly (30 r. p. m.) was supplied by the Merkle-Korff Company of Chicago, the needle bearing for the eccentric by the Torrington Company, Torrington, Conn., and the seamless, single-ply brass bellows (1.125 inch in outside diameter × 0.759 inch in inside diameter, 10 live corrugations, 1 ring burr, and 1 integral bottom) by the Fulton Sylphon Company, Knoxville, Tenn. Stainless steel bellows (with welded seam) was obtained from the Chicago Metal Hose Corporation, Maywood, Ill. Machined valve seats are as satisfactory as ground-in seats and the preferable combination is a stainless steel ball in a brass seat.

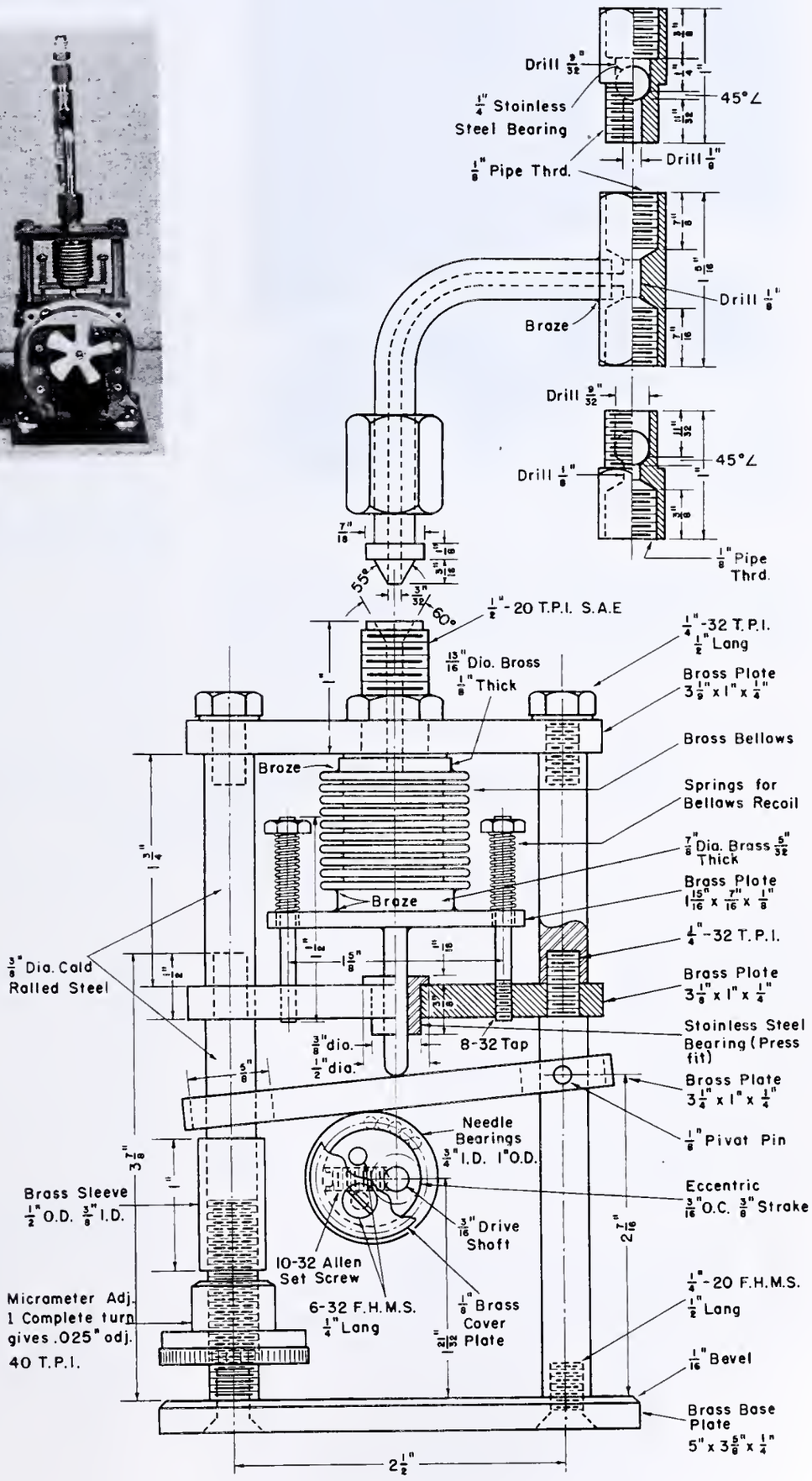

FIGURE 2. ASSEMBLY DRAWING OF PUMP

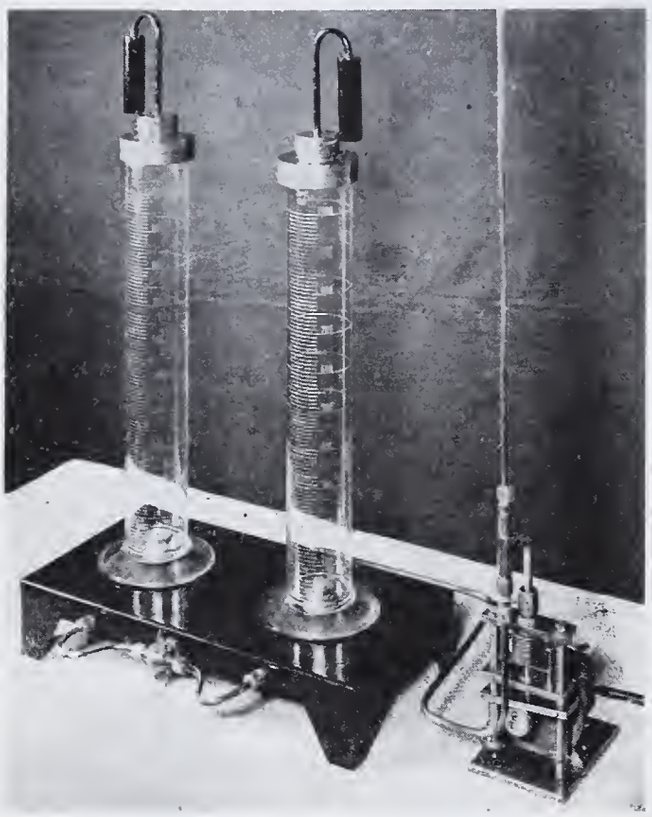

FIGURE 3. CHARGER AND PUMP

Operation

AT ATMOSPHERIC PRESSURE. This pump is designed for operation at 15 to 30 pounds per square inch gage. It will operate continuously for months with an occasional cleaning of the ball-checks. The average deviation in the pump rate at 50 cc. per hour is ±2.7 cc.

Before starting, all air should be expelled from the bellows, so as to avoid vapor-lock. The recommended procedure is to disconnect the valve assembly just above the bellows, fill the bellows with the liquid to be pumped, connect the valve assembly, and finally work the bellows up and down by hand to fill the valve assembly from the charger (Figure 3). The pump is then ready for use.

AT SUPERATMOSPHERIC PRESSURE. The pump can be easily modified to operate at 50 p. s. i. gage, with ordinary brass bellows, and at 250 to 500 p. s. i. gage, with stainless steel bellows. The modification consists in enclosing the bellows in a cylinder to prevent sidewise movement and dispensing with the two springs (since the pressure within the bellows maintains contact between the bellows and the eccentric).

A pump equipped with a $^{9}/_{16}$-inch outside diameter × $^{3}/_{8}$-inch inside diameter × 1.25-inch live-length stainless steel bellows operated at 250 p. s. i. gage for 500,000 $^{1}/_{16}$-inch strokes before failure. Operating under pressure, the brass bellows failed at the smaller diameter crease between the corrugations, whereas the stainless steel bellows failed at the welded seam.

Acknowledgment

The authors acknowledge the able assistance of John Visoskis in the development of this pump.

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Determination of Sulfur in Acid-Soluble Sulfides

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THE following method of determining sulfide sulfur is suggested in place of the familiar cadmium sulfide method. Even fairly large amounts of hydrogen sulfide can be determined conveniently, and no hydrogen sulfide is lost during the titration, as is apt to happen with the cadmium method. While some improvements still should be worked out, the method has given good results.

The principle of the method is precipitation of a heavy metal sulfide from an ammoniacal solution and direct back-titration of the excess heavy metal. Silver has been found suitable for the purpose.

The sample, containing not more than about 0.13 gram of sulfur, is weighed, transferred to a small standard sulfur flask, and covered with about 75 cc. of water and a few drops of strong alkali to minimize action of atmospheric acids. The delivery tube of the sulfur flask is extended to reach the bottom of an old 250-cc. volumetric flask containing 100 cc. of 0.1 *N* silver nitrate solution, 10 cc. of concentrated ammonium hydroxide, and about 50 cc. of water. A dropping funnel with stopcock is connected to the sulfur flask and 25 cc. of 50 per cent sulfuric acid are run in rapidly. The stopcock is closed, leaving a drop of acid as a seal. The flask is then heated slowly and gradually at such a rate that no hydrogen sulfide can escape through the ammoniacal solution. Silver sulfide precipitates at once. On further heating some water distills into the flask and heats its contents, causing the silver sulfide to coagulate and settle out. To ensure completeness of the removal of hydrogen sulfide after some minutes of boiling, a solution of about 5 grams of sodium carbonate in a little water is added dropwise through the funnel. The liberated carbon dioxide will sweep out the sulfur flask and carry residual hydrogen sulfide over into the silver solution, which should still be ammoniacal at the end.

When all hydrogen sulfide has been driven over, the delivery tube is disconnected from the sulfur flask and rinsed with little water into a standard 250-cc. flask. The silver solution is cooled to room temperature and likewise transferred into the standard flask. Rinse waters are used to bring it up to volume. A 100-cc. portion is withdrawn and titrated for excess silver.

For precise work the solution is just neutralized with nitric or sulfuric acid and sodium bicarbonate and titrated with alkali chloride and fluorescein, or by some other method. For routine work it was found sufficiently accurate to titrate the ammoniacal solution directly with, for example, approximately 0.2 *N* alkali cyanide solution, using 1 cc. of 5 per cent alkali iodide solution as indicator. Filtering is not necessary; the end point may be seen sharply as the disappearance of the stronger and yellowish turbidity caused by silver iodide so long as free silver is present. The cyanide solution must be standardized before use in terms of the standard silver solution; its precise normality, therefore, is irrelevant and the sulfur content is found in terms of standard silver nitrate.

CALCULATION:

$$\frac{0.1603 \times (\text{cc. of } 0.1\ N\ AgNO_3 \text{ used for } 100 \text{ cc.})}{\text{weight of sample}} = \%\ S$$

A Tilting Arc Flow Divider Suitable for Reflux Ratio Control[1]

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THE simple stopcock is perhaps one of the most widely used devices for control of reflux ratio of laboratory columns. Under conditions of reduced pressure, difficulty is ordinarily experienced when using the stopcock type of divider through contamination of distillate by soluble lubricants and the development of leaks at the stopcock barrel, or through obstruction by insoluble lubricant and consequent interference with "product" flow. Even very small obstructions and leaks may seriously disturb the reflux ratio. Controls of the stopcock type, and also improved controls (*3*, *6*) of this nature that require fixed settings by way of partial valve closure, provide a measure of constancy only with respect to product rate; they depend for ratio control on the maintenance of constancy of distillation rate, which of itself constitutes a difficult problem (*4*, *5*, *7*). Moreover, in the case of stopcocks, additional complications are introduced because of the lubricant. Devices in which reflux ratio settings are independent of distillation rate have obvious advantages (*1*, *2*).

The reflux divider described in this paper is of the continuously dividing type (in contrast with intermittent take-off, *2*); it has the advantage that ratio control is reasonably independent of rate; it is, moreover, not subject to difficulties arising through contact of distillate with lubricant; has a negligible holdup; and is relatively simple in construction. At low reflux ratios when continuous division of reflux may become an important factor the reflux divider meets this demand and its operation, as contrasted with the intermittent type (*2*), is thus not complicated by discontinuity of reflux to the column.

The simple flow-dividing mechanism presents a somewhat radical departure from the flow dividers commonly used. Its design is based on the observation that when a stream of liquid is delivered to some point of an "arc" (curvature circular or nearly so, the plane of curvature substantially vertical,

[1] Device (in several forms and modifications) constitutes subject matter of application for Service Patent.

TABLE I. REFLUX RATIO CONTROL

Test No.	Fraction Collected *Ml.*	Time Elapsed *Min.*	Average Throughput *Ml./hr.*	Reflux Ratio
1	0.69	38.0	79	71.5
2	0.68	26.5	113	72.6
3	0.68	26.2	115	72.6
4	0.66	24.0	125	74.8
5	0.63	20.8	144	78.3
6	0.60	13.8	218	82.4
7	0.61	13.7	219	81.0
8	0.60	13.4	224	82.4
9	0.60	12.9	233	82.4
10	0.60	10.3	293	82.4
11	0.58	9.2	326	85.2
12	0.58	3.9	766	85.2
		New Setting (Lower Ratio)		
13	1.77	37.0	81	27.3
14	1.72	29.9	100	28.1
15	1.70	27.9	107	28.4
16	1.73	27.6	109	27.9
17	1.35	15.9	189	36.0
18	1.40	13.9	216	34.7
19	1.31	13.5	223	37.2
20	1.18	9.0	333	41.4
21	1.17	8.4	356	41.7

and the surface of the arc made "even-wetting") there results a division of the incident stream into two streams which fall from the extreme ends of the arc. The relative volumes of the two streams depend upon the point of the arc where the liquid is dropped, and the angle or tilt of the arc.

Two modes of effecting the flow division are thus possible: (1) when the point on the arc at which the liquid is delivered is restricted to the center, the flow division (ratio of the two streams) is determined by the angle or tilt; and (2) when the point of contact (with the incident stream or drops) is not restricted to the center, then tilting of the arc will alter the relative length of arc extending on either side of the point of contact and correspondingly alter the reflux ratio.

The first method (restricting liquid to the center and tilting the arc to obtain the desired ratio) has been found to give better control than the second.

A small glass bead at the center of the arc facilitates the transfer of the dropping liquid to the even-wetting surface.

A wetting or spreading mechanism that has been found satisfactory consists of a close winding of glass fiber around the arc.

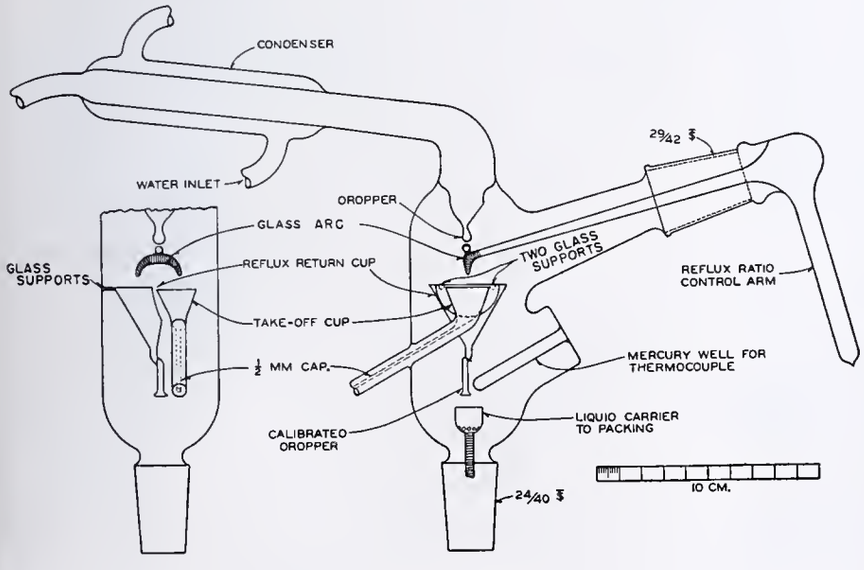

FIGURE 1

The divider proper, which with its winding of fiber glass constitutes the flow-dividing mechanism, is simply a curved segment (curvature approximately circular) of glass rod. Wire or metallic rod has also been found satisfactory. Dimensions depend on the maximum reflux rate for which it is to be used. Single segments, using rod of small diameter (about 3 mm., such as the one used here) are serviceable for liquid rates up to about 700 ml. per hour. For higher rates rod of somewhat larger diameter and with additional layers of winding may be used, or for much higher rates multiple segments, made of two or three parallel curved contacting segments with an over-all winding, have been found serviceable. The remainder of the device consists of a supporting rod sealed to a female ground-glass joint and an arm for setting the angle of the segment for the desired reflux ratio. The end of this arm is drawn out to a tip and may serve as a pointer on an arbitrary scale (not shown in the drawing). This

Table II. Reflux Ratio Control

Fraction	Time Elapsed between Fractions	Distillate Collected[a]	Average Reflux Return[b] (Throughput minus Take-off)	Take-off (Product)	Reflux Ratio	Remarks
	Hours	*Ml.*	*Ml./hr.*	*Ml./hr.*		
1	1.5	8.2	155	5.5	28	Set for approx. 30 to 1 reflux ratio
2	1.0	5.5	152	5.5	28	
3	3.0	7.2	127	2.4	53	Throughput decreased. Set for approx. 50 to 1 reflux ratio
4	16.0	39.5	127	2.5	51	
5	1.5	3.5	118	2.3	51	Throughput decreased
6	3.5	7.6	108	2.2	49	Throughput further decreased
7	2.0	3.9	93	2.0	47	Throughput further decreased
8	17.0	25.0	67	1.5	45	Throughput further decreased
9	8.0	9.5	84	1.2	70	Set for approx. 70 to 1 reflux ratio
10	16.0	19.8	85	1.2	71	
11	8.0	9.7	91	1.2	76	
12	72.0	85.2	87	1.2	73	
13	44.5	32.8	87	0.74	118	Set for approx. 120 to 1 reflux ratio
14	8.0	4.9	74	0.61	121	Throughput decreased
15	16.0	6.3	45	0.39	115	Throughput further decreased

[a] α-Pinene.
[b] Measured by calibrated dropper.

scale ranges from total reflux to total take-off. The reflux ratio obtained at any intermediate position of the arm is determined by trial and marked on the scale. The still head, in addition to having a standard-taper joint through which the flow divider is inserted and adjusted, is equipped with a thermometer well and condenser, a reflux return cup, calibrated dropper, and take-off or product cup with its capillary delivery tube as shown in Figure 1.

Some indication of the dependability of and constancy of ratio made possible by this device may be seen from the data in Tables I and II. Data in Table I were obtained on the flow divider independent of column application (divider set up on a laboratory table). Data in Table II were obtained on a column equipped with this divider, the column operating at 20-mm. pressure. Here the still head was thermally insulated by a heat-compensated enclosure (an extended part of the box housing of the column proper) provided with a window for visibility.

For the experiments in Table I, 50 ml. of turpentine were allowed to drop from a buret to the flow divider. Readings were made at comparatively short intervals, long enough to collect approximately 0.5 to 1.5 ml. of product. The rate of delivery was determined by timing with a stop watch. It can be seen from the data thus obtained that, at rates of 200 ml. per hour and above, there is very little variation in the reflux ratio at a given setting or tilt of the arc. However, a 100 per cent decrease in rate (to about 100 ml. per hour) with the same setting, gives a lower reflux ratio. The reflux ratios following such a change in rate are approximately 10 and 20 per cent lower than the original ratios of around 80 and 35, respectively. When the rates are low (100 ml. per hour and below), considerable variation does not materially change the constancy of reflux ratio. The average time interval for collecting a fraction in this series of tests (Table I) is much smaller than under conditions of actual fractionation (see Table II).

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Leakproof Stopcock for Regulation of Take-off during Distillation

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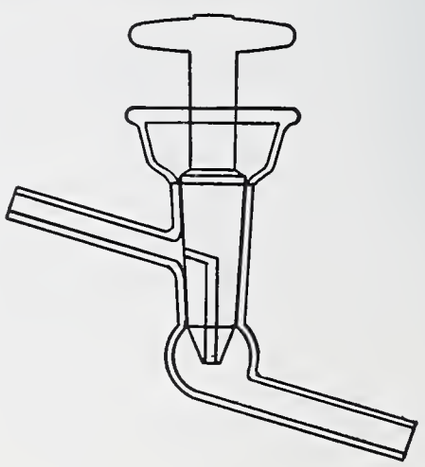
THE stopcock illustrated represents a modification of previous designs for leakproof stopcocks, useful in controlling the rate of flow in a variety of apparatus. The author has found the new stopcock particularly valuable in the regulation of take-off in distillation heads (*1*), as it embodies a number of features which make it preferable to the ordinary straight-bore stopcock.

When adjusting the take-off, the rate at which liquid is being taken is observed immediately at the drip point of the stopcock plug.

Since the rate of flow through the stopcock is governed at only one place where the hole in the barrel and the hole in the plug intersect, regulation is easier to accomplish. Furthermore, the small vertical fall in the bore of the plug acts as a siphon and thereby aids in regulation.

Mercury may be put in the well at the top. This prevents leakage during vacuum distillation and loss of volatile substances during prolonged fractionations at atmospheric pressure.

Lubrication may be accomplished effectively with the liquid being distilled.



The stopcock as described has been made according to the author's specifications by Ace Glass, Inc., Vineland, N. J.

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Stability of the Cupric-Ammonia Color System

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IN DISCUSSING the colorimetric determination of copper with ammonia Yoe (*3*) has stated that if the standards are kept in a cool place away from direct sunlight they will be unchanged for a long time. Snell (*2*) has recommended that the standards be renewed once a month, since, although they do not fade in the light, the ammonium hydroxide acts on the glass to form a flocculent precipitate. In a spectrophotometric study of this method the writer (*1*) found that there was no evidence whatever of fading or other change in color of the system over a period of 6 weeks.

TABLE I. STABILITY OF COLOR

(1.961-cm. cell)

Concentration of Copper	Transmittancy at 620 mμ		Apparent Change in Concentration of Copper
	Fresh solution	Old solution	
P. p. m.	%	%	%
40	84.3	84.0	+2.0
80	71.0	71.5	−2.0
120	59.7	59.9	−0.6
200	42.7	42.7	0.0
400	18.0	18.1	−0.3
600	7.7	7.9	−1.0

For the purpose of further study the six solutions which had been used in this test, containing 40, 80, 120, 200, 400, and 600 p. p. m. of copper in 3 *M* ammonium hydroxide, were allowed to stand 51 weeks longer in glass-stoppered Pyrex bottles in diffuse light. Spectral transmission curves were then made with the recording photoelectric spectrophotometer at Purdue University and when compared with the curves given by the corresponding freshly prepared solutions, were found to be practically identical. From the transmittancy at 620 mμ, the wave length of maximum absorption, the percentage error in the apparent concentration of copper was calculated (*1*) by use of the special color slide rule.

The results, which are given in Table I, show that the color of the cupric-ammonia system is practically unchanged after a period of 57 weeks. Any action of ammonia on the glass is negligible when Pyrex containers are used.

Acknowledgment

The writer wishes to thank M. G. Mellon of Purdue University, in whose laboratory this work was done, for the privilege of using the Purdue spectrophotometer.

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A Simple Large-Capacity Extraction Apparatus

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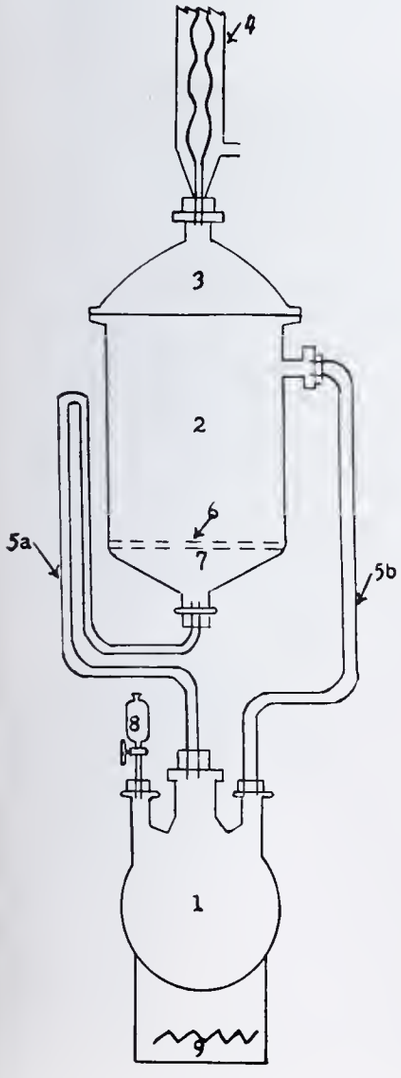

FIGURE 1. EXTRACTION APPARATUS

AN APPARATUS for extracting large quantities of material exceeding the capacity of the usual laboratory extractors can be readily improvised from parts available in any analytical laboratory, and assembled as shown in Figure 1:

1. Three-neck flask
2. Bell jar
3. Desiccator cover to fit bell jar
4. Water condenser
5*a*. Siphon tube
5*b*. Vapor conduit
6. Perforated disk
7. Cotton or glass wool
8. Separatory funnel
9. Electric heater

The siphon tube, 5*a*, can be made of block tin tubing instead of glass, for this part is easily broken in assembling. Filter paper is laid on the perforated disk, with cotton or glass wool below it to prevent loose particles of the material from being carried into the flask.

If the ground surfaces of the bell jar and desiccator top are properly ground, no lubricant is required to seal this joint. Where a lubricant is needed, one immiscible with the solvent should be applied.

Microdetermination of Lead by Dithizone

With an Improved Lead-Bismuth Separation

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DESCRIPTIONS of the dithizone method for lead determination used in this laboratory were published in 1937 (*5*) and again in 1939 (*1*) but since that time several modifications have been introduced into the procedure. Of particular importance, the bismuth test has been replaced by a lead-bismuth separation which results in a significant saving of time; this is given here in its entirety. At present, since details of the method as applied to various biological materials have been published in separate articles, it is necessary to consult at least four papers in order to obtain the complete procedure (*1–6*).

Standard dithizone solutions can be kept for months without detectable decomposition if certain precautions are observed. They should be stored in the dark, in a refrigerated space, and in glass-stoppered Pyrex containers. Whenever the chloroform used anywhere in the analysis is shaken with water, alcohol (about 0.5 per cent by volume is sufficient) should be added to it after the water is separated. Thus alcohol should be added after the chloroform is shaken with hydroxylamine solution in preparing a dithizone solution; it should be present in the bottle in which chloroform to be reclaimed is collected; it should be added to the chloroform after it is shaken with sulfuric acid in the reclaiming procedure; and the purified chloroform should be distilled into a receiver containing alcohol. The purified dithizone supplied by the Eastman Kodak Company is satisfactory for all dithizone solutions; special treatment is not necessary.

The quantity of dithizone in the standard solutions used in the final step has been increased by 25 per cent. It was found that this eliminated a tendency toward deviation of the calibration points from a straight line when the upper limits of capacity of the solutions were reached.

Although this method of lead determination was devised primarily for the analysis of biological material, it is capable of wide application. Lead in water, toothpaste, and most chemicals can be determined with little or no modification of the procedure.

The Lead-Bismuth Separation

In the various dithizone methods for the determination of lead in biological material (*1, 5, 8, 10*), the lead (and bismuth, if present) is extracted from the sample solution by shaking with dithizone in chloroform or carbon tetrachloride, the metals are stripped from the dithizone by shaking with dilute acid, and a test for bismuth is then made. If bismuth is found it is usually removed by extracting the aqueous solution with dithizone at pH 2 to 3. At this pH no lead enters the dithizone phase, but bismuth is slowly extracted, so that repeated extractions with dithizone will remove the metal completely. Bismuth is not a common constituent of biological material, yet each sample for lead determination must go through this rather tedious bismuth test in order to make the analysis specific.

Table I. Effect of pH on Lead-Bismuth Separation

(Feces 293)

	Lead Found *Micrograms*
Old method (*1*)	57, 58
pH 3.4, no bismuth added	57
pH 3.4, 100 micrograms of bismuth added	57
pH 3.6, no bismuth added	54
pH 3.6, 100 micrograms of bismuth added	55
pH 4.0, no bismuth added	50
pH 4.0, 100 micrograms of bismuth added	51

If, instead of using dilute acid—for example, 1 per cent nitric acid—to strip the metals from the dithizone solution, one uses an aqueous solution set at pH 3.4, the lead and bismuth are separated and no subsequent bismuth test or extraction is necessary. When relatively large quantities of bismuth are present a slight amount is carried over, but this can be completely extracted by the addition of one portion of dithizone solution. Elimination of the bismuth test by this procedure results in saving about 25 per cent of the working time required by the dithizone method previously described, so that a single analysis on a prepared sample can be carried out in less than 20 minutes. With 3 hours' help on the part of a technician, between 50 and 60 prepared samples can now be handled by the analyst in 7 hours.

The pH in this separation must not vary widely from 3.4, and in order to keep entrained salts from changing this factor it was found necessary to use a dilute Clark and Lubs buffer (potassium acid phthalate–hydrochloric acid). Also, in order to have the correct buffer system in operation in the final step when the photometric estimation of lead is carried out, the proper concentration of ammonium nitrate had to be present. This was accomplished by using 2 per cent nitric acid, setting the pH at 3.4 with ammonium hydroxide, adding the proper quantity of buffer, and then diluting the solution with water to twice the volume of nitric acid used. The pH of the solution is very important; if the solution is too acid some bismuth enters the aqueous phase, causing an apparently high lead recovery, while if it is too alkaline some lead will

Table II. Synthetic Samples Containing Lead and Bismuth

Sample	Lead Added	Lead Found: No bismuth added	Lead Found: 10 micrograms of bismuth added	Lead Found: 100 micrograms of bismuth added
	Micrograms	*Micrograms*	*Micrograms*	*Micrograms*
A	0 (Blank on synthetic urine ash)	0.3	0.3	0.2
B	2.5	2.8	2.7	2.8
C	25	26	26	26
D	75	74	73	74

Table III. Samples Containing Bismuth

(Micrograms of lead found)

	No Bismuth Added: Old method (*1*)	No Bismuth Added: New method	Bismuth Added: 10 micrograms	Bismuth Added: 100 micrograms
Urine 343	4.6	4.6	4.6	4.6
Urine 378	14.5	14	14	14
Feces 386	80	79	80	80
Food 920	14	14	14	14
Food 1191	37	38	37.5	37.5

stay in the dithizone-chloroform phase (cf. Table I). Considering the difference in the types of solutions analyzed, these results agree fairly well with those reported by Clifford and Wichmann (*4*) and with the rough chart proposed by Wichmann (*9*).

After this lead-bismuth separation had been used for some time a somewhat similar one was described by Kluchesky, Longley, and Kozelka in a dithizone method for the determination of bismuth (*7*). However, these workers did not use a buffer, which would make their method inapplicable to the analysis of samples of larger volume, especially where large quantities of dithizone solution must be used for extraction such as are commonly employed in the determination of lead.

In order to show the reliability of the method and the completeness of the lead-bismuth separation, the results obtained on samples with known quantities of lead added to deleaded salts simulating the ash of normal urine (*2*), some of them contaminated with bismuth, are shown in Table II. Table III shows the results obtained by the new method on samples of urine, feces, and mixed foods which had also been contaminated with bismuth. A further comparison of results by the old and the new method is included in Table IV.

The data in these tables indicate that the simplification and shortening of the old method has been accomplished with no loss of accuracy; quantities of lead below 10 micrograms can be determined with an error of less than 1 microgram, while the error with quantities greater than 10 micrograms is about 3 to 6 per cent.

When the quantity of bismuth in the sample exceeds 10 micrograms (approximately) it is advisable to shake the aqueous extract containing the lead at pH 3.4 with one 5-ml. portion of dithizone solution. The presence of this much bismuth may be recognized easily by the fact that the dithizone extract does not return to its original green color when shaken with the pH 3.4 buffer solution; the practised eye perceives the off-color readily. By using the additional extraction, the last trace of bismuth is removed from the aqueous phase.

Lead determination in samples containing relatively large quantities of bismuth may be facilitated by making use of the fact that in the original dithizone extraction the lead is completely removed long before the bismuth, and that, therefore, when the color of the initial portion of dithizone extract shows the presence of both lead and bismuth, extraction need be continued only until it is apparent that most—not all—of the bismuth has been removed from the sample solution. At this point one can be sure that all of the lead has been extracted and the sample can be discarded before the color of the last portions of dithizone extract remains an unchanged green.

Complete Procedure for Lead Determination

Preparation of Samples. *Urine.* Concentrated nitric acid (10 per cent of the sample volume) is added to the measured sample; it is evaporated to dryness on a hot plate or steam bath, and ignited at 500° C. in a silica or Pyrex evaporating dish. The ash is moistened with nitric acid, dried, and ignited for a few minutes until it is white; then it is dissolved in dilute nitric acid and is ready for analysis. Filtration is not necessary.

Feces. The sample is dried on a hot plate or steam bath to constant weight in a tared silica or Pyrex dish, placed on an electric heater at a low red heat until all volatile matter which would otherwise flame in the muffle furnace has been driven off, after which it is ignited at 500° C. The ash is weighed, dissolved in a mixture of hydrochloric and nitric acids, and is ready for analysis. Filtration is not necessary and should not be employed, since the removal of silica in appreciable quantities will result in the loss of entrained lead unless the precipitate is very thoroughly washed.

Foods. The weighed sample of mixed food is digested with concentrated nitric acid on a hot plate or steam bath until all lumps have disappeared. This may require more than a week, with repeated additions of acid. If a food homogenizer, such as a Waring Blendor, is used the time may be materially shortened, especially since aliquants may then be employed. (Unless samples can be made homogeneous by this or some similar method, the entire sample must be analyzed.) The sample is evaporated to a sirupy consistency, transferred to a silica or Pyrex evaporating dish, and concentrated sulfuric acid is added (15 ml. for an average day's mixed food). The dish is then placed on an electric heater at a low red heat and treated as described under "Feces".

Tissues. In general, the weighed sample is treated in the same way as a sample of mixed food.

Blood. The sample is transferred to a tared silica or Pyrex evaporating dish and weighed, after which concentrated nitric acid is added (5 to 10 ml. per 10 grams of blood). The mixture is evaporated to dryness on a hot plate or steam bath, placed on an electric heater at a low red heat, and ignited in a muffle furnace at 500° C. A few milliliters of hydrochloric acid are added to the ash, then nitric acid, and the dish is heated on the hot plate until the sample is dissolved. It is then ready for analysis.

Note. All glassware should be cleaned with a sulfuric acid–chromic acid mixture and with dilute nitric acid (1 + 1). It is necessary to avoid even slight contamination of samples with dust, especially during the evaporations. If reagent acids are used in the preparation of samples, lead determinations should be made on them for deduction as reagent blanks. This can be avoided by using distilled acids.

Reagents and Apparatus. *Ammonium Citrate Solution.* Four hundred grams of citric acid are dissolved in water and

Table IV. Comparison of Results by Two Methods

Sample	Lead Found: Old method (*1*)	Lead Found: New method
	Micrograms	*Micrograms*
Urine 956	23	23.5
960	3.8	4.1
962	4.5	5.5
964	6.5	6.8
978	20	21
992	29.5	29.5
996	11.5	11
998	7.5	7.5
1008	2.9	3.2
Feces 957	20	20
959	29	30.5
965	42	43
969	19.5	21
971	25	25.5
983	260	255
1516	15	15.5
1519	32	32.5
Food 1364	20.5	20.5
1372	17.5	17.5
1414	82	82
1429	11	11.5
1437	14	14
1479	24	23
1499	19.5	19
4338	32	31.5
4594	35	35
Urine 327[a]	9.5	9.5
Feces 358[a]	17.5	17.5

[a] These samples contained bismuth when received; over 200 micrograms were present.

sufficient reagent ammonium hydroxide is added to make the solution alkaline to phenol red. The solution is diluted to 1 liter with water and purified by shaking it with repeated portions of a solution of dithizone in chloroform until the dithizone retains its original green color.

Distilled Ammonium Hydroxide. Reagent ammonium hydroxide is distilled into double-distilled water which is chilled in an ice bath.

Hydroxylamine Hydrochloride Solution. Twenty grams of hydroxylamine hydrochloride are dissolved in sufficient water to make about 65 ml. and a few drops of *m*-cresol purple indicator solution are added. Concentrated ammonium hydroxide is next added until a yellow color results. Sodium diethyldithiocarbamate in water (an approximately 4 per cent solution) is added in sufficient quantity to combine with all the lead (and most other metals) present and to leave a considerable excess. After a few minutes the organo-metallic complexes and the excess reagent are completely extracted with chloroform. The absence of a yellow color in the chloroform when a portion of the chloroform extract is shaken with a dilute solution of a copper salt indicates when this point is reached. Distilled hydrochloric acid is then added to the hydroxylamine hydrochloride solution until the indicator turns pink, and double-distilled water is added to make the final volume 100 ml. It is not necessary to filter the solution.

Potassium Cyanide Solution. A practically saturated solution containing 50 grams of potassium cyanide in sufficient water to make 100 ml. is repeatedly shaken with portions of dithizone in chloroform (30 mg. per liter) until the lead is removed. Part of the dithizone dissolves in the aqueous phase but enough remains in the chloroform to color it and to indicate when the lead has been completely extracted. Most of the dithizone in the aqueous phase can be removed, if desired, by repeated extractions with pure chloroform. The strong potassium cyanide solution is then diluted with double-distilled water to the proper strength (10 grams per 100 ml.). It is not necessary to filter the solution. (If instead of the concentrated solution, the final one is shaken with dithizone in chloroform in an attempt to delead it, the increased alkalinity of this dilute solution causes the removal of all the excess dithizone from the chloroform and renders the complete extraction of the lead more difficult.)

Dithizone Extraction Solution. One liter of chloroform is shaken with 100 ml. of water containing about 0.5 gram of hydroxylamine hydrochloride, which has been made alkaline to phenol red with ammonium hydroxide. The chloroform is drained off, and 30 mg. of dithizone are dissolved in it. Approximately 5 ml. of alcohol are added to the solution if part of it is to be kept for several days. Filtration is not necessary. The quantity of dithizone solution to be used for one day is shaken with about 100 ml. of dilute nitric acid (1 ml. per 100 ml. of solution) just before use.

Standard Dithizone Solutions. These solutions are made from distilled chloroform which has been treated with hydroxylamine, as in the instructions for the dithizone extraction solution. In this case, however, the chloroform is filtered through a dry paper into a glass-stoppered Pyrex bottle, which is wrapped with heavy paper or kept in a wooden box to protect it from light. The proper amount of dithizone (5 mg. per liter for the 0- to 10-microgram range, 10 mg. per liter for the 0- to 50-microgram range, and 20 mg. per liter for the 0- to 100-microgram range) is dissolved in the chloroform, absolute alcohol is added (5 ml. per liter), and the solution is ready for standardization. It should be kept in a refrigerator.

Ammonia-Cyanide Mixture. Each liter of the mixture contains 20 grams of potassium cyanide and 150 ml. of distilled ammonium hydroxide (specific gravity 0.9), or its equivalent; it is brought to volume with double-distilled water. This solution should be kept in a cool place.

Buffer Solution, pH 3.4. Reagent nitric acid (9.1 ml.) is diluted to approximately 500 ml. with double-distilled water in a 1-liter volumetric flask, bromothymol blue indicator is added, and the pH is brought to 3.4 with distilled ammonium hydroxide. Then 50 ml. of Clark and Lubs potassium acid phthalate–hydrochloric acid buffer, pH 3.4, double strength (50 ml. of 0.2 *M* potassium acid phthalate and 9.95 ml. of 0.2 *M* hydrochloric acid, per 100 ml.), are added and the solution is diluted to 1 liter with double-distilled water.

Chloroform Recovery. Used dithizone solution is collected in glass-stoppered amber bottles containing alcohol (about 0.5 per cent of capacity of bottle). Each liter of solution is washed with about 200 ml. of water, then washed with two 100-ml. portions of concentrated sulfuric acid. Five milliliters of alcohol are added to the chloroform and it is allowed to stand overnight in the dark over lump calcium oxide. It is then distilled (through a still fitted with all-glass connections) into a glass-stoppered amber bottle containing absolute alcohol (about 1 per cent of capacity of bottle).

Apparatus. Pyrex glassware should be used throughout; containers should all be glass-stoppered. The extractions are most conveniently carried out in Squibb-type separatory funnels, 150-ml. capacity, graduated at 5-, 10-, and 10-ml. intervals to 100 ml., with the aid of rotary funnel racks (*1*). White vaseline is used to grease the stopcocks, since it is practically lead-free.

Any photometer which can be used with the cells described is usually satisfactory for this procedure. A glass filter with maximum transmission at 510 millimicrons is suitable. The neutral wedge photometer described by Clifford and Wichmann (*4*) was originally employed in this work.

Isolation of Lead. Approximately 15 ml. of the ammonium citrate solution are poured into each of the lower funnels, using the graduation marks on the funnels as a guide. The proper aliquants of the prepared samples are next added (equivalent to 50 to 200 ml. of urine, 0.1 to 0.5 gram of fecal ash, 5 to 20 grams of blood, or one-tenth of a normal day's food), together with 5 ml. of potassium cyanide solution and 1 ml. of hydroxylamine hydrochloride solution, and the mixtures are made alkaline to phenol red with distilled ammonium hydroxide. In rare instances the solutions will become very cloudy upon being made alkaline, because of the presence of an unusually large quantity of calcium phosphate; in such a case sufficient ammonium citrate solution should be added to dissolve the precipitate.

The extraction of lead is started with 5 ml. of dithizone extraction solution and the color is noted in order that the proper standard dithizone solution may be chosen in the final lead estimation. (Usually the lead is not extracted quantitatively with each portion of dithizone solution because of the various salts present. Instead of 50 micrograms of lead, which is the theoretical equivalent of the dithizone, only 40 micrograms will be extracted, as a rule, by each 5 ml. When the quantity of lead present is less than 10 micrograms this is recognized by the distinctive greenish-blue color of the dithizone.)

Another 5-ml. portion of dithizone solution is added, using the graduation marks on the funnels as a guide. Before this combined 10-ml. solution is drained into the other funnel, its color is noted, for this should indicate whether the quantity of lead present is greater than 50 micrograms, or greater than 100 micrograms. The extraction is then continued with successive 5-ml. portions of dithizone solution, the color of each portion is noted, and each is drained before adding the next, until the lead is completely extracted.

The combined dithizone extracts are washed with approximately 50 ml. of double-distilled water and the water is washed with 5 ml. of distilled chloroform. This chloroform wash should be green in color; if it is not, the presence of lead or zinc is indicated. A drop of potassium cyanide solution should then be added to the water and the funnel shaken again; if the chloroform phase does not become green the water should be washed at least once with dithizone extraction solution. All chloroform washings are added to the dithizone extract and the water is discarded. The lead is stripped from the dithizone extract by shaking with 50 ml. of buffer solution (pH 3.4); if the dithizone solution does not return to its original green color, bismuth is present. The dithizone solution is drained from the funnel, and if the presence of bismuth has been indicated, the buffer solution is shaken with one 5-ml. portion of dithizone solution. After this is drained out, 5 ml. of distilled chloroform are added and the mixture is shaken.

If the sample contains more than 100 micrograms of lead, as indicated by the color of the separate portions of dithizone extraction solution noted early in the lead isolation step, part of the buffer solution containing the lead is discarded before the 5 ml. of distilled chloroform are added. The quantity discarded should be sufficient to reduce the lead content to less than 100 micrograms; this is conveniently done by using the graduation marks on the funnel. The volume is then made up to 50 ml. by adding more buffer solution and the chloroform is added as indicated above. The funnel is allowed to stand unstoppered until the drop of chloroform floating on the surface of the aqueous phase has evaporated, and the 5 ml. of chloroform are drawn off as completely as possible without allowing water to enter the bore of the stopcock.

Final Estimation of Lead. In the following portion of the procedure direct sunlight should not be allowed to strike the solutions.

The proper standard dithizone solution (10 ml. of the 0- to 10-microgram solution, or 25 ml. of the 0- to 50- or 0- to 100-microgram solution) is added to the funnel containing the lead in 50 ml. of buffer solution; 7 ml. of ammonia-cyanide mixture are added, and the funnel is immediately shaken for 1 minute. The pressure which develops should not be released through the stopcock; instead, the stopper should be lifted in order to avoid blowing water into the funnel stem. If a number of analyses are being run, the mixed color can be developed in the whole series and photometric readings taken one after the other.

Part of the dithizone solution (2 ml. of the 0- to 10-microgram solution, or 10 ml. of the 0- to 50- or 0- to 100-microgram solution) is used to flush the stem of the funnel; the end of the funnel stem is dried, and the solution is allowed to run directly into the proper cell for the photometric reading. The 0- to 10-microgram solution is used with a 5-cm. (2-inch) cell, the 0- to 50-microgram solution with a 2.5-cm. (1-inch) cell, and the 0- to 100-microgram solution with a 1.25-cm. (0.5-inch) cell. The cells are cylindrical, with optically plane ends and have an internal diameter of about 14 mm. Since the 0- to 10-microgram cell holds the entire 8 ml. remaining in the funnel, it cannot be rinsed with part of the dithizone solution, but must be cleaned and dried with pure acetone after each sample. The other two cells, however, can be rinsed with dithizone solution at least twice, and it is rarely necessary to wash or dry them between samples.

The photometric readings are referred to calibration curves made for each standard solution in the following way:

A standard lead nitrate solution is made by dissolving recrystallized lead nitrate in 1 per cent nitric acid, so that each milliliter contains 1 mg. of lead. From this solution two dilutions in 1 per cent nitric acid are made, one containing 10 micrograms of lead per milliliter, and the other 1 microgram per milliliter. All these solutions will keep indefinitely in glass-stoppered Pyrex containers.

A standard lead solution in the proper buffer is made by taking a measured quantity of the desired standard lead solution, bringing it to pH 3.4 by addition of dilute distilled ammonium hydroxide, adding the proper amount of the Clark and Lubs buffer mentioned above, and diluting the mixture to a known volume. This solution should be made fresh each time it is used.

Measured quantities of this standard lead solution are added to clean separatory funnels, the volume in each funnel is brought to 50 ml. with the pH 3.4 buffer solution, and the dithizone solution to be standardized is added. Color development and photometric readings are then carried out as in the final estimation of lead. From these readings a standard calibration curve can be made.

Summary

Certain modifications of the photometric dithizone method previously described are discussed. The observance of certain precautions makes it possible to keep standard dithizone solutions for months without apparent deterioration. The time required for carrying out an analysis has been decreased significantly by the development of a lead-bismuth separation which permits the omission of the bismuth test.

The complete analytical procedure, including preparation of samples and purification of all reagents—previously described in several papers—is given in detail.

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A Simple Apparatus for Small-Scale Catalytic Hydrogenation

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NUMEROUS designs of apparatus for small-scale catalytic hydrogenation have been described in the literature, a good survey of which has been given recently by Johns and Seiferle (*1*). While no claim is made for great originality in the apparatus now described, the authors believe that they have combined the good features of several types of apparatus and modified them to give one which is very simple in construction and operation (Figure 1).

One of the inconveniences of most designs is the shaking mechanism, which usually is of the reciprocating type. Weygand and Werner (*2*) used an electromagnetic stirrer. The authors have substituted a medium-size Alnico permanent magnet for the electromagnet, and in place of the complicated devices for introducing the sample or catalyst have used a cup and stopcock. The type of buret used by Johns and Seiferle (*1*) has been retained, since this avoids the use of a stopcock.

As usually operated, the weighed sample to be hydrogenated is placed in the flask with a suitable solvent and the iron-cored stirrer. With the stopcock open, the flask is connected to the buret by the ground joint and held in placed with a buret clamp. The reservoir is lowered, the height of the magnet is adjusted, the stirrer is started, and the speed of the motor is regulated so

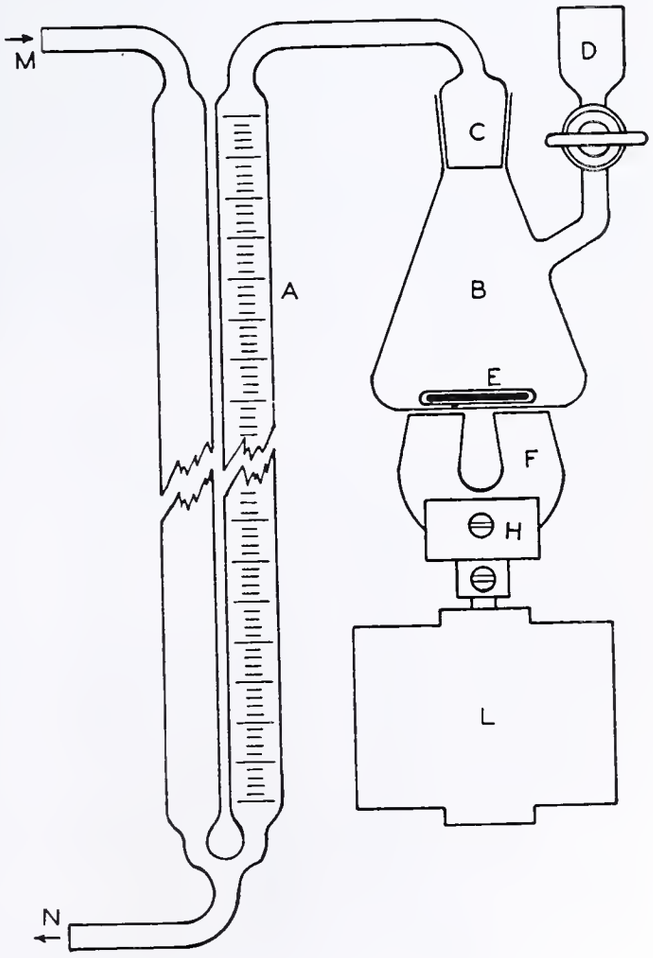

Figure 1. Apparatus

A. 50-cc. buret
B. 50-cc. flask
C. 19/38 interchangeable ground joint
D. 5-cc. cup
E. 2-cm. section of 8 d. nail sealed in glass tubing
F. Medium size Alnico magnet
H. Brass saddle with setscrews
L. 1/100 h. p. variable-speed motor
M. Hydrogen inlet
N. To reservoir for confining liquid

that good stirring is obtained without throwing the liquid too high up the sides of the flask or causing the magnet to lose the stirrer. The apparatus is swept out with hydrogen which has been saturated with the solvent used in the flask by bubbling through a wash bottle having a fritted-glass distributor. The confining liquid in the reservoir may be either mercury or solvent.

After the air has been swept out, the desired amount of hydrogen is trapped in the buret, the stopcock on the flask is closed, and stirring is continued until the volume of hydrogen as read on the buret no longer changes, at which time the stirring is stopped and the temperature and barometric pressure are noted.

After slightly reducing the pressure in the flask by lowering the reservoir, the platinum oxide or palladium oxide catalyst is introduced by the following procedure: The catalyst is weighed into a narrow glass or metal scoop and a measured volume of solvent, usually 5 cc., is drawn into a pipet. A few drops of solvent are run into the cup, the catalyst is added, and the scoop is washed with a few more drops of solvent. The catalyst is then drawn in through the stopcock and washed in with the remainder of the solvent, care being taken not to admit air. This procedure has the advantage of permitting reduction of the oxide catalyst in the presence of the compound to be reduced, which gives a more active catalyst than reduction in solvent alone. It was not found possible to have the catalyst present while the apparatus is being swept out with hydrogen, because the rate of solution and diffusion of hydrogen is so great that hydrogen is absorbed at the rate of about 0.5 cc. per minute even without stirring.

After addition of the catalyst, stirring is started and continued until hydrogenation is complete, when the volume, barometric pressure, and temperature are read again. The volume of the catalyst and wash solvent and the hydrogen absorbed by the catalyst and solvent are determined in a blank run.

While this apparatus has been used only for semimicro work and not for measuring the volume of hydrogen absorbed with great accuracy, there seems to be no reason why it cannot be adapted to micro work or why it should not yield results as accurate as most other forms of apparatus, if the corrections and calculations described by Johns and Seiferle (*1*) are applied.

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Quantitative Decomposition of Organic Bromine and Iodine Compounds by the Lime-Fusion Method

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THIS paper presents data to show that the lime-fusion method of decomposing organic compounds of chlorine (*1*) can be applied to the analysis of similar compounds of bromine (Table I) and iodine (Table II), and also describes an improvement in the bomb for heating the sample with lime and a method for testing it for leaks. Semimicrosamples have been used throughout.

The construction of the bomb has been modified by providing the contact face of the bomb chamber with a sharp ridge approximately 0.5 mm. in height. When the bomb is closed, this sharp ridge is embedded in the copper washer and a tight seal is thus easily obtained.

Bombs, especially when new, should be tested occasionally in the following way for the tightness of the seal. This test for tightness is strongly recommended to those who use the bomb for the first time. The bomb is half-filled with dry ice, sealed, and inverted in a beaker of warm water. If bubbles do not appear, it may be assumed that the method of closing the bomb is satisfactory.

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Table I. Determination of Bromine

Compound	Weight of Sample	Weight of AgBr	Br Calculated	Br Theoretical	Difference
	Mg.	*Mg.*	%	%	%
Hexabromoethane	10.87	24.27	95.01	95.23	−0.22
	12.21	27.25	94.97		−0.26
Tetrabromobutane	17.35	34.83	85.44	85.53	−0.09
	20.31	40.80	85.49		−0.04
Monobromobenzene[a]	23.58	28.11	50.72	50.91	−0.19
	17.80	21.12	50.49		−0.42
Bromomesitylene[a]	22.91	21.48	39.89	40.15	−0.24
	22.43	21.09	40.00		−0.12
1-Bromo-2,7-dimethylnaphthalene[a]	15.43	12.17	33.56	33.99	−0.43
	16.23	12.90	33.83		−0.16
1-Bromo-4-nitrobenzene[a]	14.67	13.58	39.38	39.57	−0.19
	15.80	14.64	39.43		−0.14
p-Bromoacetanilide[a]	11.92	10.53	37.59	37.35	+0.24
	11.44	10.08	37.48		+0.13
Research compound, $C_{26}H_{19}O_3Br$[a]	15.58	6.38	17.43	17.40	+0.03
	17.22	7.08	17.49		+0.09

[a] 100 mg. of KNO_3 added to ignition mixture.

Table II. Determination of Iodine

Compound[a]	Weight of Sample	Weight of AgI	I Calculated	I Theoretical	Difference
	Mg.	*Mg.*	%	%	%
p-Iodonitrobenzene	20.32	19.12	50.87	50.97	−0.10
	21.04	19.92	51.18		+0.21
p-Iodoaniline	15.86	17.02	58.01	57.98	+0.03
	20.12	21.55	57.90		−0.08
β-Iodopropionic acid	15.86	18.67	63.64	63.46	+0.18
	15.71	18.36	63.18		−0.28
Methyl-*p*-iodobenzoate	18.00	16.05	48.20	48.43	−0.23
	26.03	23.25	48.28		−0.15
p-Iodoazobenzene	16.97	12.93	41.19	41.19	+0.00
	4.92	3.75	41.20		+0.01
p-Iododiphenyl	12.91	10.79	45.18	45.31	−0.13
	14.93	12.50	45.26		−0.05

[a] 100 mg. of KNO_3 added to ignition mixture in every case.

The analytical method of determining the halogen in the ignition product is the same as that described earlier for chlorine (*1*), except that provision is made for the reduction of possible bromate and iodate. The reduction is carried out by adding 200 mg. of hydrazine sulfate (Eastman) to the water suspension of the ignition product and warming the mixture to 75° to 85° C. for 5 minutes. The calcium oxide suspension is then cooled under tap water and dissolved with stirring in the minimum amount of nitric acid.

Fine grinding of the sample and its intimate mixing with the calcium oxide have been found necessary for obtaining satisfactory results. It is also recommended that a blank be determined on the combined reagents.

The method has sufficient accuracy for the determination of the number of halogen atoms in the organic molecule.

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Preparation of Samples for Microbiological Determination of Riboflavin

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THE Snell and Strong microbiological assay for riboflavin (*10*) has been employed successfully by many laboratories on a large variety of biological materials. It is generally accepted that the values so determined compare favorably with those obtained by animal and physicochemical methods. However, it has been recognized that the microbiological method gives higher results than other methods on certain types of material, notably cereals and cereal products.

It has been reported (*1*, *9*) that the starch component of cereals, or some substance associated with it, is responsible for the high values obtained by the microbiological method. According to Scott, Randall, and Hessel (*9*) the stimulatory substance is destroyed by digestion with taka-diastase, while Andrews, Boyd, and Terry (*1*) found that taka-diastase digestion alone did not suffice to avoid the stimulating effect. Bauernfeind, Sotier, and Boruff (*2*) reported that stimulatory substances were removed from food materials by extraction with organic solvents and demonstrated that a number of known fat-soluble compounds are capable of stimulating the test organism, *Lactobacillus casei*. An ether-soluble fraction has also been obtained from blood which stimulates the growth of the organism, and a substance is present in alkali-hydrolyzed liver which inhibits it (*7*).

This paper describes a comparative study of modified procedures proposed for the riboflavin assay, a study of the effect of pure fatty acids, glycerides, and other organic materials on the test organism, and a simple and effective procedure which has been developed in this laboratory for determining riboflavin in cereal products and other materials.

Experimental

The microbiological assays were carried out as originally described (*10*), except that modifications were made in the preparation of the samples for assay.

[A minor alteration in the preparation of the yeast supplement was also introduced—viz., the first filtration after adding the basic lead acetate was omitted. Instead, the mixture was at once made alkaline with ammonia, and was then filtered. This procedure is much less laborious.

Entirely satisfactory yeast supplements have also been prepared from whole autolyzed yeast (Difco Laboratories, Detroit), rather than from yeast extract as originally recommended (*10*). These supplements have been used at a level equivalent to 20 mg. of the whole autolyzed yeast per tube.]

PROCEDURE 1, DIRECT. A weighed sample containing approximately 10 micrograms of riboflavin was suspended in 50 cc. of 0.1 *N* hydrochloric acid, the suspension was autoclaved for 15 minutes at 1 kg. per sq. cm. (15 pounds per sq. inch) pressure, and cooled, 1 cc. of phosphate buffer (pH 7.0) was added, and the mixture was adjusted to pH 6.6 to 6.8 with *N* sodium hydroxide. It was then diluted to 100 cc., and aliquots were added to the assay tubes.

PROCEDURE 2, FILTRATION. The autoclaved suspension obtained as above was adjusted to pH 4.5 (or until a heavy precipitate appeared) with 2.5 *N* sodium acetate solution, and the volume made to 100 cc. The mixture was filtered by pouring repeatedly through a fluted, No. 40 Whatman filter paper until a clear filtrate resulted. A 50-cc. aliquot of the filtrate was adjusted to pH 6.6 to 6.8, and diluted to 100 cc. for assay. In the case of a few samples it was found advantageous to filter a second time after the pH had been adjusted to 6.6 to 6.8 (cf. Table VIII).

PROCEDURE 3, FILTRATION AND ETHER EXTRACTION. This was the same as procedure 2, except that the 50-cc. aliquot of the first filtrate was shaken out three times with 30-cc. portions of ether. Without removing dissolved ether the aqueous phase was adjusted to pH 6.6 to 6.8, and diluted to 100 cc., and aliquots were added directly to the assay tubes.

SCOTT PROCEDURE. This was carried out exactly according to published directions (*9*).

ANDREWS PROCEDURE. This also was carried out as published (*1*).

MATERIALS TESTED. The fat-soluble substances to be tested for their effect on the assay were dissolved in a small amount of ethanol, and the solution was diluted with approximately 20 volumes of water. Aliquots of the suspensions so obtained were added directly to the assay tubes. The small amount of alcohol so introduced had no detectable influence on the bacterial response.

The ether-soluble fraction of cornstarch was obtained by continuous extraction of a suspension prepared by autoclaving 20 grams of commercial cornstarch for 15 minutes at 1 kg. per sq. cm. (15 pounds per sq. inch) pressure in 200 cc. of 0.1 *N* hydrochloric acid. Palmitic acid, stearic acid, and tristearin were Eastman products, and were used as received.

The oleic acid used had been purified by low-temperature crystallization from acetone, followed by distillation in vacuo. Iodine value, 89.8; calculated, 89.9. Triolein was prepared from oleic acid purified in this manner, and was subjected to molecular distillation before use. The linoleic acid was a sample which had been fractionated at reduced pressure. The acid was peroxide-free. Iodine value, 179.5; calculated, 181.1. The oleic acid, linoleic acid, and triolein were all preserved by sealing in evacuated ampoules immediately after distillation. The lecithin tested was a sample of commercial soybean lecithin which had been rendered "oil-free" by repeated extraction with acetone in a Waring blendor.

Results

The type of difficulty encountered in applying the original, direct procedure (procedure 1) to a cereal is illustrated in Table I. The drift in the values obtained for whole wheat flour is typical of cereal products in general. The same effect is seen to a greater degree in the case of cornstarch containing a known amount of added riboflavin. It is evident that both stimulation and inhibition of the bacterial response occur when the direct procedure is followed. The stimulating material was evidently not removed by the Scott procedure. However, procedure 3 and the Andrews procedure resulted in satisfactory assay values. The ether-soluble nature of the interfering material is shown by the data in the last column. Separate assays showed that the stimulation observed in this case was not caused by riboflavin in the ether extract itself.

TABLE I. RIBOFLAVIN CONTENT OF CEREAL SAMPLES

Volume of Suspension Added per Tube	Whole Wheat Flour, Procedure 1	Cornstarch[a] Containing 5 Micrograms per Gram of Added Riboflavin				Riboflavin plus Ether Extract of Hydrolyzed Cornstarch[b]
		Procedure 1	Procedure 3	Scott procedure	Andrews procedure	
Cc.	*Micrograms per gram*					*Micrograms*
1	3.9	9.5	5.4	10.8	5.4	8.2
1	3.8	9.2	5.0	9.9	5.4	8.6
2	3.0	4.2	5.2	9.5	4.9	8.2
2	3.0	5.1	5.3	9.5	4.8	7.8
3	2.7	1.9	5.0	7.9	4.9	6.4
3	2.6	1.7	5.0	8.2	5.0	5.9
4	2.3	1.8	4.9	..	5.3	4.5
4	2.3	1.4	4.9	..	5.4	4.3

[a] Cornstarch itself contained less than 0.20 microgram of riboflavin per gram.
[b] Aqueous suspension containing 5 micrograms of riboflavin and ether-soluble material from 1 gram of cornstarch.

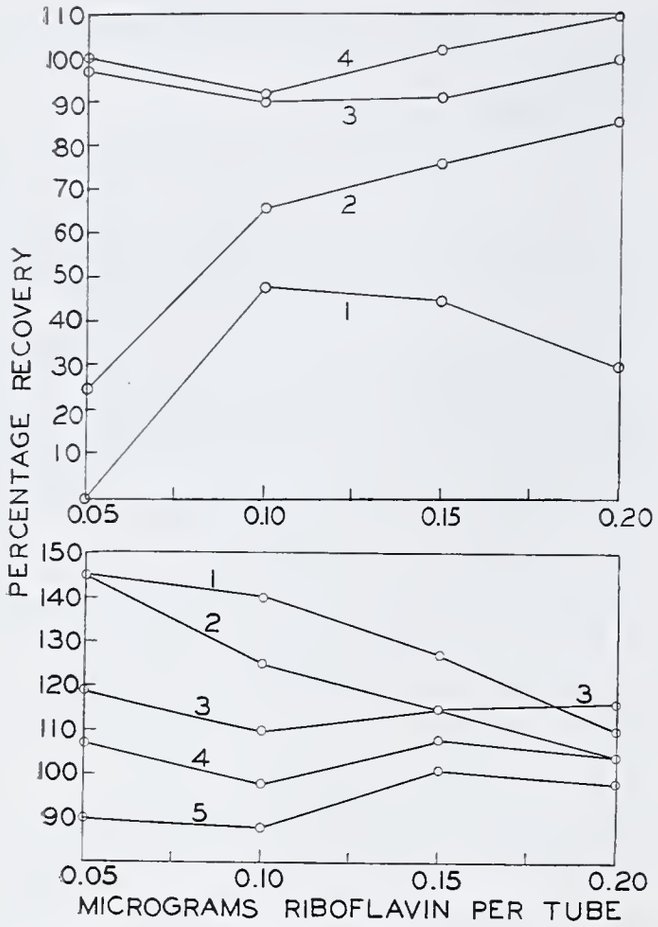

FIGURE 1. EFFECT OF FATTY ACIDS ON MICROBIOLOGICAL DETERMINATION OF PURE RIBOFLAVIN

Upper. Effect of palmitic acid. Ratio of riboflavin to palmitic acid:

1. 1 to 5200
2. 1 to 1040
3. 1 to 260
4. 1 to 26

Lower. Effect of stearic acid. Ratio of riboflavin to stearic acid:

1. 1 to 1000
2. 1 to 400
3. 1 to 200
4. 1 to 50
5. 1 to 5

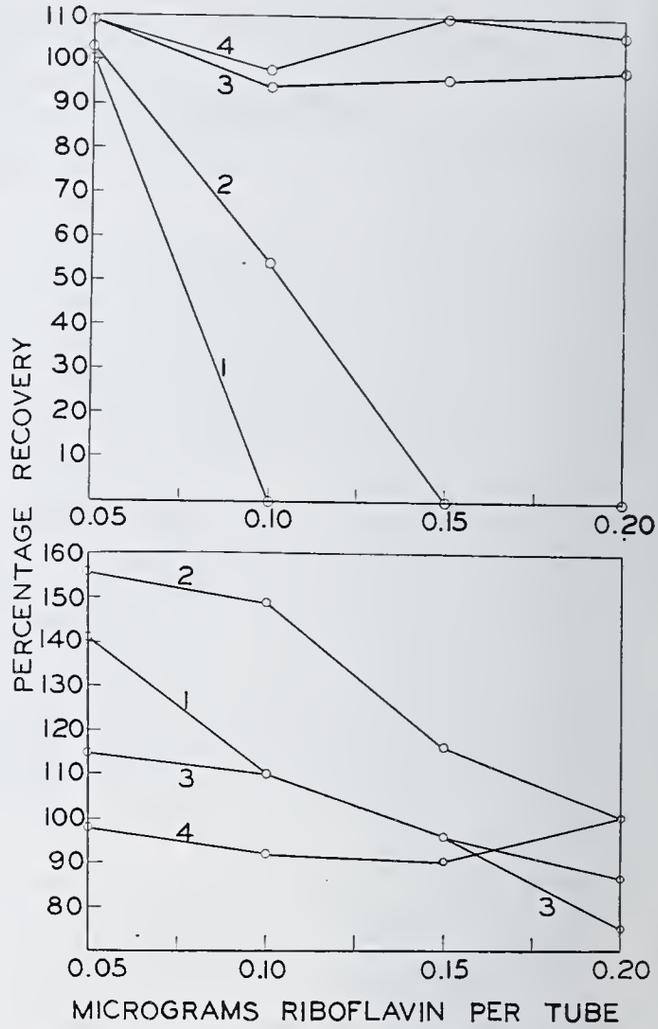

FIGURE 2. EFFECT OF FATTY ACIDS ON MICROBIOLOGICAL DETERMINATION OF PURE RIBOFLAVIN

Upper. Effect of linoleic acid. Ratio of riboflavin to linoleic acid:

1. 1 to 4000
2. 1 to 800
3. 1 to 400
4. 1 to 40

Lower. Effect of oleic acid. Ratio of riboflavin to oleic acid:

1. 1 to 4800
2. 1 to 960
3. 1 to 480
4. 1 to 96

In view of the above results and of previous work pointing to the fat-soluble nature of the interfering material (*2*, *7*), the effect of various known fatty substances was studied. Figures 1 and 2 summarize the results obtained with different mixtures of pure riboflavin with various fatty acids. The effect, as indicated by the deviation of the recovery values from 100 per cent, was found to depend both on the absolute amounts of riboflavin and fatty acid present in the tube, and on the ratio between the two. The bacterial growth was affected very markedly at ratios of 1 to 500 or 1 to 1000. At the 0.05-microgram level of riboflavin this corresponds to only 25 to 50 micrograms of the fatty acid per tube. In general, the widest deviations from 100 per cent recovery were observed at the lowest level of riboflavin tested. Oleic and stearic acids markedly stimulated the bacterial response, while palmitic acid and especially linoleic acid acted as potent inhibitors. These results corroborate, in part, the work of Bauernfeind *et al.* (*2*), who found that oleic, stearic, and palmitic acids stimulated the test organism, while linoleic acid either stimulated or inhibited, depending on the amount used. The effect of a mixture of two fatty acids was also investigated. The particular mixture tested, which consisted of oleic and linoleic acids in the ratio 1 to 0.8, gave very high recoveries when tested at a level of 3600 parts of the mixture to one of riboflavin.

The data in Table II show that in contrast to the free fatty acids neutral fats had little disturbing influence on the microbiological determination of riboflavin. Lecithin gave a moderate stimulation at high levels (Figure 3).

Although the pH of the medium (6.6 to 6.8) is such that the fatty acids tested should have been present to only a very small degree in the form of soaps, it was of interest to try the effect of substances capable of lowering the surface tension in neutral solution. The synthetic wetting agents listed in Table III proved to be inert at the concentrations tested. These Aerosols are sodium salts of various alkyl sulfosuccinic acid esters (*3*, *16*).

In order to determine whether the effect of the unsaturated acids might be attributable to peroxides or other oxidation products, a sample of oleic acid was oxidized by bubbling air through it for 15 hours at room temperature, and then tested. Substantially the same results were secured as with highly purified oleic acid. Benzoyl peroxide at concentrations of 40 to 800 micrograms per tube also was without effect.

A comparison of the different procedures as applied to various cereal samples is given in Table IV. It seems certain that the values obtained by the direct method and by the

Table II. Effect of Fats on Recovery of Known Amounts of Riboflavin

Riboflavin Added per Tube	Fat Added per Tube	Riboflavin Recovered			
		Mazola oil	Wesson oil	Triolein	Tristearin
Micrograms		%	%	%	%
0.05	200	115	112	97	132
0.10	400	103	104	96	104
0.15	600	94	99	101	110
0.20	800	89	99	99	105
0.05	40	121	106	99	99
0.10	80	100	95	98	93
0.15	120	91	96	102	99
0.20	160	82	98	101	98
0.05	4	108	112	120	108
0.10	8	92	92	100	96
0.15	12	97	98	100	100
0.20	16	103	103	110	103

Table III. Effect of Wetting Agents on Recovery of Known Amounts of Riboflavin

Riboflavin Added per Tube	Wetting Agent Added per Tube	Riboflavin Recovered		
		Aerosol AY	Aerosol IB	Aerosol MA
Micrograms		%	%	%
0.1	2000	105	116	109
0.1	1000	100	99	110
0.1	500	105	105	104
0.1	250	99	97	104
0.1	125	100	100	102
0.1	50	95	112	97

Scott method were too high, while the other procedures gave reasonably concordant results. In most cases procedures 2 and 3 gave essentially the same values.

The recovery of added riboflavin as determined by the filtration procedure is indicated in Table V, and Table VI shows that entirely consistent results at the different dosage levels were secured by this method. The validity of procedure 2 is further attested by the data in Table VII, which demonstrate that the values so obtained agree very well with results of fluorometric and animal assays on identical samples. These data show definitely that the present method is capable of yielding reliable values for composited human diets (Table VII, sample 1). That it is also applicable to mixed feeds was demonstrated by analysis of 12 individual ingredients of a chick ration. The riboflavin content of the ration as calculated from the individual analyses was 4.6 micrograms per gram, while the average of four determinations according to procedure 2 was 5.0 micrograms per gram (Table IV).

The data in Table VIII indicate that on certain types of material the original direct method yields reliable values, while many others must be handled by the modified procedure.

Discussion

In the light of the results reported in the present paper and in previous publications, it is probable that the "drift" frequently encountered in the microbiological determination of riboflavin is caused mainly by free fatty acids in the suspensions being tested. This view is consistent with the fact that the extracted solids from some types of samples cause stimulatory effects in the assay (*2*), while other solids, such as paper pulp, ground glass, and the like, are inert (*1, 2, 10*).

The stimulation and inhibition which operate to produce the observed drift may obviously be caused by one and the same substance (cf. Figures 1 to 3). It is common knowledge that a substance which stimulates an organism at one concentration may prove inhibitory or even toxic at somewhat higher concentrations.

The fact that a ratio of 960 parts of oleic acid to one of riboflavin gave a greater stimulation than either smaller or larger amounts (Figure 2, lower) is probably to be explained on this basis. The only other reports known to the authors in which oleic acid has been shown to influence bacterial growth are those of Bauernfeind *et al.* with *Lactobacillus casei* (*2*) and of Mueller with the diphtheria bacillus (*4*). In the latter case it was also found that there was an optimum concentration of the acid, and the effective quantities were of the same order of magnitude as those found to be active toward *Lactobacillus casei*.

Further evidence that the drift is attributable to fatty acids was secured in carrying out the experiments summarized in Figures 1 to 3. In working with these mixtures, which contained only pure chemicals and were entirely free from "solids", typical drifts similar to those encountered in actual assays were very frequently observed.

Since starch yields about 0.6 per cent of fatty acids on hydrolysis (*5*), cereal samples in the amounts required for the determination would supply such acids in sufficient quantities to account for the observed effects. Likewise the stimulatory and inhibitory substances found in blood and in alkaline hydrolyzed liver (*7*), although not yet identified, could well be oleic acid and linoleic acid, respectively.

From the information now available it would seem that the preparation of cereals and certain other samples for microbiological riboflavin assay should consist of two main steps, (1) a hydrolysis, and (2) a procedure designed to remove fat-

Table IV. Determination of Riboflavin in Cereal Products by Various Procedures

Sample	Procedure Used				
	1	2[a]	3	Scott	Andrews
	Micrograms of riboflavin per gram				
Whole wheat flour	2.5	1.3		1.2	0.90
Whole wheat bread	2.7	1.8	1.9	3.3	1.6
Chick ration	6.7	5.0	4.6	7.8	4.3
Wheat bran	6.0	3.6	3.3		
Farm feed 1	6.5	3.2	2.9	6.1	3.6
Farm feed 2	5.8	3.2	3.2	5.8	3.8

[a] Average of at least three assays.

Table V. Riboflavin Recovered by Procedure 2

Sample	Riboflavin Content	Riboflavin Added	Riboflavin Found	Recovery
	Micrograms per gram			%
Chick ration	5.0	5.0	10.1	102
Wheat bran	3.6	2.5	5.9	92
Whole wheat flour	1.2	1.0	2.3	110
Whole wheat bread	1.9	2.0	3.9	100
Farm feed 1	3.2	2.5	5.4	88
Alfalfa meal	10	10	20	100
Dried whey	25	20	45	100
Fish meal	6.8	6.7	12.5	85
Meat scraps	4.3	5.0	9.2	98

Table VI. Comparison of Individual Assay Data for Cereal Samples Assayed by Procedures 1 and 2

Volume of Suspension Added per Tube	Chick Ration		Wheat Bran		Whole Wheat Bread		Farm Feed 1	
	Procedure 1	Procedure 2	Procedure 1	Procedure 2	Procedure 1	Procedure 2	Procedure 1	Procedure 2
Cc.	*Micrograms of riboflavin per gram*							
1	8.8	5.2	8.1	3.9	3.1	2.2	7.1	...
1	9.1	5.3	7.3	4.2	3.1	2.0	8.0	...
2	6.1	5.0	6.1	4.0	2.6	2.0	6.7	3.3
2	6.0	5.2	6.6	3.6	2.6	1.8	6.5	3.4
3	5.3	5.0	5.6	3.5	2.3	2.0	5.9	2.8
3	5.2	4.9	5.9	3.5	2.2	1.9	5.6	3.4
4	4.8	5.1	5.1	3.5	...	1.8	4.8	2.8
4	4.8	4.8	4.1	3.6	...	1.8	5.3	3.3

soluble interfering substances. The hydrolysis should be sufficiently drastic to break up various combined forms of riboflavin and thus reduce the problem to the determination of a single compound. Although the bacterial method has been shown to measure the riboflavin contained in at least some of these combined forms (*6*), others may exist which are not so measured. Furthermore, riboflavin itself is less likely to be lost in subsequent precipitations, than, for example, riboflavin phosphate, which has been shown to have a greater affinity for at least one protein than does free riboflavin (*8*), and may well behave similarly toward others.

The removal of interfering substances from the hydrolyzate can be accomplished successfully in most cases by a careful filtration. Ether extraction has been found equally effective, but is usually complicated by emulsion formation if much solid matter is present. In any event it is time-consuming in routine work, and has been used as an adjunct to filtration in only a few cases where the filtration alone proved inadequate. Extraction of the original material with a fat solvent could obviously not be substituted for the above precautions on account of the formation of additional free fatty acids on hydrolysis. Such extraction is desirable, however, in the case of high-fat materials such as cheese, fish meal, and the like.

Table VII. Riboflavin Content of Various Materials

(Determined by the microbiological, fluorometric, and rat assay methods)

No.	Sample Description	Analyst	Method of Assay	Riboflavin Content *Micrograms/g.*
1	Fresh mixed diet	A	Microbiological, procedure 2	4.3[a]
1	Dried mixed diet	A	Microbiological, procedure 2	4.7
		B	Microbiological, procedure 2	4.1
		C	Microbiological, procedure 2	4.9
		C	Fluorometric	4.8
		D	Fluorometric	4.2
		E	Rat growth	4.8
2	Rumen solubles	A	Microbiological, procedure 2	17
		C	Fluorometric	17
3	Whole wheat flour	A	Microbiological, procedure 2	1.3
		C	Fluorometric	1.1
4	Whole wheat bread	A	Microbiological, procedure 2	1.8
		C	Fluorometric	1.5
5	Dried yeast	A	Microbiological, procedure 2	62
		C	Fluorometric	59

[a] Calculated on dry basis.

Table VIII. Riboflavin Content of Various Materials

(Determined by original and modified procedures)

Sample	Riboflavin Content Procedure 1	Procedure 2	Sample	Riboflavin Content Procedure 1	Procedure 2
	Micrograms per gram			*Micrograms per gram*	
Grapefruit juice	0.2[a]	0.3	Wheat bran	6.0[a]	3.6
American cheese	6.6	6.6	Dried whey	24	25
			B. Y. feed	66	65
Whole milk	2.1[a]	1.7	Whole oats	3.7[a]	1.7
Tomato juice	0.4[a]	0.3	Meat scraps	5.7[a]	4.3[b]
Hamburg	3.9[a]	1.3	Fish meal	7.8[a]	6.8[b]
Ice cream	7.7	2.0	Soy bean meal	6.8[a]	3.3
Yellow corn	2.2[a]	1.3	Corn gluten meal	3.4[a]	1.7
Whole barley	2.9[a]	1.6	Dehydrated alfalfa meal	11[a]	10
Wheat gray shorts	5.7[a]	3.1			

[a] Significant drift in individual values observed in these assays.
[b] A second filtration was employed after neutralization of extracts.

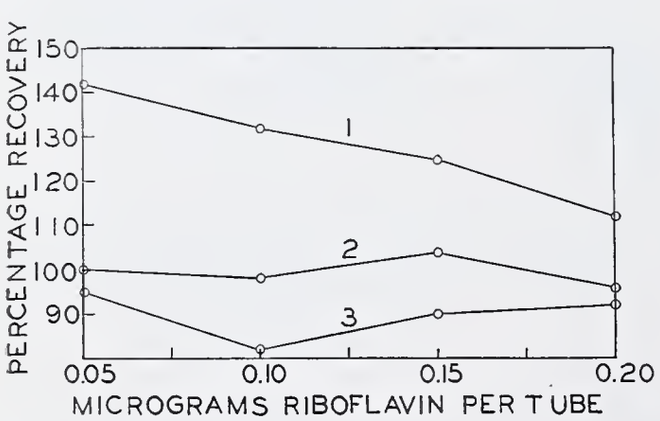

Figure 3. Effect of Lecithin on Microbiological Estimation of Pure Riboflavin

Ratio of riboflavin to lecithin:

1. 1 to 8000
2. 1 to 800
3. 1 to 80

In view of the above considerations, procedure 3 is recommended as being the most generally applicable method, while procedure 2 is regarded as being suitable for most materials, notably cereals and cereal products. The validity of these procedures is supported by (*a*) lower and more reasonable results on mixed feeds and composited diet samples, which by the direct procedure have often appeared to contain twice as much riboflavin, or more, than the sum of the constituents; (*b*) the absence of drift at different dosage levels; (*c*) satisfactory recovery of added riboflavin; and (*d*) good agreement with the results of independent assays by other methods on the same samples.

The stimulations and inhibitions studied in this paper tended to be greatest at the 0.05-microgram level of riboflavin. This observation emphasizes the desirability of calculating the riboflavin content of unknowns from tubes for which the titration values fall above this level on the standard curve. It has been the authors' experience that results from tubes containing less than 0.05 microgram of riboflavin are practically worthless, and it is the practice in this laboratory to exclude all such values from the calculation of the final assay results.

A question raised by the present paper concerns the accuracy of riboflavin values previously obtained by the original microbiological method. The agreement which has been observed repeatedly between such values and those secured in other ways, together with the direct comparisons shown in Table VIII, makes it appear probable that on many samples, especially those of relatively high potency, the older values are substantially correct. Results previously obtained on cereals, mixed feeds, composited diets, fish meals, and certain other materials by the direct method are very likely too high. In the future such materials should be assayed by a suitably modified procedure. The authors are unable at present to offer any procedure for determining riboflavin in blood which is an improvement over the method previously suggested (*13*).

The possibility of avoiding interference from fatty materials by incorporating them in the basal medium has been considered, but this procedure appears inadvisable. In the first place, the effects of various fatty acids differ not only in degree but in kind (oleic *vs.* linoleic), so that it would probably be difficult to compensate for both at the same time. Secondly, it has been the authors' experience that it is difficult to reproduce closely the effect of a given fatty acid. This may be attributable to variations in the physical state—

e. g., degree of subdivision—of the suspensions tested. Finally, the fundamentally different solubility characteristics of the fatty acids and the B-vitamins offer a ready means of separation.

Interference of the type here described has also been encountered, and often to an even greater degree (*7*), in the microbiological determination of pantothenic acid (*12*). Similar preliminary treatment of the samples appears to offer a satisfactory solution (*15*). The nicotinic acid assay (*11*), on the other hand, is but little disturbed by fatty acids (*14*).

Summary

A study has been made of the substances present in cereals and other biological materials which interfere with the determination of riboflavin by the microbiological method.

It has been shown that the interference is probably due to small amounts of free fatty acids, and methods for avoiding their effect have been investigated.

A procedure for the preliminary treatment of the sample has been worked out. The interfering substances are thereby removed, and reliable values for riboflavin are obtained on such materials as cereals and mixed diets which previously have been difficult to assay by the microbiological method.

Acknowledgment

The authors wish to express their appreciation to F. W. Quackenbush for samples of oleic acid, linoleic acid, triolein, and lecithin; to the American Cyanamid and Chemical Corporation for samples of the Aerosols AY, IB, and MA, and to John S. Andrews, Howard J. Cannon, and Miss Jean Collard for carrying out several of the analyses reported in this paper.

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Systematic Identification of the Common Metallic Coatings

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THE testing of an unknown metallic finish for identification of the metal which has been used for plating has not, to the authors' knowledge, been systematized. The series of reactions developed by the authors is designed to eliminate guesswork and to provide a simple and logical procedure for establishing the identity of the metallic coating. The method applies to the following commonly used metals: chromium, tin, nickel, gold, silver, rhodium, zinc, cadmium, copper, aluminum, and lead. This procedure is particularly adapted to a laboratory which is engaged in the testing of consumer articles where merchandise like umbrella handles, cooking utensils, fountain pens and pencils, novelty jewelry, hardware, metallic toys, trays for various purposes, etc., is submitted for the purpose of identifying the metallic coating.

Before testing is begun, any lacquer which may cover the surface of the metal must be removed, using a mixture of equal parts of ethanol and acetone on a piece of absorbent cotton. The cleaned area is then subjected to the series of spot tests described in this article. Each reagent is added on a separate spot, and no reactions are carried through beyond one step unless specifically directed. Because never more than one drop of reagent is added, it is recommended that the reagents be kept in a set of 60-ml. dropping bottles for convenience. In the special cases where a spot on a filter paper must be rendered ammoniacal, the paper is held over the mouth of a bottle of concentrated ammonium hydroxide solution for about 30 seconds.

Sometimes, the reactions of the reagents with the metal surfaces are extremely slow. In these cases it is advisable to roughen the surface by rubbing very lightly with fine emery cloth (No. 000) or sandpaper (No. 00) before adding the reagent, taking care to keep away from the base metal, since that may greatly interfere with the proper interpretation of the results. The authors have found the use of a small magnifying glass very helpful for observing reactions.

In Table I and outline, the term "colored" is used for metals which do not have the familiar "silvery" appearance of aluminum, lead, etc.

Reagents

Nitric acid, equal parts of 37 per cent nitric acid and distilled water. Hydrochloric acid, 35 per cent (concentrated). Ammonium hydroxide, 28 per cent ammonia (concentrated). Sodium sulfide, 10 per cent aqueous solution. Cacotheline. Dimethylglyoxime, 0.6 gram in 50 ml. of 95 per cent ethanol. Sodium hydroxide, 10 per cent aqueous solution.

Procedure

I. Colored plating. Add one drop of nitric acid.
  A. A blue solution is obtained: Copper, brass, or other alloys of copper
  B. Action is delayed, or no reaction occurs: Gold

TABLE I. PROCEDURE FOR CHROMIUM, NICKEL, TIN, GOLD, SILVER, RHODIUM, ZINC, CADMIUM, COPPER, ALUMINUM, AND LEAD

Colored Plating: Add Nitric Acid

Blue solution: Copper, brass, other copper alloys

Reaction delayed or does not occur: Gold (confirm with microscope)

Colorless Plating: Add Nitric Acid and Wait 2 Minutes if Reaction Is Slow

Plating Not Attacked: Rh, Al, Cr. Add NaOH

Plating attacked: Aluminum

Plating not attacked: Rh, Cr. Add HCl

Green solution: Chromium

No action: Rhodium (Pt metals)

Plating Attacked, Giving Green Solution: Cr, Ni. Add HCl, take up on filter paper, render ammoniacal. Add dimethylglyoxime solution

Red color on paper: Nickel

No red color on paper: Chromium

Plating Attacked without Giving Colored Solution: Sn, Ag, Zn, Cd, Pb: Cacotheline Test

Positive (violet): Tin

Negative: Ag, Zn, Cd, Pb. Add HNO_3, take up on filter paper, render ammoniacal, add sodium sulfide

White or no color: Zinc

Bright yellow: Cadmium

Brown-black: Ag, Pb. Add nitric acid, take up on filter paper, add NaOH

Brown-black: Silver

White: Lead

II. Colorless plating. Add one drop of nitric acid to plating and if reaction is slow, wait 2 minutes.

A. Plating is not attacked. Add one drop of sodium hydroxide to a new spot.

1. Plating is attacked: Aluminum

2. Plating is not attacked. Add concentrated hydrochloric acid and take up after a few seconds on filter paper.

a. Green solution is formed: Chromium

b. No action apparent: Rhodium and platinum metals

B. Plating is attacked by nitric acid, giving a green solution. Place hydrochloric acid on new spot, take up on filter paper, render ammoniacal, and add dimethylglyoxime.

1. Red coloration formed on paper: Nickel

2. No red color obtained: Chromium

C. Plating is attacked by nitric acid, giving a colorless solution. Perform cacotheline test. Place small amount of solid cacotheline on a small filter paper and add one drop of water. Place one drop of hydrochloric acid on the metal and touch the reverse side of the paper holding the cacotheline to the spot of acid. A red-violet color is a positive reaction.

1. Reaction is positive: Tin

2. Reaction is not positive. Place nitric acid on a new spot, take up on filter paper, render ammoniacal, and add sodium sulfide.

a. Spot remains colorless or white: Zinc

b. Spot becomes bright yellow: Cadmium

c. Spot becomes brown-black. Place nitric acid on a new spot, take up on filter paper and add sodium hydroxide.

1. Spot becomes brown-black: Silver
2. Spot remains colorless or white: Lead

Summary

A systematic procedure is described for the identification of metals used in the plating of various metallic and nonmetallic articles. The authors have used this method extensively and have found it to be dependable and accurate. Precautions must be carefully heeded to achieve the best results. Very small quantities of reagent and metal surface are used in the identification. A complete test may be run in less than 5 minutes.

Kjeldahl Nitrogen Determination

A Rapid Wet-Digestion Micromethod

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A NUMBER of wet-ashing methods published in the past few years involve the use of perchloric acid. However, they were designed for the determination of the metallic elements (*11*), phosphorus (*1*), and sulfur (*5*). In all these methods, nitrogen is invariably lost by oxidation to nitrate or even to free nitrogen (*11*), and it is necessary, therefore, to resort to a separate Kjeldahl digestion for the determination of nitrogen. The method here proposed obviates this necessity.

The digestion of plant tissues, fertilizer materials, and other organic products for a Kjeldahl determination is a long process even on a micro or semimicro scale. Many methods have been suggested for shortening the Kjeldahl digestion period; a summary of the literature in this connection is given by Gerritz and St. John (*4*). Some of these methods require the use of relatively large amounts of various salts to hasten the reactions (*4*, *10*). Periods of 1.5 hours or more have been found necessary even where catalysts such as selenium or mercury are added together with potassium sulfate to increase the reaction temperature. The use of a mercury-selenium combination has been found effective in cutting down the digestion time for whole milk (*8*), but the advantages of mercury are offset by the necessity of precipitating the mercury before distillation. Furthermore, in alkaline solution if any mercuric sulfate is present, the complex NH_2-Hg-OH may be formed, which will introduce a negative error. Therefore, on the micro scale, at least, mercury has many disadvantages which more than counteract the advantages gained by any increase in the rate of digestion with corresponding decrease in the digestion time.

In the use of the wet-ashing method herein reported for the estimation of any particular element other than nitrogen, salts containing the element in question must not be added to the sample during the course of the procedure.

Dispensing with such salts or reaction accelerators does not constitute a disadvantage, however, since the digestion time is reduced to a fraction of the previous time; rather, it is an advantage, since a reagent is eliminated, thus simplifying the procedure.

Table I. Time of Digestion and Per Cent Recovery of Nitrogen from Soybean Leaf Tissue

Sample No.	Standard Method Time	Standard Method N	Perchloric Acid Method Time	Perchloric Acid Method N
	Min.	%	*Min.*	%
1A	100	5.64	25	5.70
1B	100	5.49	25	5.61
10	110	3.40	25	3.32
14	100	3.43	25	3.46
18	80	3.26	25	3.36
116	150	3.42	25	3.48
20	130	3.03	25	3.00
30	130	3.22	25	3.14

A further advantage of the perchloric acid method is that the final stages of the digestion are carried out at a temperature below the boiling point of the digest. Thus bumping is eliminated and the reaction goes quietly and smoothly. In fact, all the digestions for the determinations reported in this paper were performed in ordinary 20 × 150 mm. rimless Pyrex test tubes and no loss of material was observed at any time.

Perchloric acid is an explosive material when used incorrectly, but there is no danger at all in the method described. The cold dilute acid is not dangerous and the very small amount used is further diluted by the sulfuric acid present to which it is added. In the many digestions performed, the results of which are presented in this report, no evidence of any explosive tendency of the perchloric acid has been observed.

Reagents Required

Concentrated sulfuric acid, nitrogen-free.

Ranker's solution, 32 grams of salicylic acid per liter of concentrated sulfuric acid.

Selenium oxychloride, 12 grams of selenium oxychloride per liter of concentrated sulfuric acid.

Sodium thiosulfate, 33 per cent, 50 grams of sodium thiosulfate monohydrate per 100 ml. of distilled water.

Perchloric acid, 35 per cent, diluted from the 70 per cent acid with distilled water.

Procedure

A suitable sample of the material to be analyzed—for example, 10 to 15 mg. of plant tissue—is weighed out and introduced into the clean dry test tube if nitrogen only is to be determined or 40 to 50 mg. if other elements are to be included as well. If nitrates are to be included in the determination 1 ml. of the salicyclic acid–sulfuric acid solution (*9*) is added, mixed with the tissue, and allowed to stand in the cold for 30 minutes. Three drops of the 33 per cent sodium thiosulfate to reduce the nitro groups, and 0.5 ml. of the selenium oxychloride solution are now added to the sample. If nitrates are not to be included in the analysis 1 ml. of sulfuric acid and 0.5 ml. of selenium oxychloride solution are added to the sample.

The test tube is heated moderately with a microburner (the standard microcombustion stand without modification will usually hold the test tubes) for a minute or two to determine whether the sample will froth excessively. If frothing does not occur the sample is heated vigorously for 10 to 15 minutes. This period depends upon the length of time necessary for the material to go into solution, char, and pass from the black stage, through the muddy brown stage, to the stage in which the solution takes on a clear ruby wine color. Some samples may not go through this sequence, but the corresponding final stage is one in which the solution takes on a clear color, which may be deep yellow or shades of red or green. In this final stage, which is produced in 10 to 15 minutes, a colorless liquid condenses on the walls of the test tube and the evolution of white fumes has decreased considerably.

The digest is now allowed to cool so that there is no danger of cracking the test tube, then cooled under the cold water tap for a few minutes more.

In order to minimize loss of the perchloric acid by volatilization from the sides of the tube, 2 drops of 35 per cent perchloric acid are now added by allowing the acid to fall directly into the digest. There is no danger of explosion if this procedure is followed.

The outside of the test tube is wiped dry and returned to the digestion rack. The burners are turned down to a low flame, since it is important that the temperature of the solution be kept below the boiling point. The mixture rapidly becomes lighter in color, going through shades of yellow and clearing in a few minutes. The digest is kept over the low flame, since after the first clearing, a yellow color reappears in the digested solution. Heating is continued at a temperature below the boiling point until this secondary color disappears and the digest becomes clear and colorless. If there is any iron in the sample a yellow tint may persist, but this usually disappears on cooling and dilution. The total time required for the digestion is usually 10 to 25 minutes.

The samples are allowed to cool, diluted with a few milliliters of distilled water, and allowed to cool again. They are now ready for nitrogen determinations or can be diluted to 25 to 50 ml. and aliquots taken for analysis.

Experimental Results

Analyses were performed on a relatively large number of samples of materials of diverse character, both by the standard methods now in general use and by the perchloric acid method here described. All the analyses were carried out on a comparative basis, involving in each case the per cent recovery of nitrogen and the time required for the digestion by the two methods. The results of these analyses are presented in the tables.

All distillations were performed with the Parnas and Wagner apparatus, using 2 per cent boric acid as the receiver with the methyl red–bromocresol green indicator employed by Ma and Zuazaga (*6*).

Table I presents the results of analyses performed on soybean leaf tissue, which proved difficult to digest. With selenium oxychloride as a catalyst, 20- to 30-mg. samples required from 100 to 150 minutes to complete the digestions by the standard method. In addition to this long time for digestion, bumping was violent and somewhat difficult to control. The time required to perform the digestions by the perchloric method averaged only 25 minutes. There was no loss of nitrogen, as is indicated by Table I.

Attempts to use perchloric acid in Kjeldahl digestions usually have resulted in a loss of nitrogen. With this in mind, the following analytical tests were made upon samples of ammonium chloride to determine whether the nitrogen could be recovered without loss when subjected to the perchloric acid treatment as here employed in a complete combustion procedure. As the data in Table II show, no nitrogen was lost.

Table II. Recovery of Nitrogen from Ammonium Chloride and Potassium Nitrate

(After subjecting samples to complete perchloric acid digestion procedure)

	Nitrogen in Sample	Nitrogen Recovered	
	Mg.	*Mg.*	%
NH_4Cl	2.46	2.46	100.0
	1.57	1.61	102.5
KNO_3	0.20	0.1970	98.5
	0.40	0.3974	99.4
	0.60	0.5912	98.5

With the same object in view similar analytical tests were carried out for the recovery of nitrogen from nitrates.

Aliquots of a standard nitrate solution were transferred to test tubes, a few drops of saturated calcium hydroxide solution were added to each, and the nitrate solution was evaporated to dryness on a steam bath. When dry, 1 cc. of the salicylic acid–sulfuric acid solution was added, allowed to react for 30 minutes, and reduced with 3 drops of the 33 per cent thiosulfate solution. The resulting solution was then subjected to the digestion process as described and the nitrogen determined.

The results are presented in Table II. Each value given in the table for nitrogen recovery from potassium nitrate represents the average determination of four aliquots. The average per cent values of nitrogen recovered are slightly below the theoretical 100 per cent of the samples. The per cent values of nitrogen recovered from individual aliquots deviated

Table III. Nitrogen Content of Pure Compounds

(Determined by perchloric acid method checked against their theoretical per cent values)

Compound	Theoretical	Experimental	Deviation from Theoretical Value
	%	%	%
Asparagine.H_2O	18.66	18.61	−0.05
Glutamine	19.17	19.14	−0.03
Tyrosine	7.73	7.86	+0.13
Benzidene	15.20	15.16	−0.04
Diphenylamine	8.28	8.16	−0.12
Tetramethyldiaminodiphenylmethane	11.01	11.03	+0.02
		Av. deviation	−0.065

Table IV. Nitrogen Content of Organic Materials

Material	Micro Perchloric Acid Method: Digestion time	Micro Perchloric Acid Method: N	Nitrogen by A. O. A. C. Official Method	Deviation from A. O. A. C. Determination
	Min.	%	%	%
Cocoa tankage	11	2.57	2.52	+0.05
Processed tankage	13	9.68	9.76	−0.05
Beetle scrap dust	13	19.02	19.02	0.00
Chicken manure	13	2.26	2.25	+0.01
Milorganite	11	5.60	5.66	−0.06
Acid fish scrap	13	8.54	8.54	0.00
Processed tankage 9b	17	6.56	6.60	−0.04
Processed tankage 8b	14	9.44	9.42	+0.02
Shuey sample 1	13	8.85	9.04	−0.19
2	12	8.50	8.55	−0.05
3	14	8.65	8.79	−0.14
			Av. deviation	0.058

both above and below this theoretical value, but all were well within the limits of experimental error of the procedure, and the average recovery values do not actually represent loss of nitrogen due to the perchloric acid treatment.

As a further check upon the effectiveness and accuracy of the method, and also upon the applicability of the procedure to ultimate analysis, a variety of pure organic compounds were analyzed for their nitrogen content and the results were checked against the theoretical nitrogen per cent values of the respective compounds (Table III). Each experimental value represents the average of duplicate determinations. These values check very closely with the theoretical values.

The average digestion time for 10-mg. samples, including the necessary cooling period, was usually less than 10 minutes.

A final test of this method was performed by analyzing a variety of organic materials, using a 10-mg. sample of each material and comparing the results with those obtained by the official A. O. A. C. method (*2*). Samples of these materials together with the analytical data determined by the official A. O. A. C. method were kindly provided by the Soils Department of the New Jersey Agriculture Experiment Station.

The results of this comparison are presented in Table IV. The average data obtained by the perchloric acid micromethod are in excellent agreement with the corresponding data by the official A. O. A. C. method. The points to be emphasized in connection with Table IV are the close agreement of the results and the difference in the average time required for the digestion procedure by the perchloric acid micromethod here described and by the official A. O. A. C. method. The average digestion time by the former was approximately 12 minutes; by the latter, it was approximately 2 hours.

The perchloric acid micromethod presents a less cumbersome procedure than most other methods, and the great saving in time and a substantial decrease in the cost of reagents are important. The final product of the digestion is a colorless solution suitable for the determination of tissue constituents except sulfur, chloride, and selenium, since no other contaminants than these three are added.

Objections have been offered to the use of selenium as a catalyst on the ground that its use may result in the loss of nitrogen (*3*, *12*). This may be due to the fact that either the digestion is not complete with this catalyst even after the digest has cleared or an actual loss of nitrogen occurs if too large a quantity of selenium is employed in the digestion.

However, with the perchloric acid micromethod here described, low nitrogen values never resulted from the use of selenium as a catalyst, and further heating after the solution had cleared was unnecessary. This fact is borne out by many trials in which the solution was distilled immediately after clearing with complete recovery of the nitrogen in the sample.

The addition of perchloric acid to the sulfuric acid at the start of the digestion as suggested in the macromethod of Mears and Hussey (*7*) invariably resulted in a loss of nitrogen, as indicated in Table V. This was also often the case if only 2 drops of perchloric acid were used. Furthermore, no significant decrease in the digestion time was obtained by this procedure.

In the proposed method, it is important to complete the digestion process at a temperature below the boiling point of the digest after the perchloric acid has been added, in order to avoid loss of the acid on volatilization and to prevent the perchloric acid from reacting too vigorously, which might result in the possible loss of nitrogen.

For some undetermined reason, 2 drops of 35 per cent perchloric acid have been found more effective than 1 drop of 70 per cent perchloric acid in oxidizing the organic matter when used as described. The use of 70 to 72 per cent perchloric acid did not materially hasten the process, but often resulted in a loss of nitrogen.

Work is now in progress to develop, if possible, a macroprocedure similar to the micromethod here reported, in the hope that the time required for the digestion of large samples may be proportionately reduced.

Table V. Loss in Nitrogen When Perchloric Acid Is Added to Sulfuric at Start of Digestion

Sample No.	Nitrogen Loss: 0.25 cc. of perchloric acid and 1.0 cc. of sulfuric acid added at start of digestion	Nitrogen Loss: Standard method
	%	%
1	1.83	2.52
2	1.54	2.72
3	1.90	2.87
4	2.08	3.11

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Diffusion Micromethod for Nitrogen

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RECENTLY it was desired to determine the nitrogen content of a number of allergen preparations. Analysis of extractable and "protein" fractions, in duplicate, brought the number of determinations in prospect to about 1800. A micromethod was definitely indicated by the cost of the materials, even if the advantage of economy of reagents, convenience, and speed had not influenced the decision. In fact, since many pollen extracts contain less than 0.1 per cent (calculated on a dry pollen basis) of nitrogen precipitable with trichloroacetic or tungstic acid, and since it was desired to do a complete analysis on a total sample not exceeding 0.5 gram, a method adapted to the determination of 100 micrograms or less was required. Choice of published methods finally lay between colorimetry using direct nesslerization, and the Borsook and Dubnoff (*1*) adaptation of the Conway and Byrne (*3*) diffusion micromethod. The advantages of the former in speed and simplicity were weighed against its more limited precision and the occasional presence of interfering substances from these materials.

Experience with the two methods showed that the advantage of the colorimetric method in speed would be overcome to a considerable extent if a satisfactory way could be found of avoiding the sample transfer from the digestion tube into the diffusion vessel, while at the same time one of the chief sources of handling difficulties and variation of results would be avoided. Needham and Boell (*6*) described apparatus for this purpose, but it has some disadvantages. The setup for large scale use is rather expensive because of the ground joints required, and the diffusion rate is inconveniently slow.

While this manuscript was being prepared a paper by Tompkins and Kirk (*8*) appeared, describing apparatus which attains the same object in a similar way, but which is different in several respects from that shown below, and especially in that the digestion tube cannot so readily be used in the centrifuge and that the diffusion rate is slower. Their paper reviews the need for an improved method. References in it and in the Conway book (*3*) include all the previous work considered except the aeration method of Folin (*4*).

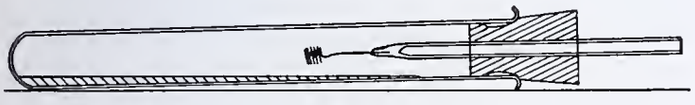

FIGURE 1. SECTIONAL VIEW OF DIFFUSION APPARATUS

The essential feature of the present method is that a plain test tube (or conical centrifuge tube, if desired) serves as both digestion and diffusion vessel. The diffusion receiver consists of a helix of platinum wire carrying a drop of absorbing solution, held in the tube by means of a grooved rubber stopper. The apparatus set up for diffusion is shown in Figure 1.

With this assembly it was apparent that convenience of manipulation would result if a concentrated solution of a weak acid were used for the absorbing solution, since then the volume of the droplet need not be measured precisely and the helix could be adjusted so as to pick up the desired volume when dipped in the solution. However, it was found that boric acid, which is commonly used for the purpose (*2*), is too weak to be suitable. A 0.02-ml. droplet of saturated solution would provide stoichiometric absorption of ammonia only up to about 20 micrograms of nitrogen. As this was considered an inconveniently small maximum sample size, search was started for a substitute. Inspection of a table of ionization constants showed that the second hydrogen of phosphoric acid has very nearly the ideal dissociation tendency, while the constant for the first hydrogen is sufficiently large that its dissociation is nearly complete at the end point near pH 4.5.

The advantage of 1.0 *M* sodium dihydrogen phosphate monohydrate over boric acid saturated at 20° C. (0.8 *M*) may be seen by inspection of the titration curves of Figure 2. They show experimental titrations of 5 ml. of each absorbent with 10.5 *N* ammonium hydroxide, delivered beneath the surface of the solution from a buret with a capillary tip. The pH values were obtained with a glass electrode pH meter (Beckman laboratory model). Also shown are the pH's of 1 to 50 dilutions, made by removing 0.02-ml. portions of the solutions at the indicated points during titration with the aid of a set of receivers and diluting each with 1.0 ml. of water for the pH reading. Those at the higher pH values were held only a few moments between removal and dilution, but probably lost appreciable amounts of ammonia in the interval, as suggested by the lines drawn on the plot to indicate various levels of ammonia dissociation in water.

Only about 0.08 mole of ammonia per mole of boric acid initially present was required to raise the pH to 6.4, the level of 0.1 per cent ammonia dissociation. The corresponding ratio for phosphate is 0.42. Furthermore, by use of a saturated solution of ammonium dihydrogen phosphate monohydrate the sample size could be increased to about 0.5 mg. of nitrogen with, of course, some loss of end-point precision for smaller samples.

In order to determine the time necessary for the diffusion, the rate constant for the apparatus was calculated from measurements on duplicate samples containing 100 micrograms of nitrogen in the form of ammonium sulfate at 0.5, 1, 2, and 3.5 hours. The constant, calculated from the equation

$$K = \frac{t}{\log A/(A - x)}$$

averaged 120 minutes (minimum 103, maximum 142). A is the total amount of ammonia and x is the amount found in the receiver after t minutes of diffusion. The constant shows the time required to transfer 90 per cent of the ammonia, and may be compared with a constant of about 50 minutes for the standard Conway (*3*) "unit", 260 minutes for the device of Tompkins and Kirk (*8*), and 700 minutes for the Needham and Boell (*6*) apparatus. (Constants were calculated from data given in the references, corrected for solution composition and to a temperature of 20° C.)

At 20° C. ammonia diffusion is 99.5 per cent complete in less than 5 hours. Amines (*2*) will diffuse more slowly, so that in analyzing digests of most substances of biological interest the recommended overnight diffusion is not unnecessarily long with the present setup. Naturally, the rate can be increased by raising the temperature, and by other means (*3*, *8*) if desired.

It seems worth while, because of the confusion that may result from the method used by Tompkins and Kirk (*8*) to determine the length of diffusion required for various size samples, to re-emphasize that the above formula has been shown (*3*) to apply generally to the diffusion of substances having low vapor pressure, such as dilute ammonia. Its significance is, of course, that for equal relative accuracy of determination the same length of time is required for all sample sizes. In unpublished work it was found that the equation has the same constant and predicts the diffusion course as well for 10 as for 100 micrograms of nitrogen. It is, therefore, not clear how Tompkins and Kirk obtained values usually within 1 per cent of theory on various samples containing less than 10 micrograms of nitrogen, when their recommended diffusion time is sufficient to transfer only about 90 per cent of the ammonia in such samples, as shown by their own rate curves.

No attempt was made to determine the limits possible with

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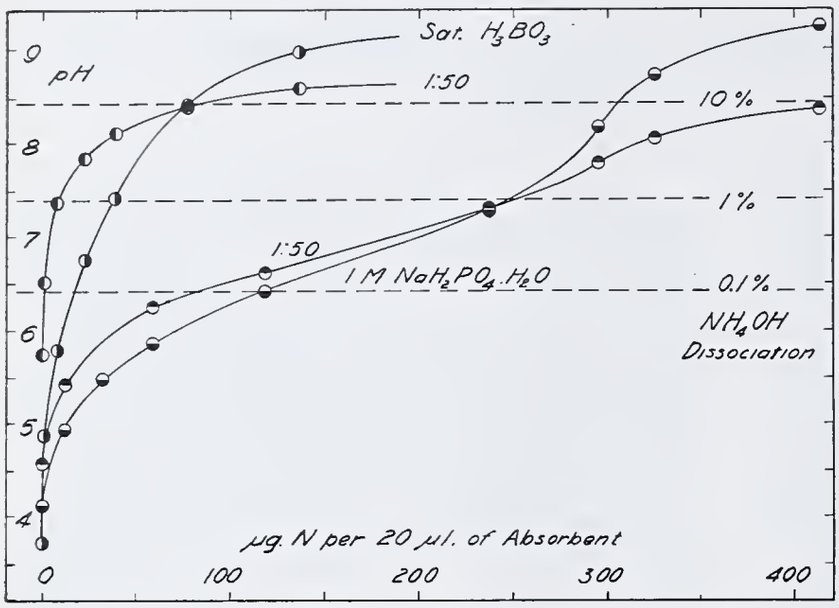

FIGURE 2. TITRATION OF ABSORBENTS WITH STRONG AMMONIA

this diffusion apparatus, but it was found that 10 micrograms can be determined without alteration of the method except to use 0.2 *M* sodium dihydrogen phosphate, if electrometric determination of the end point is used. A ground-glass joint, or possibly a stopper made of a synthetic elastomer in place of the rubber, and a smaller titration volume would no doubt permit further increases in sensitivity.

Preparation of Receivers

Five turns of 22-gage platinum wire are close-wound on a rod 2.4 mm. in diameter (13-gage), leaving a handle of straight wire 1.5 cm. long. The end of the latter is sealed into a short piece of 6-mm. soft-glass tubing inserted through the hole in a No. 0 pure gum rubber stopper.

Stoppers, obtained from the Chemical Rubber Company, Cleveland, Ohio, are treated before making up the receivers by boiling 15 minutes in 1 *N* sodium hydroxide, then boiling and rinsing in distilled water. When prepared in this way they contribute the equivalent of less than 0.3 microgram of nitrogen to the value of the blanks. A neat groove in the stopper is most easily made with the aid of an electric drill stock carrying a metal rod to hold the stopper, and a power grinding wheel, but it may also be cut by hand with a sharp knife. The groove is about 1 mm. deep and 6 mm. wide, and starts about 4 mm. from the small end of the stopper. It is effective in preventing the stopper from forcing itself out of the tube or moving so as to bring the helix in contact with the tube wall.

The helix is cleaned either by immersing for a few minutes in hot cleaning solution or by heating to incandescence for a second in a flame. It is then adjusted by pulling with fine forceps or spreading the center turns with a knife blade, until the volume of the drop it carries is within 10 per cent of 0.02 ml. (determined by titration). Uniformity is of more importance than the absolute size of the droplet.

The helices are recleaned at frequent intervals in order to maintain a uniform drop size. Platinum wire was chosen because of the ease of cleaning, although Nichrome wire which was also tried will work, as, no doubt, would other materials, including glass.

Reagents

Digestion solution, 18 *N* sulfuric acid (nitrogen-free) containing 0.1 gram of selenium dioxide and 0.1 gram of copper sulfate per 100 ml.

Hydrogen peroxide, 30 per cent, conforming to A. C. S. specifications.

Sodium hydroxide, 50 per cent by weight, which has stood or been centrifuged until clear.

Absorption solution, 1.0 *M* monobasic sodium phosphate (Merck reagent).

Standardized acid, of strength suited to the microburet and sample size.

Procedure

Samples are pipetted (*1*) or weighed into ordinary 125 × 15 mm. Pyrex test tubes taking care to keep the upper wall as clean as possible, 0.2 ml. of acid digestion solution is added to each, and excess water is removed overnight in an oven at 100° C. If the sample volume is less than 0.5 ml., the preliminary drying may be omitted if the first heating is done cautiously.

Digestion is carried out in a sand bath heated by a Tirrill burner so adjusted as to cause refluxing to about 4 cm. from the bottom of the tubes. Ebullition and bumping do not occur. The tubes are covered with glass caps cut from the bottoms of soft-glass vials 21 mm. in diameter, to prevent concentration of the digests and to permit operation outside the fume hood.

No attention is required during digestion as a rule, but if the digests are not nearly colorless in about 4 hours the flame is removed for 10 minutes, and then a small uniform drop of 30 per cent hydrogen peroxide is placed in each tube, including the blanks. Refluxing is continued for at least 30 minutes after the straw color is gone.

When digestion is complete the tubes are placed in a rack and allowed to cool, then 0.6 ml. of water is added to each, and all are stirred by shaking the rack. After further cooling each in turn is treated as follows:

Three-tenths milliliter of 50 per cent sodium hydroxide is run down the side of the tube without agitation and so as to avoid wetting the rim and 1 cm. of the upper wall of the tube. Although the solutions become warm, the large difference in specific gravity prevents complete mixing which would cause loss of ammonia. On warm days it may be necessary to cool the tube in a beaker of ice water. It is left undisturbed while the receiver is prepared.

The latter is removed from the 1.59 × 12.7 cm. ($^5/_8$ × 5 inch) test tube in which it is kept, and rinsed with distilled water, flamed, and rerinsed. The water is shaken off the helix and wiped off the stopper with cleansing tissue. The helix is dipped in 1.0 *M* sodium dihydrogen phosphate solution, deeply enough to cover it, but not so deeply that the platinum-glass junction is wet with the solution. The stopper is lubricated with a drop of glycerol and then inserted in the tube, taking care that the helix does not touch the wall. If it does it must be rerinsed and recharged. Contact may be avoided by placing the rim of the tube against a notch in the bench edge, holding the receiver in the other hand in a position coaxial with the tube, and sliding the tube up over the receiver, which is held motionless while the tube is twisted to seat the stopper.

The digest is now overneutralized by shaking the tube while cooling with a stream of tap water. The entire wall of the tube is coated with a film of alkali by inverting and rotating, and the assembly is left overnight on its side on a paper towel or blotter placed on a level surface.

When ready to titrate, the receiver is carefully removed by reversing the insertion procedure. The stopper is wiped free of alkali and the helix is immersed in 1 ml. of water containing either indicator or a slight excess of quinhydrone, depending on whether the end point is to be detected colorimetrically or electrometrically.

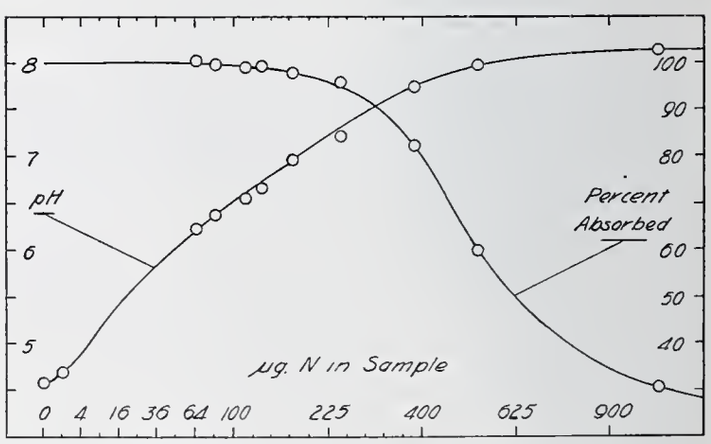

FIGURE 3. pH OF DILUTED ABSORBENT AND ABSORPTION EFFICIENCY FOR VARIOUS AMOUNTS OF AMMONIUM ION

The solution is titrated with standardized acid from a microburet to the pH given by 0.02 ml. of the buffer solution diluted in the same way. The end point lies between pH 4.3 and 4.7. Blanks are, of course, included with each set of determinations.

With electrometric titration the end point can be determined to 0.02 pH, or 0.2 per cent of the 100 microgram "optimal" sample. Indicator end point error is about three times as great, but could be decreased by decreasing the titration volume (for a discussion of limitations see Conway, *3*). Drying and digestion introduce greater variation, but duplicate determinations on protein solutions almost always agree within 1 per cent and agree with larger samples run by the semimicromethod (*7*) to within the same limit.

An important advantage of electrometric titration, in addition to greater precision, more warning of end-point approach, and no need to back-titrate if the end point is overrun (small excesses can be read from a previously prepared titration curve), is that the size of the droplet of absorbent solution can be checked by comparing the pH before titration with the amount of acid required to reach the end point. [A pH meter with continuous (nonballistic) indication of balance is more convenient and rapid than the ballistic type, especially for titration purposes.] In this way it is easy to be sure that sufficient buffer was present to ensure stoichiometric absorption of the ammonia.

In Figure 3 is shown the normal relation between pH and sample nitrogen content, also the completeness of absorption, for a considerable range of amounts of ammonia added as ammonium sulfate solution.

Table I gives the results of a series of determinations done on an ammonium sulfate solution containing 0.500 gram of nitrogen per liter (2.358 grams of the salt, recrystallized from water, and dried in vacuo over fresh calcium chloride.) A semimicro-Kjeldahl (*7*) determination done in triplicate on the same ammonium sulfate solution and referred to the same independent standardization of acid and alkali gave a value of 0.4982 ± 0.0005 mg. per ml.

Since the main point of novelty in the method and consequently of interest in these determinations was the diffusion procedure, the samples were not carried through the digestion steps prior to the diffusion or distillation.

The actual titration volumes are given and the analytical results which were calculated from the averages, as well as the standard errors of the individual determinations and of the averages and results. All the data obtained under the described conditions for this solution of ammonium sulfate are reported, except for a single determination obtained with pipet B, which was 163.8 microliter and was discarded as probably in error through a fault in pipetting technique.

The difference between the value obtained with pipet E and the semimicro-Kjeldahl value, 0.0016 ± 0.0008 mg. per ml., is of doubtful significance, but the differences between the larger sample and the smaller, 0.0031 ± 0.0010, and between the larger sample and the semimicrodetermination, 0.0057 ± 0.0010, are significant. All the differences are in the direction to be expected if transfer of the ammonia is incomplete, and they probably may be taken as a measure of this incompleteness for correction of other analyses, if the highest accuracy is wanted. The diffusion time was ample to ensure the establishment of an effective equilibrium between the evolving and absorbing solutions.

Notes

The oven used at first for removing excess water previous to digestion had been employed in organic preparation work for several years. Despite repeated cleanings, including sluicing out with water, and baking out at high temperature, large and variable blanks which were easily traced to the drying operation were obtained until a new oven was constructed. In the latter the heat is radiated downward from an element in the ceiling, resulting in rapid evaporation without danger of loss by bumping.

The sand bath is a flexible and inexpensive substitute for the drilled aluminum bar first used in this laboratory as a digestion rack and is considerably easier to obtain at the present time.

It consists of a 20-cm. (8-inch) hemispherical iron bath with 18 notches cut with tin snips in the periphery. Enough fine sand to make a layer about 10 cm. (4 inches) in diameter is placed in it and the tubes are leaned into the notches with their bottoms immersed to about the level of the digest, around the outer edge of the sand layer.

The sand bath also makes a very satisfactory digestion rack for six 30-ml. Kjeldahl flasks as used in the semimicro or micromethod, but must then be placed in the fume hood and heated with a Méker-type burner.

A titration stand is an even greater convenience for microthan for macroanalytical work. That used in this laboratory may be of interest because of its simple construction. It is shown in Figure 4.

Table I. Determinations of a Standard, Ammonium Sulfate Solution

(0.5 mg. of N per ml.)

Sample		Titrations with 0.0495 N Acid	Standard Error[a]	Analytical Result	Standard Error[a]
		μl.		*Mg. N/ml.*	
Blanks		0.4	0.12		
		0.3			
		0.6			
		0.4			
		0.5			
		0.7			
		0.5			
		0.5			
		0.6			
		0.6			
		0.3			
		0.4			
		0.5			
		0.4			
		0.4			
	Av.	0.47	0.031		
Pipet E, 0.1645 ml. (82 μg.)		118.0	0.55 (0.46%)		
		117.6			
		117.8			
		118.3			
		117.4			
		117.4			
		118.7			
		118.7			
		118.1			
		118.7			
		118.6			
		118.9			
		118.8			
		118.8			
	Av.	118.27	0.147	0.4966	0.0006
Pipet B, 0.2261 ml. (113 μg.)		162.3	0.86 (0.53%)		
		160.7			
		161.8			
		160.7			
		160.1			
		161.5			
		160.3			
		162.5			
		161.7			
		162.1			
	Av.	161.37	0.272	0.4935	0.0008
Blanks[b]		3.7[b]	1.3		
		1.5			
		1.0			
		3.0			
	Av.	2.30	0.63		
Pipet B (11.3 μg.)[b]		165.8[b]	1.62 (0.98%)		
		165.9			
		165.5			
		164.8			
		166.1			
		166.2			
	Av.	165.72	0.66	0.5012	0.0028

[a] Standard error of individual observation, s, = $\sqrt{\Sigma d^2/(n-1)}$, and standard error of average = $s/\sqrt{n}$, where d is deviation of each observation from average and n is number of observations.

[b] In these analyses 0.2 M NaH_2PO_4 was used as absorbent. Ammonium sulfate solution and titration acid were 1 to 10 dilutions of standards, each made with same pipet and volumetric flask.

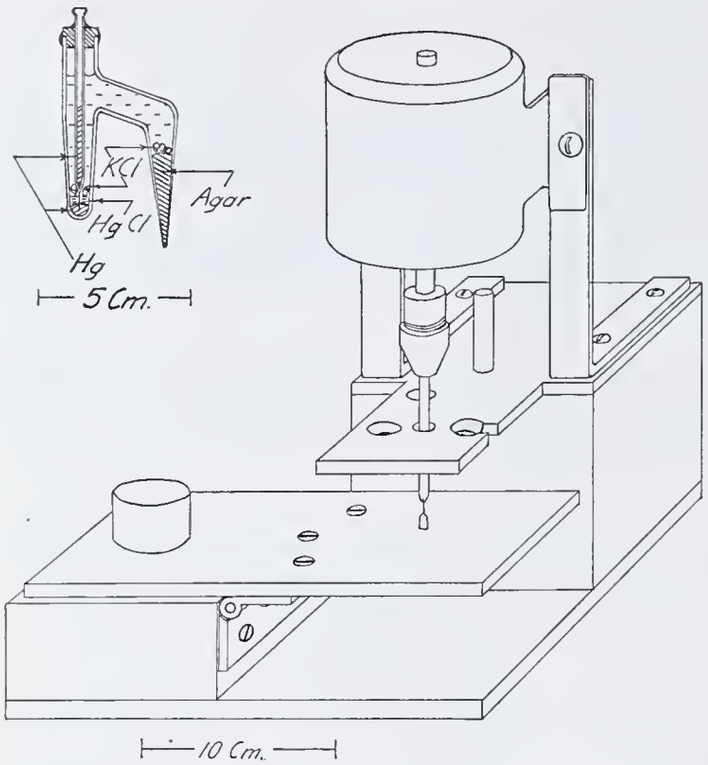

FIGURE 4. TITRATION STAND AND CALOMEL HALF-CELL

The stirring motor is a 1400 r. p. m. shaded-pole induction motor, with a chuck from a small hand drill attached to hold the stirring paddle. The latter is an old platinum foil electrode, 3 mm. wide by 5 mm. high, sealed into a piece of soft-glass tubing. The motor is mounted on two shelf brackets fastened to a block of wood which also carries a rod to support the electrode terminal block and a piece of Bakelite in which holes for the electrodes and stirrer and a slotted hole to carry the receiver have been cut as shown.

The titration vessel, consisting of a beaker 10 mm. high and 15 mm. in diameter, is supported on a counterweighted hinged shelf of Masonite. Electrodes are held in the holes in the Bakelite sheet by means of small soft wood wedges.

The construction of the calomel half-cell and agar bridge is also illustrated in Figure 4. It is made of Pyrex glass, except for the internal platinum electrode, which is sealed into a small soft-glass tube. A fiber of acid-washed asbestos sealed into the tip may be substituted for the agar bridge, if desired. When not in use the electrode is stored with the agar bridge immersed in saturated potassium chloride in a squat bottle closed with a one-hole rubber stopper.

The quinhydrone electrode consists of a short piece of 22-gage platinum wire sealed into a piece of 8-mm. soft-glass tubing drawn down at the tip to about 2-mm. inside diameter. It was substituted for the small glass electrode used by Borsook and Dubnoff because of the fragility of the latter. For these titrations pure quinhydrone provides an entirely satisfactory half-cell.

An easily constructed microburet is illustrated in Figure 5. Its outflow is started and stopped by immersion and withdrawal of the tip from the liquid being titrated, eliminating the need for a stopcock or any kind of positive displacement device.

It is made from a Kahn serological pipet, 0.2 ml. graduated in microliters. A constriction 4 cm. long and 2 mm. in diameter is drawn, as near the tip as possible, pulling the tip with a handle or forceps. The farthest point of the constriction is further drawn until it has a diameter of about 0.2 mm., and it is broken at the narrowest point. The entire tip is bent at an angle of about 75° away from the side carrying the graduations.

The pipet is filled with water and placed in a horizontal position with the tip downward. The orifice is adjusted by breaking off very short pieces of the tip until the delivery rate is between 0.1 and 0.2 microliter per second, with the tip just immersed.

It is now placed on the titration stand as shown. The height of the water column in the test tube, which is held in place by a spring brass clamp so that it can be moved up or down, is adjusted with the tube in its uppermost position until the delivery rate is about 100 microliters per minute. A mark is made on the tube at the level of the water surface. In this way two constant delivery speeds which can be used as needed are available: a rapid "approach" speed, and a slow "end-point" speed. The former will determine to some extent the calibration of the buret, and should therefore be maintained reasonably constant.

The buret is adjusted in height until it is horizontal when the tip is immersed in 1 ml. of water in the titration beaker. It is filled by immersing the tip, placing a finger firmly on the rubber connecting tubing, and rolling to the left. The meniscus is brought to the zero point by immersing the buret tip in a beaker of water, then the buret rack is moved to the titration stand, and the titration is started by immersing the tip and raising the test tube. When the end point is nearly reached the test tube is lowered (if a drop hangs across the inner tube a drop of oil may be floated on the water) and the end point is approached by alternately immersing and withdrawing the tip. The tip height adjustment lever is of sufficiently thin metal that it can be sprung out of the way for reading if the meniscus is near it.

The buret is calibrated by weight of water or by titration. Four or more points should be taken along its length, as these pipets are seldom found to have perfectly uniform bore.

A reservoir for the standard acid may be attached to the buret by a stopcock, one-way mercury valve, or other device, just behind the graduations, if greater convenience in filling is wanted.

A convenient indicator is 0.0015 per cent of bromocresol green plus 0.0002 per cent of methyl red. The combination, which has been in use in this laboratory for several years, was recently recommended (*5*) for the increased sharpness of end point it affords (in the pH range within which both indicators change color—i. e., 4.4 to 5.4), but no mention was made of another equally important advantage it has over the commonly used methylene blue–methyl red combination (Tashiro's reagent)—namely, that it gives considerably more warning of end-point approach.

Optimal proportions of the two dyes vary with the end-point pH and should be adjusted, with the aid of a solution buffered at the desired end point, so as to give a gray or neutral tint. Suitable proportions for the usual macro- or micro-Kjeldahl procedure with the end point at about pH 5.4 are 0.0001 per cent methyl red and 0.002 per cent bromocresol green.

The indicator is made up in alcohol to 100 times the specified final concentrations.

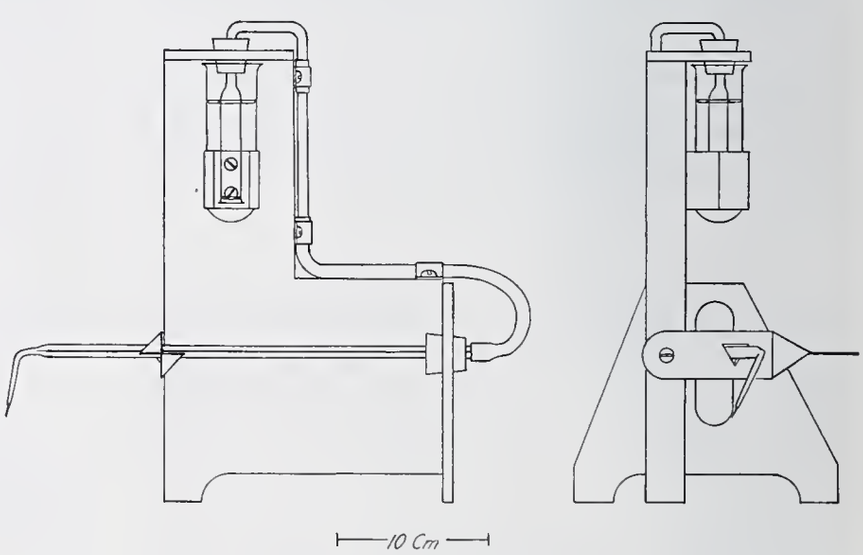

FIGURE 5. MICROBURET AND STAND

Summary

A micro-Kjeldahl method for nitrogen in 10- to 100-microgram amounts is based on the diffusion principle developed by Conway. In terms of the time required for manipulations it is more rapid than any other of like precision. The apparatus required is inexpensive and is easily made from readily available materials.

Two pieces of accessory apparatus described are a simple microburet and an easily made titration stand. The advantages of an indicator to replace the commonly used methylene blue–methyl red combination are pointed out.

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Spectrographic Analysis of Rat Tissues for Ingested Vanadium

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A spectrographic method for the quantitative estimation of vanadium in limited amounts of biological material is presented. Chromium is employed as an internal standard in the arc spectrum and use is made of standard reference curves established by plotting the ratios of line blackening produced by standard solutions of vanadium and chromium against the concentrations of vanadium. The spectrum lines, vanadium 3185.406 Å. and chromium 3188.0 Å., were measured by means of a nonrecording densitometer.

From a number of ashed tissues analyzed for vanadium, blood samples taken from rats fed three different levels of this element were chosen to illustrate the applicability of the method.

DURING the course of a study of the distribution of ingested vanadium in rat tissues, the following method for determining very small amounts of this element in limited amounts of biological material was developed.

Preparation of Rat Tissues for Analysis

Tissues from rats fed sodium metavanadate were dissected out under ether anesthesia, washed thoroughly in physiological saline to remove the blood, and wet-ashed, after combining with similar organs of other rats on a diet containing the same level of vanadium. Ashing was begun in micro-Kjeldahl flasks over microburners, and after complete digestion the samples were transferred to weighed silica dishes and brought to dryness. A little perchloric acid was used in the final stages of digestion of fatty tissues which were difficult to ash with nitric acid alone.

Method of Analysis

Analyses were made by means of a Bausch & Lomb high-dispersion, Littrow-type quartz spectrograph. The arc was operated on a 220-volt direct current line with a large 24-step resistor in series, thereby affording a much steadier arc than is possible on a 110-volt line. A voltmeter connected across the electrode terminals made it possible to determine the potential of the arc gap at any time. A condensing lens was used in concentrating the light from the sample. Although this practice is not generally used in quantitative spectroscopy, it seemed desirable in the present case because the smallest detectable quantities of vanadium were sought. A Bausch & Lomb nonrecording densitometer modified according to Scribner (*4*) was used to read the plates.

The most sensitive group of vanadium lines in the arc spectrum, 3185.406, 3183.99, and 3183.415 Å., was selected for measurement. Consideration was given to such factors as current, slit aperture, type of electrodes, interelectrode distance, size of sample, depth of electrode well, creeping of the sample, internal standard, burning time, and background.

Duffendack, Wolfe, and Smith (*3*) noted that fluctuations in the relative intensities of line pairs are insignificant above an arc current of approximately 12 amperes. In the present study it was observed that amperages of 12 to 15, while producing a somewhat steadier arc than lesser currents, caused too much background on the photographic plate for densitometer readings. At 10 amperes this background could be cut down sufficiently to read the spectrograms and at the same time remain in a range which did not exceed a tolerable change in the relative intensities with variation in current. The voltage across the arc with this current was 50.

In practically every case, background was the factor which in the end greatly influenced the choice of procedure.

A slit width of 30 microns was selected as a compromise between one which allowed lines of sufficient width for measurement and one which did not cause excessive background. A slit length of 1 mm. was found satisfactory and practical in that it permitted taking 60 spectrograms on one 10 × 25 cm. (4 × 10 inch) plate. By simple shielding, the range of light from the densitometer could be readily narrowed to accommodate spectrograms of this width.

The vanadium impurity in ordinary carbon electrodes excluded their use. Purified "Hilger spectro" vanadium-free copper electrodes were found unsatisfactory, since, at the current used,

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they melted and occluded a portion of the unburned sample. Also, the sensitivity of vanadium was less in the copper arc. National Carbon Company, Inc., high purity graphite electrodes 0.47 cm. (0.187 inch) in diameter were found to be vanadium-free and were used throughout these experiments. They were cut in approximately 1.25-cm. (0.5-inch) lengths for lower electrodes and were discarded after one use to avoid any possible danger of contamination. The upper electrode was left as a long stick and except for thorough wiping with filter paper between spectrograms, it was used continuously until completely burned down.

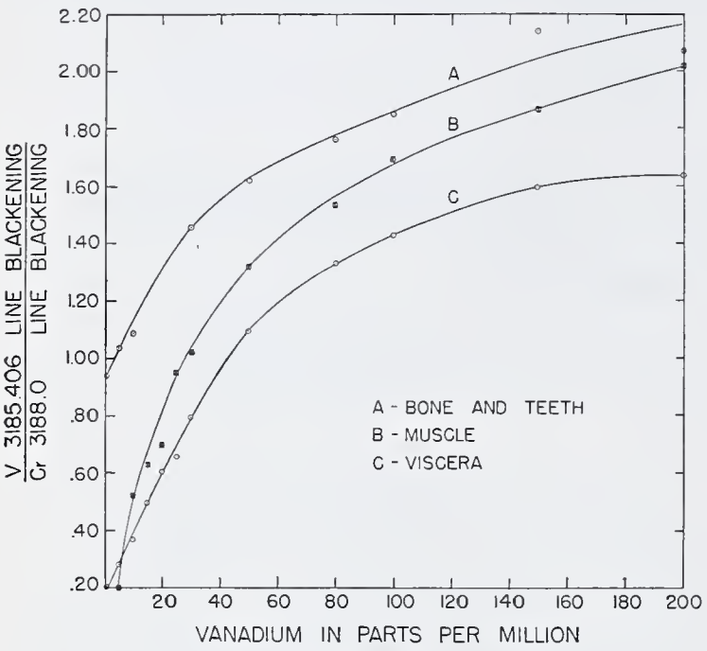

FIGURE 1. STANDARD REFERENCE CURVES

An interelectrode distance of 4 mm. was found to give maximum blackening of the vanadium lines. This distance was manually maintained during the arcing period with the aid of a projected image of the electrodes on a wall several feet away.

Two-milligram samples, sufficient to detect quantities of vanadium ranging from 1 to 5 p. p. m. and yet small enough to permit several spectrograms from limited quantities of material, were introduced into the wells of lower electrodes which had been lathe-drilled to a uniform depth. Experience proved that the more shallow the well and the thinner the wall of the electrode the less was the interfering background on the spectrogram. Kerosene was placed in the drilled electrode before introduction of the sample to prevent creeping and penetration of the salts during arcing. Halowax, frequently used for this purpose, was found to inhibit the vanadium lines.

Drop-plate exposures at 5-second intervals showed that vanadium came off very rapidly and the faintest traces were seldom detected after a 15-second period. In order to allow a margin of safety, 20-second exposures were adopted. Obviously, prolonged exposure of the sample after complete volatilization of the vanadium only added to objectionable background interference in reading the plates.

Use was made of special Eastman II-0 photographic plates which are highly sensitive in the spectral region in which the most sensitive vanadium lines occur and at the same time offer considerable contrast. These were developed with Eastman's high contrast developer formula at a temperature maintained between 17° and 20° C. and subsequently were hardened and fixed according to Eastman formulas.

To obviate the criticism of using absolute blackening which may vary from plate to plate or even from spectrogram to spectrogram on the same plate with the same concentration of vanadium, the internal standard procedure was adopted in which relative blackening between a pair of lines, one of which is held constant and both of which have been subjected to identical spectrographic conditions, is the criterion of measurement. Brode and Steed (*2*) have employed chromium lines 3118.65 and 3132.053 Å. as an internal standard for vanadium lines 3092.13 and 3130.27 Å., respectively, in the spark spectrum. No reference to an arc spectrum standard for vanadium has been noted. A consideration of cobalt, titanium, and chromium resulted in the selection of chromium as the most desirable standard for vanadium. Drop-plate spectrograms of chromium showed that its burning time in the arc did not exceed that of vanadium, and the position of its line at 3188.0 Å. was ideal for measurement in comparison with the sensitive arc lines of vanadium at 3183.415, 3183.99, and 3185.406 Å.

It was found by trial that 0.03 ml. of a chromium oxide solution containing 0.1 per cent chromium, when arced with 2 mg. of biological ash as described produced a desirable degree of blackening of the chromium line at 3188.0 Å. By introducing this constant amount of chromium into the sample under analysis the vanadium-chromium ratio of line blackening served as a relative measure of the vanadium present in the sample. "Line blackening" is used in the same sense as that employed by Brode (*1*). Reference curves were established with known material under controlled conditions.

Recognition of inter-ion effect on line blackening led to the use in the preparation of these curves of a base ash obtained from tissues of normal rats receiving ordinary stock ration. These had previously been shown to be vanadium-free by spectrographic analysis. In the present case this seemed more desirable than preparing a synthetic ash which, obviously, is not ideal.

Standard vanadium solutions were prepared from sodium metavanadate of determined purity in such concentrations that by the addition of from 0.02 to 0.05 ml. to a 2-mg. sample of this biological ash it was possible to prepare mixtures containing exact amounts of vanadium varying from 1 to 200 p. p. m. The distilled water used in making these solutions was tested after evaporating 2 liters and found to be vanadium-free.

It was impractical and seemed unnecessary to establish reference curves for every tissue to be tested. Instead, one curve was obtained from a composite sample of rat viscera, another from bone and teeth, and a third from muscle ash alone. The order of procedure for preparing the reference samples for arcing was as follows: After introduction of a previously weighed 2-mg. sample of rat ash into the well of an electrode which had been treated with kerosene, vanadium solutions of known concentration were added to the ash. After they had dried (at low temperature to prevent excessive creeping) 0.03 ml. of 0.1 per cent chromium solution was added and when this had dried the electrode was ready for arcing.

An average of sixteen spectrograms was made to establish each point on the curves below 50 p. p. m. of vanadium; above 50 p. p. m. an average of five spectrograms was taken. The vanadium-chromium ratios used in establishing these reference curves are based upon the vanadium line at 3185.406 Å. which lies nearest the chromium line.

Results and Discussion

The standard reference curves for each of the three types of ashed rat tissue are shown in Figure 1. By interpolation from these reference curves, ashed blood, for example, from rats receiving 0.25 mg. of vanadium per 10 grams of diet and giving a vanadium-chromium line blackening ratio of 0.931 was shown to contain 35 to 40 p. p. m. of vanadium. At higher levels of vanadium intake, 2 and 4 mg. per 10 grams of diet, ratios of 1.046 and 1.315, respectively, were obtained. These indicate corresponding vanadium contents of 45 to 50 p. p. m. and 75 to 80 p. p. m.

Acknowledgment

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Reduction of Ash Content of Residual Oils with Boric Acid

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A practical method for de-ashing residual oils resulting from distillation of crudes in the presence of sodium hydroxide has been developed. The oils are treated with boric acid at about 400° F. and the acid is recovered from the resulting sludge by acidification with sulfuric acid or sulfuric acid sludge. The treated oils are free of moisture and easily pass general specifications developed for industrial fuels.

PETROLEUM crude oils containing appreciable quantities of naphthenic acids are frequently distilled in the presence of sodium hydroxide in order to improve the quality of distillate fractions and minimize corrosion of equipment. The sodium salts resulting from this treatment are concentrated in the residuum of the still, which is commonly referred to as "soda tar", increasing its ash content. However, soda tar having a high ash content is not satisfactory for use as a fuel because on burning it deposits an alkaline residue that has a deleterious action on conventional furnace refractories. Hence, the reduction of the ash content of the soda tar is of considerable economic importance.

Several methods were proposed for lowering the ash content of petroleum oils, such as washing with hot water under pressure (*3*) or treatment with acid and alcohol (*2*), but most of them are not commercially applicable because the use of aqueous reagents leads to formation of emulsions which are difficult to resolve (*4*). For this reason in the search for new methods capable of removing sodium from the still residua the use of water solutions in conventional plant equipment has not been considered desirable.

Removal of sodium without simultaneous removal of naphthenic acid radicals present in the naphthenates may be accomplished by the use of acids stronger than naphthenic, followed by the separation of the resulting sodium salts from the treated residuum. One of the important considerations in selecting the acid is its ability to form a sodium salt which is insoluble in the oil and which might be easily separated by settling on account of a high gravity differential. The acid should have little effect on the oil itself, be available in commercial quantities, and be capable of easy regeneration because fuel oil is cheap and application of expensive treating methods for its improvement is impractical.

In the search for a refining agent capable of meeting the above requirements, boric acid has been found to be outstanding (*5*). It easily displaces naphthenic acids from their salts, has for all practical purposes no effect on the oil constituents, and the sodium borates formed vary in specific gravity from 1.73 to 2.37 which ensures a quick rate of settling.

The reactions involved in the de-ashing process employing boric acid may be represented as follows:

$$\underset{\text{(oil-insoluble)}}{\text{Boric acid}} + \underset{\text{(oil-soluble)}}{\text{sodium naphthenate}} \longrightarrow \underset{\text{(oil-insoluble)}}{\text{sodium borate}} + \underset{\text{(oil-soluble)}}{\text{naphthenic acid}}$$

$$\text{Sodium borate} + \text{sulfuric acid} \longrightarrow \text{sodium sulfate} + \text{boric acid}$$

Assuming that sodium borate ($NaBO_2$) is the main reaction product, 680 grams (1.5 pounds) of boric acid (H_3BO_3) are required for de-ashing a 159-liter (42-gallon) barrel of fuel oil of an equivalent sodium hydroxide content of 453.6 grams (1 pound) per barrel. However, an excess of about 50 per

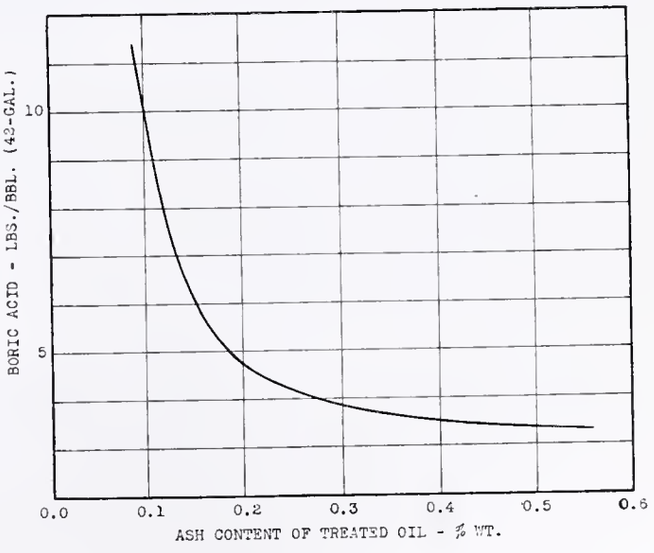

FIGURE 1. RELATION OF ASH CONTENT OF TREATED FUEL TO QUANTITY OF BORIC ACID USED

Oil contained 2.05 pounds of sodium hydroxide per 42-gallon barrel

Table I. Boric Acid Consumption

(Oil contained 2.05 pounds of NaOH equivalent per 42-gallon barrel)

Operating Conditions	
Temperature, ° F.	400
Oil flow, gallons per hour	0.5
Pressure, pounds per square inch	250
Mixing time, minutes	30
Settling time, minutes	180
Purity of boric acid	99.5

	Properties of Treated Oil				
Boric Acid *Lb./bbl.*	S. F. V. at 122° F.	Gravity ° *A. P. I.*	B. S. & W. *% Vol.*	Ash *% Wt.*	Boric Acid per NaOH Equivalent *Lb./lb.*
None	600	15.5	0.4	1.00	..
14.5	549	16.0	0.4	0.07	7.00
7.9	555	15.9	0.3	0.12	3.85
4.6	559	15.9	0.3	0.21	2.25
3.3	575	15.5	0.2	0.56	1.60

cent of boric acid is necessary for a more or less complete de-ashing of the oil, as shown by the experimental data presented in Table I and Figure 1.

The ash content of samples was determined by the A. S. T. M. method of analysis of grease, D-128-37, which expresses the percentage of ash in the form of sulfates.

The equivalent sodium hydroxide content was determined as follows:

Approximately 5 grams of sample were dissolved in 50 cc. of benzene and mixed with 10 cc. of 95 per cent ethyl alcohol and 10 cc. of water. The mixture was heated with vigorous shaking to 120° F. and titrated with 0.1 *N* hydrochloric acid solution until the appearance of a permanent end point employing methyl orange in the alcohol-water phase as an indicator.

The soda tar samples were obtained from a commercial plant operating on a Coastal type of crude. In most of the experiments viscosity of the tar was reduced by blending with a gas oil of 36° A. P. I. gravity. Properties of soda tar and blended materials are given in Table II.

Table II. Properties

	Soda Tar	Fuel Oil Blend
Saybolt Furol viscosity	1325–1400 at 210° F.	300–830 at 122° F
Gravity,° A. P. I.	11.3–11.6	15.5–17.0
Ash content (A. S. T. M. D-128-37), % wt.	1.0– 1.6	1.0– 1.1
Alkalinity, pounds of NaOH per 42-gallon barrel	2.0– 2.8	2.0– 2.4

Fluctuations in the ash content of the samples depended on the amount of sodium hydroxide added in the distillation process.

The initial experiments were made in a batch unit shown in Figure 2 and consisting of a 1.5-gallon jacketed autoclave equipped with a mechanical stirrer operated at 400 r. p. m. The soda tar was charged to the autoclave, solid boric acid (commercial grade of 99.5 per cent purity) was added, and the mixture was heated and agitated. The oil was allowed to settle at elevated temperatures and cooled to permit separation of the treated oil from the solidified borate–boric acid layer by decantation. The solidified layer was removed by dissolving in hot water. Compressed nitrogen was employed for maintaining pressure in the autoclave during treating and settling.

Typical results obtained from these treats are shown in Table III in comparison with those obtained by de-ashing the oil with dilute sulfuric acid. They indicate that the boric acid method is capable of reducing the ash content to a considerably lower figure than that obtained with sulfuric acid even with low viscosity oils. Moreover, the bottom sediment and water content of the sulfuric acid–treated oils showed a very considerable increase after treatment, while that of the boric acid–treated oils was frequently lower but never above the original figure.

The highly encouraging results obtained with the batch treats prompted the building of a continuous unit which is shown diagrammatically in Figure 3.

The unit consisted of a feed pump, a preheating coil, a mixing chamber, and a settling chamber. The oil was continuously passed through the heating coils and into the mixing chamber where it was agitated with the boric acid. A small amount of the oil charge was added to the boric acid to form a slurry in order to facilitate pumping. The oil was separated from the boric acid sludge in a settler. The treated oil overflowed from the settler through coolers into suitable receivers where continuous removal of the boric acid sludge was ensured by leaching with a small stream of water introduced into the bottom of the settler.

Table III. De-ashing with Boric Acid by Batch Method

	Boric Acid Method			Sulfuric Acid Method
Properties of untreated oil				
Gravity, ° A. P .I.	17.0	15.7	11.6	16.4
Viscosity				
S. F. V. at 122° F.	297	700	...	309
S. F. V. at 210° F.	..	..	1325	..
Ash content, % wt.	1.03	1.13	1.02	0.52
B. S. & W., % vol.	0.4	0.4	0.2	0.2
Treating conditions				
Temperature, ° F.	400	400	360	350
Pressure, pounds per square inch	180	180	180	250
Mixing time, minutes	30	30	15	35
Settling time, minutes	60	60	120	270
Properties of treated soda tar				
Gravity, ° A. P. I.	17.3	16.0	12.0	16.4
Viscosity				
S. F. V. at 122° F.	231	640	...	299
S. F. V. at 210° F.	..	..	...	..
Ash content, % wt.	0.03	0.06	0.09	0.17
B. S. & W., % vol.	0.2	0.2	0.2	2.1

Typical results obtained with this continuous unit are given in Table I.

The results of this work showed that the treating and settling temperatures should be maintained at about 400° F. At this temperature the boric acid is in a molten state (melting point of boric acid is 365° F.), which permits its thorough mixing with the oil. Higher temperatures offer no advantage but necessitate the use of higher pressures owing to the presence of small quantities of water in the system. Temperatures lower than 400° F. increase the settling time, particularly with the high-viscosity oils. The pressure required for preventing the escape of water from the aqueous sludge and for avoiding dehydration of boric acid to boric anhydride is about 163,000 kg. per sq. meter (235 pounds per square inch) at 400° F. The use of higher pressures serves no useful purpose.

The time required for completion of the reaction is governed to a great extent by the degree of dispersion of the molten

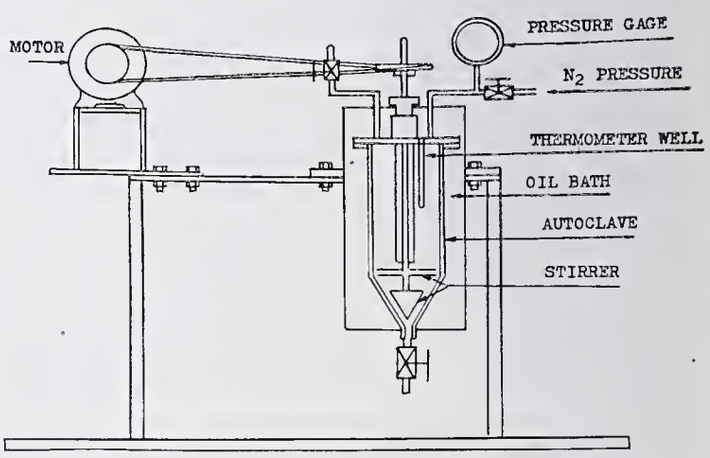

Figure 2. Laboratory Batch-Treating Unit for Processing Soda Tar with Boric Acid

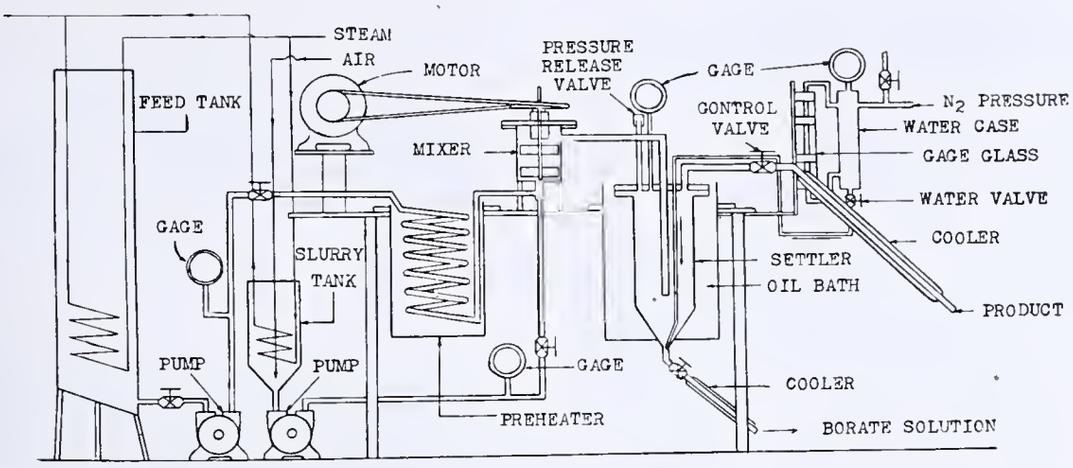

FIGURE 3. LABORATORY CONTINUOUS UNIT FOR PROCESSING SODA TAR WITH BORIC ACID

TABLE IV. EFFECT OF MOISTURE CONTENT OF RECOVERED BORIC ACID ON PROPERTIES OF TREATED OILS

Operating Conditions

Temperature, ° F.	400
Oil flow, gallons per hour	0.5
Pressure, pounds per square inch	250
Mixing time, minutes	30
Settling time, minutes	180
Boric acid added, pounds per barrel (42)	5.2

Moisture Content of Recovered Boric Acid	Properties of Treated Oil: S. F. V. at 122° F.	Gravity	B. S. & W.	Water by Distillation	Ash
% Wt.		° A. P. I.			% Wt.
Untreated fuel	831	15.1	0.25	0	0.95
None	707	15.7	0.20	0	0.15
35	763	15.3	0.9	0.8	0.18
40	774	15.4	1.0	0.9	0.23

TABLE V. ASH CONTENTS OF OILS TREATED WITH FRESH AND RECOVERED BORIC ACID

Purity of Boric Acid, % Wt.	Lb. Acid/Lb. NaOH Equivalent	Ash Content of Treated Oil, % Wt.
99.5	2.25	0.21
90.0	2.30	0.24

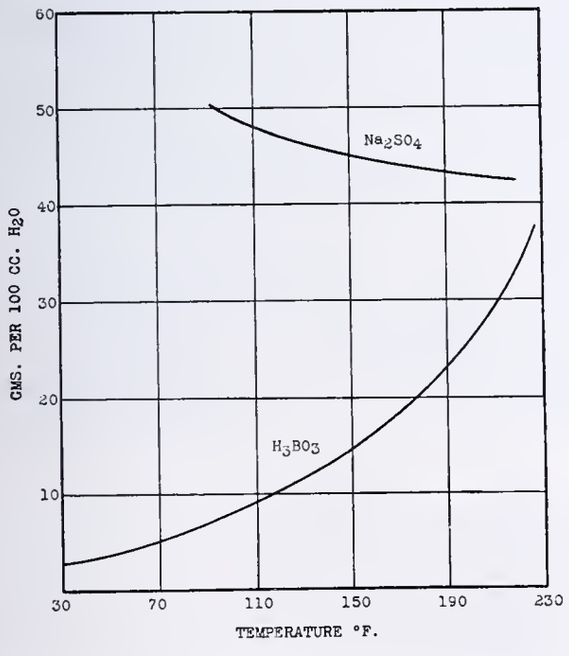

FIGURE 4. SOLUBILITY OF BORIC ACID AND SODIUM SULFATE IN AQUEOUS SOLUTIONS

boric acid in the oil, which in turn depends on the type and degree of mixing. The results presented in Table VI show that with the mixer employed in this work about 30 minutes were required to obtain optimum results.

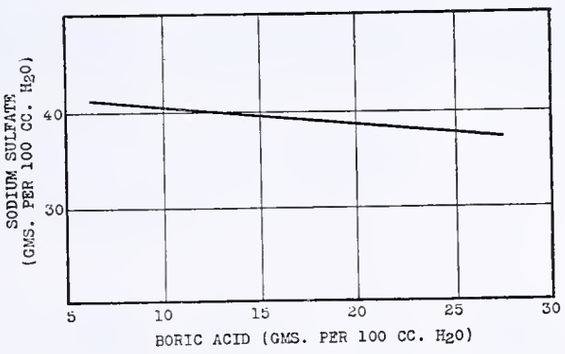

FIGURE 5. SOLUBILITY OF BORIC ACID IN SOLUTION CONTAINING SODIUM SULFATE AT 210° F.

The settling time is influenced by the viscosity of the mixture at the settling temperature and by the degree of dispersion. Data indicate that under the conditions employed, the settling time of 180 minutes was ample for oils varying in Saybolt Furol viscosity from 600 at 122° F. to 1425 at 210° F.

The quantity of water necessary for leaching boric acid sludge from the settling chamber in a continuous unit is about 3 pounds per pound of boric acid used. The use of excessive quantities of water should be avoided in order to facilitate the subsequent recovery of boric acid.

TABLE VI. METHODS OF AGITATING BORIC ACID–OIL MIXTURES

Operating Conditions

Temperature, ° F.	400
Pressure, pounds per square inch	250
Oil flow, gallons per hour	0.5
Settling time, minutes	180
Boric acid, pounds per barrel	3.7
Viscosity, S. F. V. at 122° F.	600–831

Method	Mixing Time	Speed of Stirrer	Ash Content: Untreated	Ash Content: Treated
	Min.	*R. p. m.*	*Wt. %*	
Pump circulation	0.16	1100	1.10	0.74
Paddle agitation	2	900	1.10	0.64
	2	1600	1.10	0.57
	30	400	1.00	0.33[a]
	30	700	0.95	0.26
	30	1100	0.95	0.24

[a] Estimated from Figure 1.

The recovery of boric acid is accomplished by acidifying the boric acid sludge with sulfuric acid or sulfuric acid sludge, followed by recrystallization. Approximately 1.3 pounds of 95 per cent sulfuric acid or its equivalent are necessary for each pound of sodium hydroxide removed from the oil. The acidified spent boric acid solution containing sodium sulfate is kept at about 200° F. for approximately 60 minutes in order to effect separation of oily matter with which it is usually contaminated. After the oil is skimmed off, the solution is cooled to about 90° F. to precipitate most of the boric acid. This temperature was chosen to prevent simultaneous crystallization of sodium sulfate. The solubility characteristics of boric acid in water and in sodium sulfate solutions are illustrated in Figures 4 (*1*) and 5, respectively.

The precipitate is separated from the remaining solution by filtration. This procedure yields wet boric acid containing approximately 30 per cent by weight of the mother liquor. The recovered acid on a moisture-free basis is thus composed of 91 to 92 per cent boric acid and 8 to 9 per cent sodium sulfate. The presence of water and sodium sulfate in the recovered acid does not interfere with the de-ashing process, as shown by experimental data given in Tables IV and V.

The mother liquor is concentrated to 50 to 55 per cent of its original volume and the precipitated sodium sulfate is separated by filtration. The remaining liquid is recycled in order to increase the boric acid recovery. Total recovery of boric acid is approximately 90 per cent by weight of that originally charged to the unit.

Acknowledgment

The authors wish to express their appreciation to J. B. Rather, in charge of the General Laboratories of the Socony-Vacuum Oil Company, Inc., for permission to publish this manuscript.

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Determination of Selenious Acid and Selenites

A Rapid Volumetric Method

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ALTHOUGH adequate colorimetric methods have been developed for the determination of minute quantities of selenium in organic matter, the present methods for the determination of selenious acid and selenites are not entirely satisfactory. Of the available methods, the method of Norris and Fay (*8*), titration with standard thiosulfate, described by Coleman and McCrosky (*3*) appears to be most commonly used. Coleman and McCrosky (*4*) have described the quantitative oxidation of colloidal selenium to selenite with a number of oxidizing agents. The gravimetric determination of selenium described by Lenher and Kao (*7*), involving sulfur dioxide reduction of selenite to selenium, is long and is subject to the same criticism in so far as the determination of selenite in the presence of selenate is concerned.

The method described here was developed to provide a rapid determination of selenite in salts or solutions of selenious acid. This determination can be used in the presence of the corresponding selenates. The method consists in the oxidation of the selenite ion to selenate ion in nitric acid solution by an excess of standard bromate solution, which is in turn back-titrated with standard arsenite solution, using Fast Red B indicator.

That a bromate in nitric acid solution would oxidize selenite to selenate was shown by Blumenthal (*1*) in 1913. The use of bromic acid in volumetric analysis was suggested by Kratschmer (*6*) in 1885. Smith (*10*) has demonstrated the stability of bromate solutions in the presence of nitric acid and bromine. Shaver and McCrosky (*9*) described a method by which selenium is oxidized quantitatively to selenite by bromate in hydrochloric acid solution. An electron beam sectrometer was used to determine the end point of the titration. Feit and Kubievschky (*5*) described a method by which an excess of bromate in acid solution was used as a volumetric oxidant, boiling off the bromine formed in the reaction and back-titrating the excess of bromate iodometrically. The method described is a modification and improvement on this method.

Preparation of Reagents

ARSENITE SOLUTION. Prepare a 0.1 *N* solution by dissolving 4.95 grams of arsenious oxide in 50 ml. of 1 *N* sodium hydroxide solution. Neutralize to litmus with 1 *N* sulfuric acid and dilute to 1 liter.

POTASSIUM BROMATE SOLUTION. Prepare a 0.1 *N* solution by dissolving 3.34 grams of potassium bromate and make up to 1 liter, using 50 ml. of concentrated nitric (sp. gr. 1.42) acid and 950 ml. of distilled water.

STANDARD SELENIOUS ACID SOLUTION. Standard selenious acid solutions were prepared by two methods. (1) Resublimed selenium dioxide (*2*) was dissolved in distilled water and the selenium content determined gravimetrically. (2) Reprecipitated selenium was weighed out, oxidized to selenious acid with concentrated nitric acid, and diluted quantitatively to a known volume.

PRIMARY STANDARD SELENIUM. Resublimed selenium dioxide was reduced by sulfur dioxide to selenium, which was washed with water containing sulfur dioxide, then with alcohol and ether, successively displacing each other to avoid oxidation. The selenium was dried at 105° C.

FAST RED B INDICATOR SOLUTION. The indicator solution was prepared by dissolving 0.5 gram of Fast Red B in 100 ml. of distilled water.

Procedure

DETERMINATION OF ARSENITE-BROMATE RATIO. Measure about 35 ml. of the arsenite solution into a titration flask, and add 10 ml. of concentrated hydrochloric acid and 2 drops of Fast Red B indicator solution. Add potassium bromate solution, from a buret, until a fading of the indicator warns that the end point is being approached. Add another drop of indicator and titrate dropwise until the red color is destroyed. Calculate the ratio of arsenite to bromate.

Standardization of Potassium Bromate Solution. Weigh out about 0.12 to 0.15 gram of reprecipitated selenium and oxidize with 1 ml. of concentrated nitric acid. The solution may be heated to hasten the dissolving of the selenium, but boiling must be avoided to prevent the sublimation of selenium dioxide. Dilute with 25 ml. of water and add a calculated excess of potassium bromate solution. Boil until all the bromine has been expelled. Cool the flask somewhat and add a quantity of arsenious acid solution calculated to be sufficient to reduce the excess bromate. Add 10 ml. of concentrated hydrochloric acid and 2 drops of Fast Red B indicator solution. Titrate with potassium bromate until the red color is destroyed. Calculate the selenium and selenium dioxide titer of the bromate solution.

$$BrO_3^- + 3AsO_2^- \rightarrow Br^- + 3AsO_3^-$$

Determination of Selenious Acid. Weigh or aliquot a sample of selenious acid containing about 0.10 to 0.30 gram of selenium dioxide, add a calculated excess of potassium bromate solution, and boil until bromine-free. Add from a buret a quantity of arsenious acid calculated to be sufficient to reduce the excess bromate. This may involve a preliminary determination to find the quantities of bromate and arsenite required for a given sample. Add 10 ml. of concentrated hydrochloric acid and 2 drops of Fast Red B indicator solution and titrate with bromate to a colorless condition. The volume of solution used should be kept to about 50 ml. and the final titration should be carried out at or below a temperature of 80° C.

$$2BrO_3^- + 2H^+ + 5SeO_3^{--} \rightarrow Br_2 + 5SeO_4^{--} + H_2O$$

Table I. Determination of Selenite

HNO_3 in 50 Ml. of Solution	SeO_2 Found	Error
Ml.	*Gram*	*Gram*
0.5	0.3134	−0.0056
0.6	0.3188	−0.0002
0.7	0.3192	+0.0002
0.8	0.3185	−0.0005
0.9	0.3192	+0.0002
1.0	0.3189	−0.0001
1.1	0.3188	−0.0002
1.2	0.3194	+0.0004
1.3	0.3189	−0.0001
1.4	0.3189	−0.0001
1.5	0.3191	+0.0001

Experimental

Effect of Nitrate Concentration. Blumenthal (*1*) found that nitric acid must be present if bromate is to oxidize selenite to selenate. Smith (*10*) has shown that nitric acid does not decompose boiling solutions of bromate. To determine the limiting concentration of nitric acid needed for the oxidation, 25-ml. aliquots of standard selenite solution containing 0.3190 gram of selenium dioxide per aliquot were analyzed using varying amounts of concentrated nitric acid (Mallinckrodt, C. P. analytical reagent, specific gravity 1.42, 70 per cent). Selenite content was determined as previously described with results shown in Table I.

It is evident that only a small amount of nitric acid is necessary for the reaction and that large amounts of acid do not interfere. It was found convenient to add the nitric acid directly to the bromate solution before standardization as has been described.

Results of Analysis of Selenite Solutions. Samples of selenious acid were prepared by weighing reprecipitated selenium, dissolving it in nitric acid, and diluting. Aliquots were analyzed with results as follows:

SeO_2 Taken	SeO_2 Found
Gram	*Gram*
0.2507	0.2505
	0.2507
0.1009	0.1010
	0.1010
	0.1010
0.3190	0.3189
	0.3188
	0.3192

A sample of selenious acid was prepared from resublimed selenium dioxide and was analyzed gravimetrically by precipitating and weighing the selenium. The results compared with the volumetric determination as follows:

Gravimetric	Volumetric
Gram	*Gram*
0.1000	0.1000
0.1000	0.0996
	0.1002

Certain cations, which form insoluble selenates, were found to interfere with the determination. Barium and lead selenites could not be analyzed by this method. Other cations which were easily oxidized and reduced, such as ferric and chromic ion, interfered. Colored ions, such as nickelous and cupric ion, made it difficult to observe the end point of the determination. However, if an additional amount of indicator were added analyses of these selenites could be carried out satisfactorily. In the presence of 0.4 to 0.5 gram of copper selenate, aliquots containing 0.1009 gram of selenium dioxide were analyzed, using an excess of the indicator to make the end point observable: taken, 0.1009 gram; found, 0.1011, 0.1011, and 0.1009 gram of selenium dioxide.

Colorless cations which form soluble selenates did not interfere with the determination. Anions forming soluble salts with the cations present in solution were found not to interfere, with the exception of the halide ions, which were required to be absent from the original sample because of the reaction of halide ions with bromate ion. An excess of nitrate ion did not interfere. A solution of selenious acid containing 0.3240 gram of selenium dioxide was analyzed, 2.0-gram portions of several salts being added to the respective aliquots with results as follows:

Salt Added	SeO_2 Found
	Gram
K_2SO_4	0.3240
$MgSO_4$	0.3236
$CdSO_4$	0.3242
$Al_2(SO_4)_3$	0.3236
$MnSO_4$	0.3238
$Ca(NO_3)_2$	0.3236
$Zn(NO_3)_2$	0.3242
$Sr(NO_3)_2$	0.3246
$Cu(C_2H_3O_2)_2$	0.3240
Na_2HPO_4	0.3244

Addition of large quantities of alkali and alkaline earth cations, except barium, did not interfere nor did the presence of selenate, sulfate, or tellurate ion.

Discussion

This method affords a means of determining selenites and selenious acid in the presence of selenates. It is rapid and accurate, comparing favorably in accuracy with the gravimetric methods. The determination is particularly well adapted to the analysis of selenious acid solutions and has been found useful for the determination of purity of selenates which contain selenites.

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Rapid Determination of Very Small Gas Flows

A Soap-Bubble Method

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IN PRODUCING very small gas concentrations by the dilution method, and for other purposes, it is necessary to meter small volumes of gas at low rates (1 to 10 ml. per minute). The usual gas-displacement measuring devices require long periods of time or the use of very small gasometers when measuring these small flow rates. The following method eliminates the above difficulties, is simple to use, and requires no elaborate equipment. It may be used to calibrate needle valves, capillary flowmeters, or other indicating devices.

The principle of the method is the formation and measurement of soap bubbles measured at a capillary tip. The apparatus necessary consists of a small capillary tip, a 3-way stopcock, a stop watch, a magnifying glass, and an illuminated scale. The latter was simply a piece of thin cross-section ruled paper mounted on a cardboard frame and illuminated from behind by a 15-watt lamp. The arrangement of apparatus is shown in Figure 1.

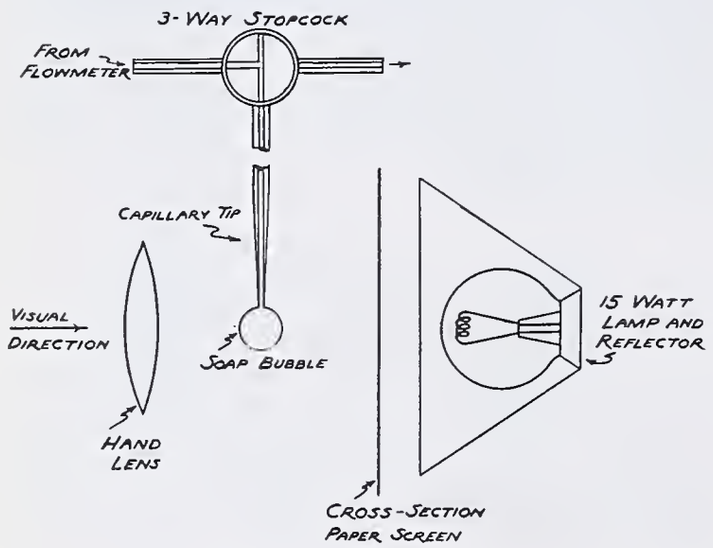

FIGURE 1. APPARATUS

In order to measure flow or calibrate flowmeters the desired flow position or manometer reading is set with air by-passing the capillary tip. A small beaker (5-ml. is adequate) is then filled with soap solution and brought in contact with the tip. As soon as the capillary tip has drawn up the soap solution, the small beaker is removed. The soap solution may be freshly prepared from commercial castile soap or a permanent mixture used (*1*). Both soap solutions employed gave comparable results in measuring the flow of gases, or mixtures of density close to that of air which are relatively insoluble in soap solutions.

With soap solution in the capillary tip the stopcock is turned towards the tip for flow measurement. The instant the bubble begins to form the stop watch is started and then stopped at any desired size measurement. Bubbles as large as 25 mm. in diameter are easily formed. The bubbles were found to be very stable (1.5 to 2 minutes) and elastic. A bubble could be formed by a syringe filled with air and collapsed by evacuating it. This could be repeated three times and all diameters checked for the same syringe air volume.

Timing the bubble just as it starts to form was simpler and gave results comparable to the exact method which consists of starting the watch at a definite diameter, stopping it at another, and then calculating the volume from differences in sphere volumes. Since the volume of a sphere varies as the cube of the diameter, the volume of the small spheres (less than 2 mm.) at the start is negligible in comparison to their final volume. If a large diameter is used initially, both measurements should be made.

TABLE I. COMPARISON OF ACTUAL WITH CALCULATED VOLUMES

Calibrated 5-Ml. Syringe Volume *Ml.*	No. of Measurements	Mean Diameter *Mm.*	Calculated[a] Volume *Ml.*	Ratio of Calculated to Actual Volume
1.00	4	12.1	0.91	0.91
1.96	6	15.5	2.25	1.15
2.94	4	17.8	2.92	0.99
3.90	5	20.2	4.34	1.11
5.00	4	22.2	5.76	1.15

Average deviation from mean, 10.2%.

[a] Based on volume of sphere $= \frac{\pi D^3}{6}$.

For calibration purposes a series of five or more readings is made for each flow and averaged. The time for each reading is small—for example, at 7 ml. per minute each reading took about 32 seconds. Thus a series of readings can be obtained in a short time. Care should be taken to have the measuring station free from cross drafts and air currents which cause the bubble to oscillate. A cardboard screen can be placed around the measuring station.

A comparison of actual volumes with calculated volumes is shown in Table I. A calibrated 5-ml. syringe was used for the source. The average deviation from a sphere for the five bubble sizes measured is about 10 per cent. This difference is due to errors in diameter measurement, departure from sphericity, and errors in setting syringe volumes (hand adjustment was employed). In use, volumes can be determined by measuring the diameter or by calibrating the measuring station by means of a syringe.

Resistance measurements showed that the back pressure created by the bubble itself was less than 2 mm. of water. In order to reduce the back pressure produced by capillary action on the tip walls below 12 mm. of water (before the bubble is formed), the tip should be 1.5 mm. or more in diameter.

Measurements can be carried out using larger openings, but the departure from sphericity is greater. Flows as high as 2 to 4 liters per minute were measured using a small funnel (30-mm. opening). Bubbles as large as 75 mm. in diameter can be formed. For these measurements, a calibration of the bubbles using their horizontal diameter was made by means of a 100-ml. syringe. Repeated measurements on given volumes using the syringe were found to check within 4 per cent, when the horizontal diameter was measured. However, because of the large departure from sphericity direct calibration for large bubbles is recommended—that is, the measuring station should be calibrated first by using a syringe.

All the measurements in this study were made using air or nitrogen at room conditions (760 mm. of mercury and 25° C.); however, the method should be applicable to other gases.

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Accurate Determination of Calcium without Reprecipitation

In the Presence of Aluminum, Iron, Magnesium, Manganese, Phosphorus, Sodium, and Titanium

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IT IS well known that calcium can be precipitated as the oxalate without interference by moderate amounts of iron or aluminum, if the pH is sufficiently low (*1*, *3*, *7*). A search of the literature, however, fails to reveal an adequate study of the effect of variations in the quantity of the accompanying elements on the accuracy of the calcium determination. The literature also lacks a satisfactory report on the minimum pH at which calcium oxalate can be quantitatively precipitated. Perciabosco's (*8*) statement that complete precipitation is obtained at a pH of 1 seems hardly credible. Shohl's (*9*) limit of 4.0 is based on insufficient data; he failed to study any pH between 4.0 and 2.8. The minimum allowable pH will vary slightly with the accuracy required and with the conditions of precipitation, such as the quantity of calcium and of accompanying elements, the excess of oxalate added, the volume, etc.

From the data of the authors, presented elsewhere (*5*, *6*), it can be computed that a negligible amount of calcium (0.0005 millimole) will remain in 200 ml. of solution containing 8.0 millimoles of excess oxalate at an ionic strength of 0.36 and a pH of 3.70. These conditions were chosen for the determination of calcium, and a formate buffer was selected.

A major source of error in the determination of calcium in a limestone is the postprecipitation of magnesium. This contamination may be diminished by decreasing the digestion. On the other hand, too short a digestion may cause an incomplete yield of a nonfilterable precipitate. These difficulties were avoided by slow precipitation of calcium oxalate and digestion for 5 minutes in a hot, strongly acid solution, rapid cooling of this solution, adjusting the pH to 3.70, and a final digestion of 30 minutes at 25° C.

Reagents

A standard solution of calcium was obtained by dissolving a weighed quantity of Merck's reagent calcium carbonate (low in alkali and chloride) previously dried for 3 hours at 110° C. in a slight excess of hydrochloric acid and diluting in a volumetric flask.

Solutions of aluminum, ferric, ferrous, magnesium, manganese, and sodium chlorides, and ammonium monohydrogen phosphate were prepared from reagent grade salts. The first five solutions were made 0.01 *M* with hydrochloric acid to prevent hydrolysis. The concentrations of manganese and sodium chlorides were calculated from the weights of salts taken. All the other solutions were standardized by appropriate methods. The solution of ferrous chloride was passed through a silver reductor (*10*) just before use. A solution of titanic chloride was prepared by slowly pouring distilled titanium tetrachloride into 0.4 *M* hydrochloric acid, and was standardized by a gravimetric determination of titanium.

A wash solution 0.020 *M* with ammonium hydrogen oxalate and 0.010 *M* with ammonium oxalate was prepared by dissolving 0.020 mole of ammonium oxalate and 0.010 mole of oxalic acid in a liter of water.

A solution of 2.0 *M* formic acid was prepared by dilution of the chemically pure concentrated acid. Similarly 0.50 *M* oxalic acid and 2.0 *M* ammonium formate were prepared from weighed quantities of the chemically pure solids. Since these three solutions are used to control the pH of the precipitation, they should have the recommended concentrations within 10 per cent. Or the concentrations may be determined and appropriate changes in the volumes applied.

Procedures

DETERMINATION OF CALCIUM IN A SOLUTION. The solution should contain preferably about 2 millimoles of calcium. If elements such as iron or phosphorus are present, the solution should contain enough hydrochloric acid to prevent the formation of a precipitate, but a large quantity of acid should be avoided.

Add 12 ml. of 2.0 *M* formic acid, dilute to 140 ml., and heat to 95° C. Add with stirring 20 ml. of 0.50 *M* oxalic acid through a funnel with the stem constricted so that about 85 seconds are required for the addition. Rinse the funnel with 2 ml. of water. Digest the precipitate 5 minutes at 85° to 90° C. Set the beaker in a larger vessel of cold water, so that it cools to 25° C. in 15 minutes, then add with stirring 34 ml. of 2.0 *M* ammonium formate through the same funnel. Rinse the funnel with 5 ml. of water, and digest the precipitate 30 minutes at 25° C.

Filter the solution through a mat of asbestos supported on a perforated porcelain disk in a funnel. Wash the precipitate three times by decantation with 20-ml. portions of the wash solution, then transfer the precipitate to the filter, using a total of about 150 ml. of the wash solution. Finally wash the precipitate and funnel with three 8-ml. portions of water.

Transfer the precipitate along with the asbestos and porcelain disk to a 400-ml. beaker, and titrate with standard permanganate according to the method of Fowler and Bright (*2*). Stir the solution occasionally for 5 to 10 minutes after the addition of sulfuric acid before starting the titration.

After the solutions have been prepared, duplicate determinations of calcium by this method require less than 2 hours.

In some cases, instead of titrating the precipitate with permanganate, one of the following gravimetric methods was applied: (1) The precipitate was dried at 110° C. for 2 hours and weighed as calcium oxalate monohydrate. (2) The precipitate was ignited for 1.5 hours at 485° C. and weighed as calcium carbonate (*11*). (3) The precipitate was ignited in a covered platinum crucible to constant weight over a Meker blast burner, cooled over sodium hydroxide, and weighed quickly.

DETERMINATION OF CALCIUM IN A LIMESTONE. Ignite a weighed sample of about 300 mg. in a platinum crucible. To the cool residue add cautiously 2 ml. of water, then 2 ml. of 6 *M* hydrochloric acid. Stir the mixture, crush any lumps, and transfer to a 400-ml. beaker. Repeat the addition of water and acid to the crucible, warm the crucible gently, and transfer the contents to the beaker, rinsing thoroughly. Heat the solution in the beaker until the sample is completely dissolved except for a residue of silica. Dilute the solution to about 100 ml. Add a slight excess of bromine (as judged from the color) and 10 drops of 0.1 per cent bromophenol blue. Now add 6 *M* ammonia dropwise with stirring until the solution turns blue or until the precipitation of ferric hydroxide indicates that the solution is nearly neutral. Add 12 ml. 2.0 *M* formic acid, dilute to 140 ml., and continue as in the foregoing procedure, using the volumetric method.

In the case of argillaceous limestones, sodium carbonate must be added before the ignition.

Results

COMPARISON OF METHODS FOR TREATMENT OF PRECIPITATED CALCIUM OXALATE MONOHYDRATE. In a series of experiments, known quantities of about 2 millimoles of calcium were taken, and calcium oxalate was precipitated as described above. The precipitates were treated by one of the foregoing procedures. The results are shown in Table I.

EFFECT OF THE QUANTITY OF CALCIUM. In another series of experiments, varying known quantities of calcium were taken and determined as above. The results are shown in

Table I. Comparison of Methods for the Treatment of Calcium Oxalate

Method	No. of Determinations	Relative Mean Deviation	Relative Mean Error
		Parts per thousand	
$KMnO_4$	6	±0.6	−1.8
$CaC_2O_4.H_2O$	6	±0.5	−0.8
$CaCO_3$	4	±0.3	−1.0
CaO	2	±3	+5

Table II. Effect of Quantity of Calcium

Ca Taken	Method	Ca Found	pH
Millimoles		*Millimoles*	
0.2004	$CaC_2O_4.H_2O$	0.1944 0.1930	3.74
0.4996	$KMnO_4$	0.5036 0.5020	3.72
0.999	$CaC_2O_4.H_2O$	0.997 1.005 0.997	3.73
3.998	$KMnO_4$	3.989 3.984	3.67

Table III. Effect of pH

HCOOH Taken	$HCOONH_4$ Taken	pH	Mean Error
Millimoles	*Millimoles*		*Millimoles* × 10^3
67	4.4	3.97	−3
69	59	3.47	+1
70	123	3.17	−2
71	438	2.54	−2
72	1430	1.65	−17

Table II. The values in column 4 were obtained by measuring the pH of the filtrate with a Beckman pH meter.

Effect of pH. In another series of experiments, the quantities of ammonium formate and formic acid were altered so as to vary the pH. Two millimoles of calcium were taken in all cases, and the precipitates were weighed as calcium oxalate monohydrate. The results are shown in Table III, where each entry represents the mean of two determinations which differed from each other by 0.001 millimole or less.

Effect of Accompanying Ions. A known quantity of calcium, approximately 2 millimoles, was taken in each case, mixed with the indicated quantity of other ion, and determined as above by titration with permanganate. The results are shown in Table IV.

Determination of Calcium in Limestone. Since the quantities of other elements that are found in a limestone sample sufficient to yield 2 millimoles of calcium are far below the amounts that introduce serious errors as indicated in Table IV, good results should be obtained when the foregoing method is applied to a limestone. The results of such analyses of two limestones from the National Bureau of Standards are shown in Table V. When the calcium was determined as calcium oxide or calcium carbonate, the silica was removed by the standard procedure before the determination of calcium. The filtrate in these determinations had pH values between 3.69 and 3.80. The quantity of sodium carbonate shown in column 3 was mixed with the sample before the ignition.

Discussion

From Table I it appears that the method gives excellent results when the precipitate is treated volumetrically or weighed as calcium oxalate monohydrate or calcium carbonate. High and less precise results are obtained by weighing as calcium oxide. However, from Table IV it is clear that the weighing as calcium oxalate monohydrate also gives high and erratic results when magnesium is present in the sample. Probably traces of occluded magnesium oxalate hinder the drying process.

Table II indicates that the quantity of calcium may vary from 0.5 to 4.0 millimoles.

Table III indicates that minor variations of the pH from the recommended value of 3.70 have no effect. The minimum pH for quantitative precipitation of calcium as the oxalate is about 2.5.

The effect of phosphate on the calcium determination depends on the method of treating the precipitate. When the sample contains about equimolar quantities of calcium and phosphate, sufficient calcium phosphate is coprecipitated to give an appreciable negative error by the volumetric procedure (Table IV). This error becomes negligible, however, if a gravimetric method is used because the calcium phosphate contributes to the weight of the precipitate.

In the analysis of an argillaceous limestone (sample 1 contained 18.15 per cent silica), the sodium introduced as carbonate for the ignition is later coprecipitated as sodium oxalate and causes a slight positive error. This error is insignificant if only 25 mg. of sodium carbonate are used. Since potassium is less coprecipitated by calcium oxalate than sodium (*4*), it is probable that potassium carbonate would be a better flux for this purpose.

Summary

A rapid and accurate method for the determination of calcium is described. The effect of seven elements commonly associated with calcium on the accuracy of this method has been investigated. This method is recommended for limestone and similar material but not for sand or other substances very low in calcium.

Table IV. Effect of Accompanying Ions

Ion	Quantity of Ion	No. of Determinations	Mean Error	Mean Deviation	pH
	Millimoles		*Millimoles* × 10^3		
Al^{+++}	0.500	2	− 2	1	3.72
	1.00	1	+ 1		3.67
	2.00	2	+ 2	3	3.66
Fe^{+++}	0.050	1	0		
	0.100	1	+ 6		
	0.200	1	+11		
	0.300	1	+10		
	0.400	1	+11		
	0.500	3	+11	3	3.72
	1.00	1	+11		3.73
	1.50	1	+ 9		3.71
	2.00	2	− 1	1	3.66
Fe^{++}	0.100	2	+11	1	3.74
Mg^{++}	0.500	1	0		3.70
	2.00	1	+ 1		3.68
	5.00	2	+ 1	1	
	5.00[a]	2	+11	8	
Mn^{++}	0.005	1	− 1		
	0.010	2	+ 4	1	3.70
	0.020	1	+ 5		
	0.030	1	+ 8		3.70
PO_4^{---}	0.500	1	− 6		3.70
	1.00	1	− 4		3.70
	2.00	2	−13	1	
	2.00[a]	2	+ 1	1	
	2.00[b]	2	− 4	1	
Na^+	0.200	1	+ 2		
	0.500	1	+ 6		
	1.00	1	+10		3.70
	5.00	1	+17		3.69
Ti^{++++}	0.010	1	+ 1		3.72
	0.030	1	+ 5		3.69
	0.050	1	+11		3.67
	0.070	1	+21		3.60

[a] Weighed as $CaC_2O_4.H_2O$. [b] Weighed as $CaCO_3$.

Table V. Analysis of Limestones

Sample No.	Correct CaO	Na_2CO_3 Taken	Method	No. of Determinations	Mean CaO Found	Mean Deviation
	%	*Mg.*			%	%
88	30.49	0	$KMnO_4$	5	30.30	0.06
		0	CaO	2	30.37	0.01
		0	$CaCO_3$	1	30.30	
1	37.65	0	$KMnO_4$	2	37.15	0.24
		25	$KMnO_4$	6	37.74	0.04
		150	$KMnO_4$	4	37.86	0.10

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Color Reactions of 1,10-Phenanthroline Derivatives

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MANY examples of the influence of slight changes of structure on the behavior of organic reagents may be found among the benzene arsonic and arsinic acids, a score of which have been prepared and studied. Differences between 8-hydroxyquinoline and its derivatives, such as ferron, have also been reported. The oxidation potential of the ferrous *o*-phenanthroline complex is 1.14 volts compared with 1.25 for the corresponding nitro derivative, nitro-ferroin, and the range of high-potential, oxidimetric indicators is thus extended (*3*). Dimethylglyoxime, after 35 years of useful service as the nickel reagent, may soon be rendered obsolete by 1,2-cyclohexanedionedioxime.

Since reactions involving formation of the metal complexes appear to be susceptible to slight changes in the reagent used, it is of interest to study the effect of various substituents whenever possible. These effects are usually unpredictable. Accordingly, a comparison of 1,10-phenanthroline and five of its derivatives was made with respect to their reactions with iron, copper, and molybdenum. (The various phenanthroline derivatives were prepared by Frederic Richter at the University of Illinois. The methods will be described in a paper by him and G. Frederick Smith.)

Apparatus and Solutions

Transmittancy measurements were made with a General Electric recording spectrophotometer set for a spectral band width 10 mμ. pH values were measured with a glass electrode meter calibrated with Clark and Lubs buffers.

One-tenth per cent solutions of the following compounds were prepared by dissolution in 95 per cent ethanol and dilution with redistilled water: 1,10-phenanthroline and its 5-bromo, 5-chloro, 5-methyl, nitro (position in ring uncertain), and 5-nitro-6-methyl derivatives. The last two are practically insoluble in water and require about 80 per cent alcohol for complete dissolution. The final alcohol content was 5 per cent by volume for the four other reagent solutions.

A standard ferric nitrate solution prepared from iron wire of reagent grade contained 0.01 mg. iron per ml. and sufficient nitric acid to prevent hydrolysis. Measured quantities of this solution were reduced with a 10 per cent solution of hydroxylamine hydrochloride to obtain known concentrations of ferrous iron. The merits of this reducing agent have been established (*1*).

Twenty per cent ammonium acetate solution was used as a buffer and pH adjustments were made with 6 *N* solutions of ammonium hydroxide and hydrochloric acid.

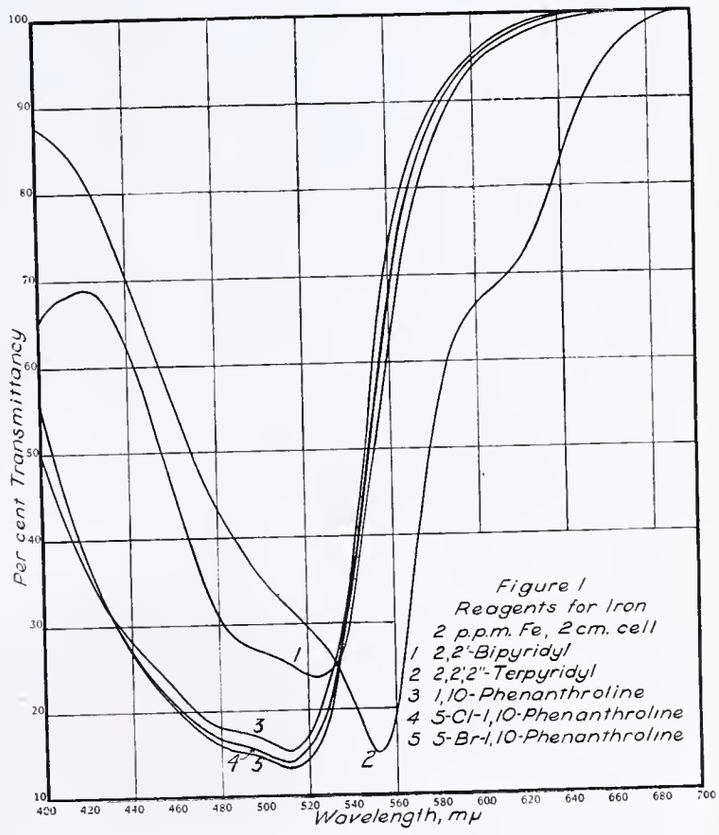
Copper sulfate solution containing 0.05 mg. copper per ml. was prepared by dissolving copper wire in nitric acid, evaporating with sulfuric acid, and suitably diluting with redistilled water.

A standard molybdate solution, made by dissolving 1.503 grams of molybdic oxide in 10 ml. of 10 per cent sodium hydroxide solution and diluting to 1 liter, contained 1 mg. of molybdenum per ml.

A solution of chlorostannous acid contained 110 grams of C. P. "stannous chloride" and 170 ml. of concentrated hydrochloric acid per liter.

Determination of Iron

COLOR REACTION. Substitution of various groups in 1,10-phenanthroline does not materially affect the hue of the ferrous complexes except in the case of the nitro derivative which has a more purplish color than the others (Figures 1 and 2). A weak absorption band at 480 mμ characteristic of the other compounds is absent. An extra band at 440 mμ is exhibited by the 5-methyl but not by the 5-nitro-6-methyl derivative. The latter compound gives a slightly lower transmission for 2 p. p. m. of iron than the others, although the difference is too small to be significant. In more highly concentrated solutions—e. g., 0.025 *M* in iron—the nitro derivative appears visually to be the most sensitive. For solutions of 50 ml. containing 2 p. p. m. of iron, 2 to 3 ml. of reagent solution provide an excess for development of the maximum color and larger amounts do not affect the intensity, provided the acidity is not less than the lower limits specified in Table I.



Effect of pH. In the determination of iron with 1,10-phenanthroline, pH variations between 3 and 9 do not influence the color. The derivatives studied are similar to the parent compound in this respect. Limits between which pH variations have no effect on the color reaction are shown in Table I.

Table I. Comparison of 1,10-Phenanthroline Derivatives

Reagent	Applicable pH Range	Wave Length of Maximum Absorption, Mμ	Molecular Extinction Coefficient
1,10-Phenanthroline	3–9	510	11,100
5-Chloro-1,10-phenanthroline	2.7–8.9	512	11,700
5-Bromo-1,10-phenanthroline	3–8	512	12,100
5-Methyl-1,10-phenanthroline	2.5–10	516	11,500
Nitro-1,10-phenanthroline	2.5–9.5	510	11,500
5-Nitro-6-methyl-1,10-phenanthroline	3–8	512	12,400

Effect of Iron Concentration. All the compounds are highly sensitive in detecting iron in low concentrations. In this respect they are a slight improvement over phenanthroline itself but not sufficiently so to commend their use. Beer's law is valid in the range 0.1 to 5 p. p. m. of iron for all the reagents studied. This is the optimum working range for determinations with a 1-cm. transmission cell.

Stability of the Color. Solutions of 50 ml. containing 0.1 to 5.0 p. p. m. of iron with an excess of both the color-forming reagent and hydroxylamine hydrochloride buffered with 0.2 gram of ammonium acetate were allowed to stand under ordinary laboratory conditions in glass-stoppered bottles. In 30 days there was no change in the case of the methyl derivative. After 3 months, decreases of several per cent in transmission at 400 mμ appeared. The nitro and bromo compounds changed appreciably in 24 hours, accompanied by slight fading. In 10 days, the nitromethyl compound underwent a change in hue, but the fading was only slight at the wave length of maximum absorption. The chloro compound darkened slightly, the transmission decreasing about 2 per cent in 2 weeks for concentrations greater than 1 p. p. m. The bromo compound faded overnight, producing a green suspension. A small amount of the green material precipitated on further standing. It was found to be soluble in ethanol and showed its maximum transmission at 560 mμ with absorption bands at 415 and 660 mμ. Solutions of ferrous complexes of each of the reagents studied showed some tendency to decrease in transmission in the red, thus becoming more green. The time required varied from 1 day in the case of the bromo derivative to 3 months in the case of the methyl derivative and the parent compound. Standards for visual comparison in the determination of iron with *o*-phenanthroline are reliable for at least 6 months, however (*1*). Blank solutions of the reagents containing no iron became noticeably darker at 400 mμ on standing 1 to 2 weeks.

Effect of Diverse Ions. The extent of interference by diverse ions is dependent only slightly on the reagent used. The effects of cobalt, copper, chromium, carbonate, orthophosphate, pyrophosphate, and cyanide were determined using the various reagents. These are representative of the ions which interfere with *o*-phenanthroline. In each case, the ion in question was added to the iron before reducing. Interference was found in all cases.

Determination of Copper

Ammoniacal cupric solutions containing 1,10-phenanthroline yield an insoluble, brown, complex cuprous salt when treated with hydroxylamine. If the copper concentration is sufficiently low, precipitation is delayed and the intensity of the color is determined by the amount of copper present. Precipitation can be avoided by adding an organic solvent miscible with water and conformity to Beer's law thereby achieved.

As a reagent for copper, no advantage over 1,10-phenanthroline was observed in any of its derivatives. The color reaction occurs immediately on the addition of hydroxylamine and the reddish-purple hue varies somewhat according to the reagent. A flocculent precipitate forms with several of the reagents, but this dissolves immediately on dilution with the solvent, while the hue undergoes a striking change from purple to orange. The optimum working range for a cell thickness of 1 cm. is 0.5 to 10 p. p. m. Beer's law is valid and the color is stable for 24 hours.

The phenanthrolines have no value as precipitants for copper.

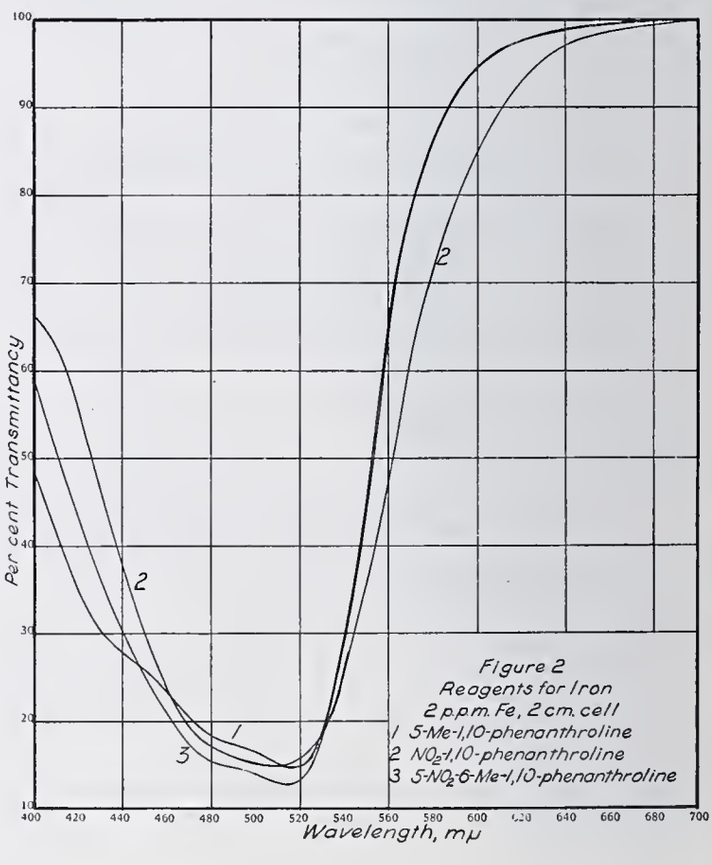
Determination of Molybdenum

A sensitive color reaction involving reduction of molybdate ion in the presence of 2,2′-bipyridyl with chlorostannous acid has been described (*2*). Development of the color was attributed to reaction of a "lower oxide of molybdenum" with bipyridyl.

1,10-Phenanthroline and its derivatives give a reddish-purple color similar to that obtained with bipyridyl. Bipyridyl is the least sensitive of the reagents tried. None of the reagents produced a color sufficiently stable to be useful for the determination of molybdenum. Various organic solvents miscible with water and present in concentrations of 50 per cent did not appreciably retard the fading.

Under the most favorable conditions for carrying out the test with the phenanthroline derivatives, fading proceeded at the rate of an increase of 5 to 10 per cent in transmission within the first 5 minutes. Reproducibility is poor. Satisfactory transmission curves could not be obtained even in the short time of only 3 minutes required for operation of the recording instrument used.

In order to produce the color, the reagent must be added before the chlorostannous acid. Ten milliliters of reagent and 10 ml. of chlorostannous acid are suitable for developing a color with 0.5 mg. of molybdenum in 50 ml. of solution. Concentrated hydrochloric acid (0.5 ml.) prevents the color



development if added before the reagent, but if added immediately after, 10 ml. may be present.

Although the reaction described may be used for detecting molybdenum, even in the presence of iron, it should be regarded as unsound for quantitative purposes.

Summary

A spectrophotometric study of the ferrous complexes of five derivatives of 1,10-phenanthroline indicates that these compounds may be used successfully as color-forming reagents for the determination of iron. Advantages over several methods in use were observed, including sensitivity and freedom from pH effects. With the exception of the methyl derivative, they do not form complexes of exceptional stability and are not preferred over the parent compound.

1,10-Phenanthroline and its derivatives form insoluble, highly colored, cuprous complexes which can be measured colorimetrically in the presence of suitable solvents. These reagents are not satisfactory precipitants for copper.

Molybdate ion in the presence of 1,10-phenanthroline, or its derivatives, may be reduced with chlorostannous acid to give a reddish hue capable of detecting 0.5 p. p. m. of molybdenum. The color is not sufficiently stable for quantitative purposes.

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Abstracted from a thesis presented by M. L. Moss to the Graduate School of Purdue University in partial fulfillment of the requirements for the degree of doctor of philosophy, May, 1942.

Effect of River Mud Deposits on Determination of B. O. D. of Supernatant Liquor

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During an investigation of conditions along the Illinois Waterway it was noted that B. O. D. results obtained varied widely at certain points, depending upon whether the initial dissolved oxygen of the diluted sample was calculated or actually determined. It had been found previously by Ruchhoft and Moore (*1*) that the presence of river mud in suspension caused considerable discrepancy in dissolved oxygen and B. O. D. results, which was attributed to the presence of highly reducing substances in such muds.

Table I. Discrepancy in B. O. D. Results Obtained by Two Different Methods on Illinois Waterway

Station 1		Station 2	
Determined initial	Calculated initial	Determined initial	Calculated initial
10.6	20.8	5.8	8.8
6.8	15.8	5.8	8.9
7.9	17.4	6.4	9.1
8.4	17.6	7.3	9.7
10.4	18.0	8.8	11.0
12.5	19.6	9.5	11.2
16.9	26.7	...	...
12.6	20.9	...	...
12.3	21.5	...	...
10.6	24.5	...	...
13.9	25.7	...	...
16.1	27.0	...	...
16.8	29.8	...	...
Av. 11.9	22.0	7.3	10.4

No experiments were carried out, however, on the effect of anaerobic mud deposits upon the B. O. D. of the supernatant liquor. It is the purpose of the present paper to indicate such effects and to show that the discrepancy in the B. O. D. as determined by the two methods varies with the time during which the supernatant liquor is in contact with these deposits.

Experimental

In the first experiments, fresh domestic sewage was allowed to stand under anaerobic conditions for varying periods of time and the B. O. D. was determined by two methods: (1) the proper dilution was made up and from the dissolved oxygen content of the dilution water and per cent concentration of the sewage the initial dissolved oxygen was calculated, and (2) the proper dilution was made up and aerated vigorously and the initial dissolved oxygen was determined by the Winkler method.

As these first experiments failed to show any discrepancy in the B. O. D. results, it was decided to simulate river conditions by placing a deposit of river mud in bottles, then filling with dilute sewage and allowing the bottles to stand for varying periods of time. At the desired intervals samples of the clear supernatant were removed and B. O. D. determinations were made as above described. At the same time, controls on the same diluted sewage were put up from bottles containing no mud deposits.

Results

In Table I are given results observed in samples obtained at two points on the Illinois Waterway. It made a material difference upon the 5-day B. O. D. whether or not the initial dissolved oxygen was actually determined or calculated, the former giving approximately 60 per cent of the B. O. D. value obtained by the latter method. In the lower B. O. D. range (taken from a point approximately 11 miles further downstream) less discrepancy is found (approximately 30 per cent), which denotes a better stabilized mud deposit in this portion of the river. These same discrepancies have been noted by the Sanitary District of Chicago (*2*) in its extensive study of the Illinois Waterway.

In Table II are given the results obtained in using a strong domestic sewage, undiluted and diluted, when it was allowed to stand anaerobic for varying periods of time and the B. O. D. was determined by the two methods. These results check well within the limits of error and only in the case of the 48-hour anaerobic sample of undiluted sewage is any appreciable difference found. It can be concluded that the mere existence of anaerobic conditions in diluted sewage is not the important factor contributing to the B. O. D. discrepancies as found on the Illinois waterway.

Table II. Effect of Period of Anaerobiosis upon B. O. D. Determination

(No mud present)

Anaerobic, Hrs.	100 Per Cent Sewage		Dilute Sewage	
	Determined initial	Calculated initial	Determined initial	Calculated initial
Fresh sewage	353	350	79	80
24	353	376	73	70
48	347	393	70	71
72	323	339	64	63

Owing to the fact that no discrepancies were found in B. O. D. determinations when sewage was allowed to stand anaerobic by itself, the effect of mud deposits upon the supernatant water was next tried (Table III). With a well stabilized river mud the B. O. D. results obtained by the two methods check about as closely as when no mud deposit is present. This indicates that such mud deposits have very

little effect upon the B. O. D. of the supernatant. However, when a strong mud deposit (high B. O. D.) was present, discrepancies were found which increased with the time period of anaerobiosis. When a liquor having a lower B. O. D. was used as supernatant with a strong river mud, greater discrepancies in the B. O. D. were found by these procedures, the discrepancy increasing as before with length of time the sample was anaerobic.

Table III. Effect of Mud Deposits upon B. O. D. Determination

	Control, No Mud Present		Weak River Mud Deposit Present[a]		Strong River Mud Deposit Present[b]	
Anaerobic, Hrs.	Determined initial	Calculated initial	Determined initial	Calculated initial	Determined initial	Calculated initial
48	42	42	33	35	34	41
72	40	38	28	29	30	39
96	40	43	26	30	28	44
120	41	46	22	26	26	46
					Very Strong Mud[c]	
48	11.9	11.8	..	..	12.8	18.0
120	14.3	14.0	..	..	11.4	19.8
168	14.6	16.0	..	..	8.1	15.8
216	16.6	17.2	..	..	7.3	21.5

[a] Weak mud, low organic content and low B. O. D.
[b] Strong mud, high organic content and high B. O. D.
[c] High volatile matter content and very high B. O. D.

In the anaerobic decomposition of river mud deposits various chemical compounds are formed, some of which are highly reducing, such as hydrogen sulfide. It was thought that perhaps some correlation between the oxidation-reduction potential of the supernatant liquor and the dissolved oxygen values as determined by the Winkler method could be found. These values were determined after various periods of anaerobiosis (Table IV).

Table IV. Effect of Mud Deposits upon Dissolved Oxygen and E_h of Supernatant

	E_h of Supernatant		10 Per Cent Dilution of Supernatant — Mud Present			10 Per Cent Dilution of Supernatant — No Mud Present		
Anaerobic, Hrs.	Mud present	No mud present	Determined D.O.	Calculated D.O.	E_h	Determined D.O.	Calculated D.O.	E_h
24	−0.012	+0.114	7.67	7.83	+0.221	7.85	7.83	+0.271
48	−0.023	+0.035	7.16	7.41	+0.097	7.38	7.41	+0.147
72	−0.031	+0.003	7.14	7.52	+0.082	7.43	7.52	+0.101
96	−0.033	−0.015	7.24	7.89	+0.061	7.63	7.89	+0.073
120	−0.044	−0.027	7.30	7.93	+0.054	7.80	7.93	+0.062

The discrepancy in dissolved oxygen values of a 10 per cent dilution of the supernatant increases with the period of anaerobiosis and at the end of 120 hours is 0.63 p. p. m. where a mud deposit was present, and only 0.13 p. p. m. where no such deposit was in the bottle. During this experiment the E_h of the 10 per cent dilutions of the supernatant with no mud present were higher than those of dilutions of supernatant over the mud deposit. However, at the end of 120 hours the discrepancy between the oxidation-reduction potential of the dilutions of the two supernatants had largely disappeared. This same relation held for the straight supernatant without any dilution.

Discussion

The discrepancies found in B. O. D. results when the initial dissolved oxygen is calculated or actually determined are not a matter of simple anaerobic conditions prevailing in the sample, but apparently other factors are contributory. No doubt when a sample to be incubated has been anaerobic for some time there is a loss of volatile constituents which contribute to the oxygen demand upon vigorous aeration. However, any attempt to account for this discrepancy on this basis failed and a very slight difference was found in the amount of iodine consumed by an aerated and nonaerated sample of anaerobic supernatant liquor. However, the presence of a strong river mud does have a decided effect upon the B. O. D. of the supernatant, depending upon the strength of the mud deposit in terms of B. O. D. or rather upon the degree of stabilization of such deposits.

Neither can these discrepancies be accounted for on the basis of the oxidation-reduction potential developed in the supernatant. It would be natural to conclude that in the anaerobic decomposition of river mud deposits the highly reducing substances formed would affect the E_h of the supernatant. This is true, as shown by the fact that the E_h becomes more negative as the period of anaerobiosis is increased. The same holds for supernatants which have not been in contact with river mud and at the end of 120 hours the two values are approximately the same; even though there is little difference in the E_h of the supernatants after 120 hours of anaerobic conditions, the sample which is in contact with mud shows a much greater discrepancy in the determined and calculated D. O. values made on dilutions of these supernatants. This denotes the presence of other substances formed during the anaerobic decomposition which adversely affect dissolved oxygen results but have only a slight effect upon the oxidation-reduction potential.

Therefore, in the determination of the B. O. D. of river water which is in contact with mud deposits containing quantities of very slightly stabilized organic matter, the initial dissolved oxygen content of the incubated sample should be calculated and not actually determined by the Winkler method.

This procedure gives the B. O. D. of the liquor plus the oxygen demand of products resulting from anaerobic decomposition of the sludge deposit which have diffused into the supernatant liquor. These experiments suggest that the oxygen demand of the diffusion products of an anaerobic mud into an anaerobic supernatant is oxidized very rapidly chemically when the supernatant water is aerated. The experiments seem to indicate that the B. O. D. of an anaerobic river sample obtained after determination of the initial D. O. is for the supernatant water only. The discrepancy between the results obtained by the two methods used in the experiments represents the oxygen requirement contribution of the river mud deposit which was satisfied upon reaeration of the anaerobic supernatant. These two methods might be applied simultaneously to indicate sections of a polluted stream in which slightly stabilized mud deposits contribute to the total B. O. D.

Summary

River mud deposits have a decided effect upon the determination of the B. O. D. of the supernatant liquor, depending upon their degree of stabilization and length of time the anaerobic supernatant is in contact with them.

No correlation was found between the oxidation-reduction potential of the supernatant and the interference in dissolved oxygen determinations by the Winkler method.

It is recommended that in the determination of the B. O. D. of supernatant liquor in contact with a highly unstabilized mud deposit the initial dissolved oxygen be calculated and not determined by chemical methods, and that simultaneous use of calculated and determined initials be used to estimate the oxygen demand contribution of the mud on oxygen-depleted river samples.

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Presented before the Division of Water, Sewage, and Sanitation Chemistry at the 104th Meeting of the American Chemical Society, Buffalo, N. Y.

Analysis of Cellulose Derivatives[1]

Determination of Free Hydroxyl Content

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A method for the determination of free or unsubstituted hydroxyl groups in cellulose derivatives has been developed, which makes use of the acetic anhydride in pyridine reagent of Verley and Bölsing. Acetylation at 75° to 80° C. for about 24 hours using 0.5-molar reagent in generous excess gave results on the samples of cellulose acetate, acetate propionate, and acetate butyrate tested, which agreed well with hydroxyl values calculated by difference from the acyl analyses. The effects of time, temperature, anhydride concentration, and excess were studied and optimum ranges for each were established.

Equations and graphs are presented with which per cent hydroxyl can be converted to hydroxyls per glucose unit of cellulose.

The method is useful for checking the accuracy of other analytical procedures, analyzing multicomponent derivatives whereby one component is calculated by difference, and correlating free hydroxyl with certain useful physical properties. Most commercial cellulose esters have essentially three replaceable hydroxyl groups per glucose unit of cellulose and this assumption is used in many calculations. Certain very low viscosity samples, however, contain more than three hydroxyls and the deviation from three may be used for the calculation of an average degree of polymerization of such samples.

CELLULOSE derivatives are usually characterized analytically by measurement of the substituted groups. The amount of free or unsubstituted hydroxyl may be calculated by difference from these data. For many purposes, however, direct measurement of free hydroxyl would be desirable. Reinhart and Kline (*15*) showed that certain vital properties of airplane lacquers vary directly with the hydroxyl content of the cellulose ester. Multicomponent esters, ethers, or ether esters may sometimes be analyzed conveniently by measuring directly free hydroxyl and one component and calculating another by difference.

In the first paper of this series (*4*) the difficulty of establishing the accuracy of methods for the analysis of total acyl in cellulose esters was discussed. By comparing the free hydroxyl values found by direct measurement with those calculated from acyl analyses, a measure of the accuracy of these methods may be obtained. In such calculations the assumption is made that there are exactly three replaceable hydroxyls per glucose unit. This is justified in the case of ordinary cellulose esters of medium high viscosity. In very low viscosity esters, however, the average hydroxyl content (free plus substituted) per glucose unit is measurably greater than three, and this deviation may be used to calculate an average degree of polymerization or molecular weight.

[1] This article and the one following are the third and fourth in this series on "Analysis of Cellulose Derivatives". For the first, see IND. ENG. CHEM., ANAL. ED., 13, 369–74 (1941), and for the second, *Ibid.*, 14, 292–7 (1942).

[2] Present address, United States Coast Guard.

Measurement of alcoholic hydroxyl groups by the acetylation method has been widely applied, and the literature to about 1930 well reviewed by Meyer (*12*). Verley and Bölsing's acetic anhydride in pyridine reagent (*21*) was applied originally to various alcohols and phenols. This basic procedure has since been adapted to special groups of compounds, including sugars and sugar alcohols by Peterson and West (*14*), castor oil and phenols by Marks and Morrell (*11*), lipids and hydroxy fatty acids by West, Hoaglund, and Curtis (*23*), glycol and glycerol solutions by Shaefer (*16*), mixtures of fats, fatty acids, and alcohols by Normann and Schildknecht (*13*), and phenols, sugars, etc., on a semimicro scale by Freed and Wynne (*3*).

Other methods used for determination of hydroxyl include the acetyl chloride method which was described by Smith and Bryant (*17*) and modified by Kaufmann and Funke (*7*) and by Christiansen, Pennington, and Dimick (*2*), the Karl Fischer method as described by Bryant, Mitchell, and Smith (*1*), and Grignard reagent method of Zerewitinoff (*25*).

The use of the acetic anhydride in pyridine reagent is extended in this paper to certain cellulose derivatives. The procedure makes use of the following reactions:

$$R - OH + (CH_3CO)_2O + C_5H_5N \rightarrow CH_3COOR + CH_3COOH.NC_5H_5$$

$$(CH_3CO)_2O + H_2O + 2C_5H_5N \rightarrow 2CH_3COOH.NC_5H_5$$

Samples and blanks are run in parallel and the excess reagent is destroyed with water. The difference in titers between sample and blank is directly equivalent to hydroxyl. The pyridine serves as a solvent, produces a vigorous and practically irreversible reaction, yet does not interfere with titration of the acids with standard alkali. This reagent is particularly suitable for use with cellulose esters because they are soluble in pyridine over a wide range of composition.

A variation of this method, which is useful in special cases, is to acetylate the compound completely, using acetic anhydride or acetyl chloride with or without pyridine, and then isolate the product. The increase in ester content, as found by saponification, is a measure of the hydroxyls which were present in the original compound (*12*).

The determination of free hydroxyl in cellulose derivatives involves certain difficulties. The sample usually is limited in its solubilities, and the fully acetylated derivative may be even more limited, particularly in solubilities in water-miscible solvents. Water must be added to decompose the excess reagent and to provide a medium in which a titration with alkali can be made. This results in the precipitation of the ester. The conditions must be controlled so the precipitate comes out in a soft, fine, and easily penetrable form; otherwise acid or alkali may be held mechanically and a serious error introduced in the titration. The hydroxyls of cellulose are not extremely reactive towards acetic anhydride and pyridine, for Hess and Ljubitsch (*6*) found that 30 to 40 days' reaction at 40° to 55° C. was required to acetylate cellulose and certain regenerated celluloses This is an extreme case for ordinary cellulose derivatives can be completely acetylated much more easily. It does show the necessity, however, for working out the conditions of time, temperature, and reagent excess, and these conditions can be expected to vary with the degree of substitution of the sample. The work described in the following sections was confined to samples having not more than 1.25 free hydroxyls per glucose unit of cellulose.

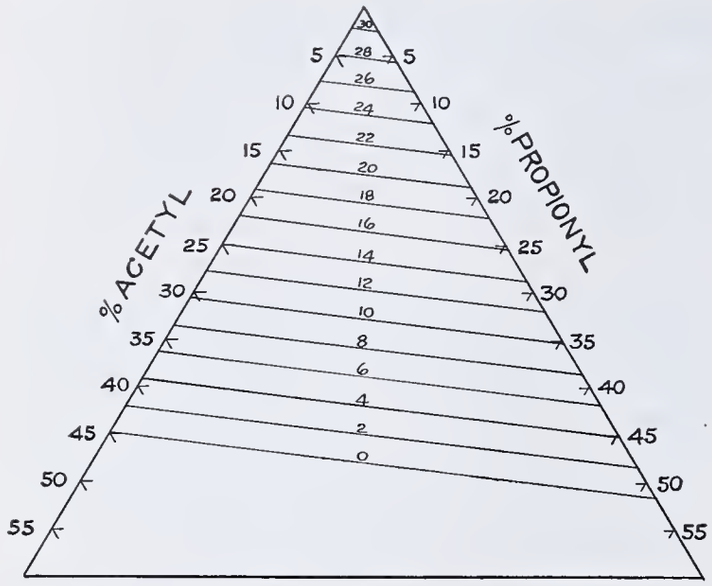

FIGURE 1. RELATIONSHIP BETWEEN PER CENT OF FREE HYDROXYL AND COMPOSITION OF CELLULOSE ACETATE PROPIONATE

Reagents and Apparatus

Acetic anhydride, commercial grade, at least 97 per cent.

Pyridine, reagent grade. A good commercial grade, if available, can be purified by careful distillation, and the fraction boiling at 115.5° to 116.5° C. taken. The water content can be measured by the acetyl chloride method (*18*) or the Karl Fischer method (*19*), and is preferably in the order of 0.1 per cent. Wilson and Hughes (*24*) have described purification methods and specifications for a suitable grade of pyridine. They found that 0.3 to 0.5 per cent of water is necessary to prevent an interfering side reaction between pyridine and acetic anhydride at boiling temperatures. However, in this work, the conditions were adjusted to avoid this side reaction, so a lower water content has been satisfactory.

Reagent consisting of 50 ml. of acetic anhydride and 950 ml. of pyridine. If the water content of the pyridine is greater than 0.1 per cent, an additional 5 ml. of anhydride should be added for each 0.1 per cent of water in the pyridine. This solution should preferably be allowed to stand for 24 to 48 hours in an all-glass container before it is used.

Standard 0.5 *N* sodium hydroxide solution.

Erlenmeyer flasks, 250-ml., connected by means of ground-glass joints to suitable reflux condensers. Short water-cooled spiral condensers were used, but many other types should be acceptable. Corks fitted with bent-over glass tubes may be used with ordinary Erlenmeyer flasks, if a small loss in accuracy can be tolerated.

Electrometric titration apparatus. A Beckman industrial model pH meter fitted with extension glass and calomel electrodes was used for these experiments, but other similar apparatus should be equally applicable.

Procedure

The sample to be tested is thoroughly dried, usually for 2 hours at 100° to 110° C., and cooled in a desiccator. One-gram samples are weighed out and placed in 250-ml. Erlenmeyer flasks, and 40 ml. of reagent are added to each sample and also to other flasks to serve as blanks. Since this reagent is of approximately 1 *N* concentration, it must be measured very accurately. The flasks are then fitted with reflux condensers using ground-glass joints, and heated for about 24 hours at 75° to 80° C.

After acetylation the excess anhydride is decomposed and the ester precipitated in a soft fine form. In the case of cellulose acetate and other esters of moderate water resistance, this can be accomplished by carefully adding water in a fine stream with rapid shaking or stirring. Acetate butyrates of high butyryl content and similar esters of high water resistance must be precipitated by the following technique, which is generally applicable:

Approximately 5 ml. of distilled water are added through the condenser and mixed by swirling. Heating is continued for a few minutes to complete the decomposition of anhydride, and the flask is cooled, and removed from the condenser. The condenser is then rinsed down with 100 to 150 ml. of distilled water which is caught in a 600-ml. beaker. The reaction dope, thinned if necessary with pyridine, is poured slowly into the water with very vigorous stirring. This precipitate, as specified above, must be soft, fine, and easily penetrable. If it is lumpy or hard the determination must be repeated.

The solution in the beaker is then titrated with standard alkali to an end point preferably determined electrometrically. In the case of experiments reported below, both samples and blanks were titrated to a pH of 9.0 as determined by a Beckman pH meter. A slightly less sharp and accurate end point is obtained using phenolphthalein indicator.

Calculations

The result may be conveniently expressed as per cent of free hydroxyl (equivalent weight 17) as calculated by the following equation:

$$\frac{(\text{Ml. of NaOH for blank} - \text{ml. of NaOH for sample}) \times \text{NaOH normality} \times 1.7}{\text{sample weight}} = \%\ \text{free hydroxyl} \quad (1)$$

Figures 1 and 2 show the relationship between per cent of free hydroxyl and composition of cellulose acetates, propionates, butyrates, and acetate-propionate and acetate-butyrate mixed esters.

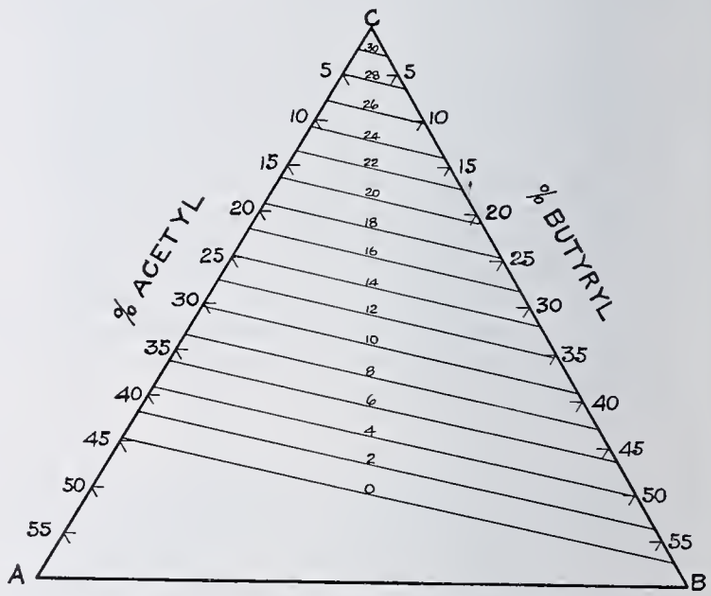

FIGURE 2. RELATIONSHIP BETWEEN PER CENT OF FREE HYDROXYL AND COMPOSITION OF CELLULOSE ACETATE BUTYRATES

It is often necessary to express free hydroxyl content in terms of the number of free groups per glucose unit of cellulose. This value can be read from Figure 3 or 4, in the case of a simple ester, by first reading the acyl content corresponding to the observed per cent of free hydroxyl on Figure 1 or 2. The desired value is then read at the same point on Figure 3 or 4, as the case may be—for example, using larger and more accurate charts of this type, 3.0 per cent of free hydroxyl is found to be equivalent to 40.5 per cent of acetyl in cellulose acetate, and this acetyl content corresponds to 0.47 free hydroxyl per glucose unit. This conversion can also be made by a simple calculation.

The case of mixed esters is more complicated, for in order to determine the number of free hydroxyls per glucose unit, at least two out of three of the following must be known: per cent of free hydroxyl, apparent acetyl (saponification value calculated as acetyl), acetyl content, and propionyl or butyryl content, as the case may be. Figures 1 and 3 may be used for acetate propionates, or Figures 2 and 4 for acetate buty-

rates, to obtain this value graphically, or calculations involving the following equations may be applied.

Let a = weight per cent of acetyl in sample
p = weight per cent of propionyl in sample
b = weight per cent of butyryl in sample
h = weight per cent of free hydroxyl in sample
N_a = number of acetyl groups per glucose unit
N_p = number of propionyl groups per glucose unit
N_b = number of butyryl groups per glucose unit
N_h = number of free hydroxyl groups per glucose unit

Case of Cellulose Acetate Propionates

$$\% \text{ apparent acetyl} = \frac{4300\,(N_a + N_p)}{162 + 42\,N_a + 56\,N_p} \quad (2)$$

$$a = \frac{4300\,N_a}{162 + 42\,N_a + 56\,N_p} \quad (3)$$

$$p = \frac{5700\,N_p}{162 + 42\,N_a + 56\,N_p} \quad (4)$$

$$h = \frac{1700\,(3 - N_a - N_p)}{162 + 42\,N_a + 56\,N_p} \text{ assuming only 3 replaceable hydroxyls per glucose unit} \quad (5)$$

Equations 3 and 4 solved as simultaneous equations (*5*) give:

$$N_a = \frac{162}{\frac{4300}{a} - 42.2\frac{p}{a} - 42} \quad (6)$$

$$N_p = \frac{162}{\frac{5700}{p} - 55.7\frac{a}{p} - 56} \quad (7)$$

$$N_h = 3 - N_a - N_p \text{ (by difference)} \quad (8)$$

$$N_h = \frac{h\,(162 + 42\,N_a + 56\,N_p)}{1700} \quad (9)$$

Case of Cellulose Acetate Butyrates

$$\% \text{ apparent acetyl} = \frac{4300\,(N_a + N_b)}{162 + 42\,N_a + 70N_b} \quad (10)$$

$$a = \frac{4300\,N_a}{162 + 42\,N_a + 70\,N_b} \quad (11)$$

$$b = \frac{7100\,N_b}{162 + 42\,N_a + 70\,N_b} \quad (12)$$

$$h = \frac{1700\,(3 - N_a - N_b)}{162 + 42\,N_a + 70\,N_b} \text{ assuming only 3 replaceable hydroxyls per glucose unit} \quad (13)$$

Equations 11 and 12 solved as simultaneous equations give:

$$N_a = \frac{162}{\frac{4300}{a} - 42.4\frac{b}{a} - 42} \quad (14)$$

$$N_b = \frac{162}{\frac{7100}{b} - 69.4\frac{a}{b} - 70} \quad (15)$$

$$N_h = 3 - N_a - N_b \text{ (by difference)} \quad (16)$$

$$N_h = \frac{h\,(162 + 42\,N_a + 70\,N_b)}{1700} \quad (17)$$

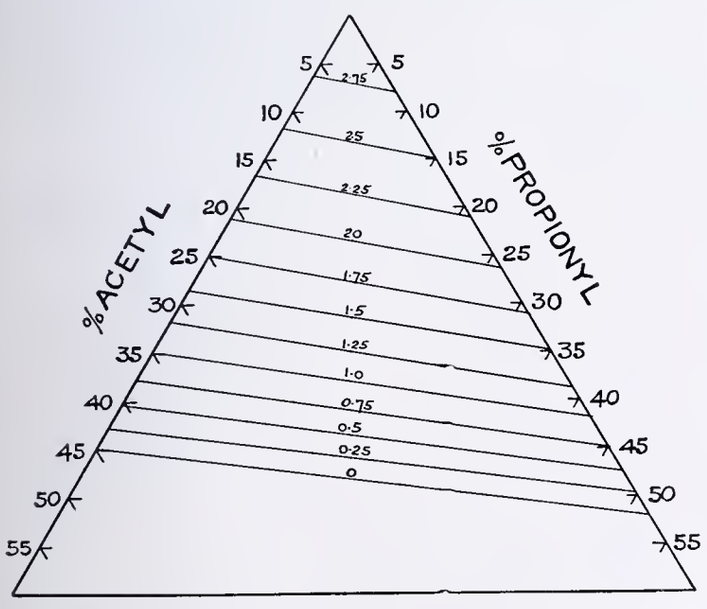

Figure 3. Relationship between Number of Free Hydroxyls per Glucose Unit and Composition of Cellulose Acetate Propionates

Free hydroxyl, apparent acetyl (*4*), and acetyl and propionyl or butyryl (*10*) are readily measured. If any two of these are known, the rest of the data can be found by calculation. The known data are substituted in the proper pair of the above equations, which are then solved for the unknowns. For example, if free hydroxyl and apparent acetyl are known for an acetate propionate, Equations 2 and 5 can be solved for N_a and N_p, and N_h obtained by Equation 8 or 9. If acetyl and butyryl are known for an acetate butyrate, Equations 14 and 15 give values for N_a and N_b, and h can be calculated by Equation 13 or N_h by Equation 16 or 17.

Table I. Effect of Time of Reaction on Free Hydroxyl Values

	Cellulose Acetate, Sample E	Cellulose Acetate, Sample C	Cellulose AP, Sample H	Cellulose AB, Sample T	Cellulose AB, Sample U
	%	%	%	%	%
	Analysis				
Acetyl	31.5	40.5	29.3	29.5	17.6
Propionyl	..	..	14.5	..	..
Butyryl	..	..	..	17.5	28.3
	Free Hydroxyl Calculated				
	9.36	3.03	2.07	1.13	3.54
	Free Hydroxyl Observed				
Time of Reaction at 75°–80° C. *Hours*					
6.5	8.76	2.90	1.93	1.12	3.45
18	9.18	2.98	1.89	1.16	3.47
24	9.23	..	1.94	..	3.55
27	9.15	2.99	1.98	1.17	3.41
36	9.14	2.99	1.98	1.06	3.32
48	9.17	3.04	1.93	1.14	3.42
74	8.98	2.96	1.73	1.18	3.41

Study of the Method

Effect of Time of Reaction. The acetylation of free hydroxyls in cellulose derivatives is not, of course, instantaneous, as shown by the following data. After long times of reaction, changes or side reactions sometimes occur which lower the values observed. It is necessary, therefore, to hold the reaction time within certain limits. In the experiments described in Table I the basic procedure, as given above, was followed, varying only the time of acetylation.

It is apparent from these data that a maximum value is reached in from 18 to 24 hours, and reaction time beyond about 48 hours is of no benefit.

Effect of Temperature. Table II gives similar data showing the effect of temperature. The reaction at room temperature is not complete after 48 hours and therefore is too slow to be practical. It is also rather slow at 40° C., particularly in the case of esters more difficult to esterify.

A temperature of 75° to 80° C. gave satisfactory results, while reactions carried out at the boiling point of the mixture gave very erratic and unsatisfactory data. Any convenient temperature between 40° and 80° C. might be used, provided that the time were adjusted to assure a complete reaction.

Effect of Anhydride Concentration and Excess. A cellulose acetate sample having 32.1 per cent of acetyl (1.24 free hydroxyls per glucose unit or 8.93 per cent hydroxyl) was acetylated at 75° to 80° C. for 20 to 24 hours using reagents of varying anhydride concentrations, as shown in Table III.

Table II. Effect of Temperature

(Analysis of sample as in Table 1)

Reaction Conditions		Cellulose Acetate, Sample E	Cellulose Acetate, Sample C	Cellulose AP, Sample H	Cellulose AB, Sample T	Cellulose AB, Sample U
Hours	° *C.*	%	%	%	%	%
		Free Hydroxyl Calculated				
		9.36	3.03	2.07	1.13	3.54
		Free Hydroxyl Observed				
48	25–30	7.28	2.40	1.99	1.00	..
24	40	8.39	2.72	1.98	1.09	3.31
48	40	8.89	3.02	2.13	1.15	3.26
24	75–80	9.23	2.99	1.94	1.17	3.55
48	75–80	9.17	3.04	1.93	1.14	3.42

The first column gives the approximate molar concentration of acetic anhydride, the second column shows the approximate excess of reagent furnished, while the third column shows this value corrected for the effect of about 0.07 per cent of water in the pyridine used for this experiment. It is apparent that small amounts of water consume appreciable amounts of reagent, and this factor should not be ignored. The results obtained are expressed both in per cent hydroxyl and in per cent esterification of the value calculated by difference from the acetyl content.

Table III. Effect of Anhydride Concentration

Anhydride Concentration	Approximate Reagent Excess Nominal	Actual	Hydroxyl	Esterification
M	%	%	%	%
0.14	0	−22	5.93	66.4
			5.92	66.3
0.27	100	75	8.26	92.5
			8.18	91.6
0.39	200	170	8.86, 9.02	99.2, 101.0
			8.73, 8.67	97.8, 97.1
0.52	300	270	8.84, 8.98	99.0, 100.6
			9.04, 8.98	101.6, 100.6
0.64	400	370	8.70	97.5
0.78	500	470	8.63	96.6
			8.51	95.3

The data show that 200 to 300 per cent excess gives good results. Concentrations of anhydride higher than about 0.5 molar not only give lower results but also involve very long titrations and are therefore less convenient and accurate.

Table IV shows the calculated reagent excess for different hydroxyl contents of a 1 gram sample using the 40 ml. of 0.5 molar reagent called for in the procedure. If the sample to be tested contains more than 9 or 10 per cent hydroxyl the sample size should be reduced, particularly if the water content of the pyridine is as high as 0.1 per cent.

Side Reaction. Dry pyridine when boiled with dry acetic anhydride was found by Wilson and Hughes (*24*) to form a resinous condensation product which is not decomposed by water. This reaction occurred to a greater extent in the blank than in the sample, so the blank titer was low and the result also was low. The presence of 0.3 to 0.5 per cent of water inhibits this reaction, but results in a considerable increase in titer. The difference between a blank held at room temperature and one at the acetylation temperature used is an indication of this undesirable side reaction. This difference has been found to be insignificant for reagents of 0.5 molar or lower concentration and for reaction temperatures of 80° C. or lower. It is of the order of 0.5 ml. of 0.5 *N* alkali for the 0.66 and 0.78 molar reagents shown in Table III, and offers at least a partial explanation for the low results obtained. The erratic results obtained in the experiments tried at the boiling temperature, using pyridine with about 0.1 per cent of water, were also undoubtedly due to this reaction.

Isolation of Cellulose Triacetate. A sample of hydrolyzed cellulose acetate, when analyzed for its free hydroxyl content, should be converted to the triester. In the first experiments to isolate such a product, however, the analysis showed low acetyl contents, even though the hydroxyl values were satisfactory. It was found that pyridine is held tenaciously by the precipitate, thereby lowering the actual sample weight, but reprecipitation from methylene dichloride–methanol, 90 to 10 by weight, into an excess of methanol followed by a boiling treatment with distilled water gave a product of satisfactory analysis. For example, 5 grams of cellulose acetate of 32.1 per cent acetyl were acetylated under the specified conditions, precipitated into water, washed, dried, reprecipitated from methylene dichloride–methanol into methanol, washed, boiled with distilled water, and dried. Analysis of the product gave values of 44.7, 44.8, 44.8, and 45.0 per cent acetyl compared with the theoretical value of 44.8 per cent for cellulose triacetate. This difficulty of removal of pyridine should be taken into account when hydroxyl is measured by the method of acetylation and analysis for the increase in ester content.

Application of the Method

Test of Accuracy. It has been difficult to establish the accuracy of cellulose ester analytical methods, because compounds of exactly known composition have not been available, nor can they be synthesized. When, however, the acyl content plus the hydroxyl content corresponds to three hydroxyls per glucose unit in medium or high viscosity cellulose esters, a useful indication of the combined accuracy of the two methods is obtained. Table V gives the results of analyses of representative samples of cellulose acetate, acetate propionate, and acetate butyrate. The results are given both in per cent and in terms of groups per glucose unit of cellulose. Comparisons are shown in both units between the observed hydroxyl contents and the hydroxyl contents calculated by difference from the acyl analyses.

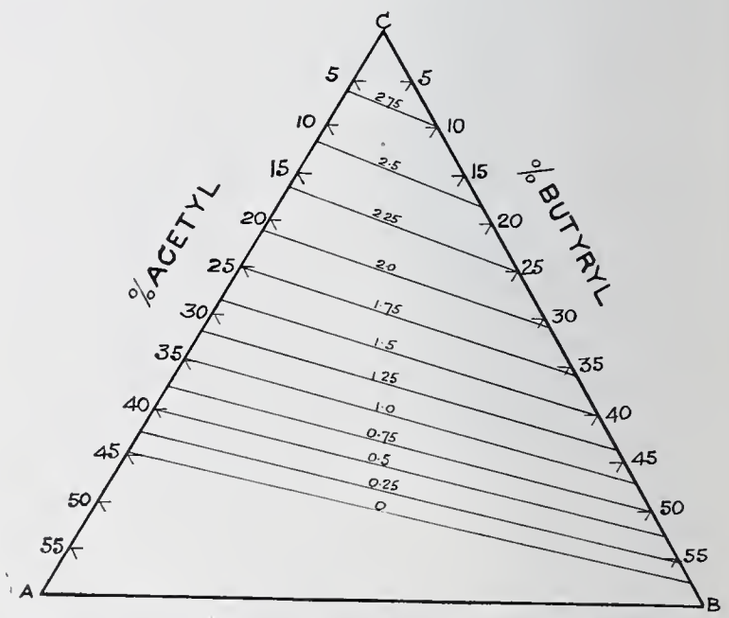

Figure 4. Relationship between Number of Free Hydroxyls per Glucose Unit and Composition of Cellulose Acetate Butyrates

Table IV. Excess Reagent for Different Hydroxyl Contents

Hydroxyl %	Approximate Reagent Excess %
1	3300
2	1600
3	1100
4	750
5	580
6	470
7	380
8	330
9	280
10	230

The maximum deviation from three hydroxyls measured per glucose unit in the twelve samples of normal viscosity was 0.018 group, which corresponds to an error of 0.1 per cent free hydroxyl or 0.25 per cent acyl as apparent acetyl. The average deviation for these samples was 0.006 group per glucose unit, which corresponds to an error of 0.03 per cent free hydroxyl or 0.08 per cent acyl as apparent acetyl. These values thus give an indication of the over-all accuracy attained.

Average Degree of Polymerization. When the average number of hydroxyls per glucose unit is greater than three by more than can be accounted for by experimental error, it is possible to calculate an average degree of polymerization or molecular weight based on the following assumptions. Cellulose is assumed to consist of chains of anhydro-glucose units each having three reactive hydroxyl groups, and the glucose units at each end of the chain have four such hydroxyls. Thus, chains of n units would contain $3n + 2$ hydroxyl groups, and the average number of hydroxyls per unit would be:

$$\text{Average OH} = \frac{3n + 2}{n} \tag{18}$$

This expression may be solved for n, the average degree of polymerization.

$$n = \frac{2}{\text{average} - 3} \tag{19}$$

The average number of hydroxyls can be calculated from analytical data using the equations given above, and typical values are given in Table V. Acetate butyrate sample W, for example, was made to have an extremely low viscosity, and analysis showed it to have an average of 3.111 hydroxyls per glucose unit. When this value is substituted in Equation 19 a value of 18 is obtained for n.

This method, like other end-group methods, measures a number-average degree of polymerization (*8*), which may be accurate for a sample having uniform chain lengths, but gives only an average value when the chain lengths vary considerably.

The degree of polymerization of acetate butyrate sample W was measured by the Staudinger method (*20*) for comparison. The relative viscosity of a solution of 0.1 gram of sample in 100 ml. of acetone was measured at 25.0° C. and the calculations were made as follows:

$$dp = \frac{\text{specific viscosity}}{K_m} = \frac{\text{relative viscosity} - 1}{K_m} = \frac{0.036}{15.8 \times 10^{-4}} = 23 \tag{20}$$

The agreement with the value obtained above, 18, is as good as would be expected, considering that viscosity measures a weight-average property of the material, and the K_m value was obtained by the osmotic pressure method (*22*) which measures a number-average property.

Molecular weight is the product of the degree of polymerization and the weight of the repeating unit. In the case of the sample cited it is thus

$$18\,(162 + 42 \times 0.926 + 70 \times 2.167) \text{ or about } 6340$$

Values for n corresponding to different average hydroxyl values are as follows:

Average Hydroxyls per Glucose Unit	n (or dp)
3.000	∞
3.005	400
3.010	200
3.020	100
3.050	40
3.100	20

It is apparent from these values that the extreme range of n ordinarily encountered is equivalent to a maximum deviation of about 0.1 hydroxyl group, so the method would be useful only for low values for n. This difficulty, however, is shared by many other similar methods.

Analysis of Multicomponent Cellulose Derivatives. It is occasionally convenient to analyze a cellulose derivative containing several substituents by determining one directly, measuring free hydroxyl, and calculating another by difference. For example, an acetylated cellulose ether might be analyzed by determining acetyl by saponification and free hydroxyl by this procedure, and calculating the alkoxyl group by difference. The composition of a mixed ester such as an acetate butyrate can be determined from its apparent acetyl

Table V. Analyses of Representative Cellulose Esters

Sample	Acetyl %		Groups per Glucose Unit of Cellulose: Acetylated hydroxyls	Free hydroxyls by difference	Observed hydroxyl groups	Observed total per C_6	Hydroxyl: Calculated by difference %	Hydroxyl: Observed %
			Cellulose Acetates					
A	32.1		1.761	1.239	1.243	3.004	8.93	8.96
B	38.3		2.304	0.696	0.691	2.995	4.57	4.58
C	40.5		2.525	0.475	0.472	2.997	3.03	2.99
D	42.1		2.690	0.310	0.307	2.997	1.91	1.90
			Cellulose Acetate Propionates					
		Propionyl %	Acetyls	Propionyls				
H	29.3	14.5	1.933	0.722	0.345	0.330	2.985	2.07	1.98
I	30.2	16.5	1.099	0.865	0.036	0.040	3.004	0.21	0.23
J	15.0	30.5	1.018	1.566	0.416	0.425	3.009	2.42	2.47
K	14.1	33.9	1.003	1.822	0.175	0.157	2.982	0.97	0.87
			Cellulose Acetate Butyrate					
		Butyryl %	Acetyls	Butyryls				
S	30.9	16.2	2.160	0.686	0.154	0.152	2.998	0.87	0.86
T	29.5	17.5	2.060	0.740	0.200	0.205	3.005	1.13	1.16
U	17.6	28.3	1.209	1.177	0.614	0.616	3.002	3.54	3.55
V	2.6	49.8	0.203	2.301	0.496	0.495	2.999	2.54	2.54
W	11.3	43.6	0.926	2.167	−0.093	0.018	3.111	..	0.09

content (saponification value calculated as acetyl) and free hydroxyl, using Equations 10 and 13 to obtain values for N_a and N_b and then substituting these in Equations 11 and 12. The values given in Table VI were calculated in this way from the analytical data given and are compared with analyses by the partition method (*10*). These data show that satisfactory agreement between the methods is attainable.

Correlation with Physical Properties. Free hydroxyl content of cellulose acetates, acetate propionates, and acetate butyrates in both units may be correlated with melting point, specific gravity, sorption of moisture, and solubilities in solvents or plasticizers by comparing Figures 1, 2, 3, and 4 with the triangular diagrams published by Malm, Fordyce, and Tanner (*9*).

Summary

The free or unsubstituted hydroxyl content of cellulose derivatives can be measured by acetylation with acetic anhydride in pyridine under controlled conditions.

The effects of time and temperature of reaction and of anhydride concentration and excess have been measured, and the procedure has been set up to utilize the optimum conditions.

The results obtained may be expressed as per cent hydroxyl, or if certain other analytical data are known the hydroxyl content may be expressed in terms of free hydroxyl groups per glucose unit of cellulose. Equations are given for making these calculations for cellulose acetates, acetate propionates, and acetate butyrates.

The method has been found useful in checking the accuracy of other analytical methods, correlating the hydroxyl content with various physical properties of cellulose esters, and analyzing multicomponent derivatives, one component of which may be calculated by difference. The deviation from three hydroxyls per glucose unit, as found by analysis, may be used to calculate an average degree of polymerization, in the case of very low viscosity cellulose derivatives.

Table VI. Mixed Ester Composition Using Free Hydroxyl Data

Sample	Observed Apparent acetyl[a]	Observed Free hydroxyl	Calculated Acetyl	Calculated Butyryl	Observed by Partition Method Acetyl	Observed by Partition Method Butyryl
	%	%	%	%	%	%
X	35.0	0.14	1.3	55.6	0.8	55.6
V	32.6	2.51	2.4	49.8	2.6	49.8
	32.5	2.57	2.3	49.8	2.5	49.9
Y	31.2	3.96	3.8	45.2	3.9	45.1
	31.2	3.99	3.8	45.2	4.0	45.0

[a] Apparent acetyl is total saponification value calculated as acetyl, equivalent weight 43 (*4*).

Acknowledgment

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Determination of Small Amounts of Combined Sulfur

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A method has been developed for the determination of very small amounts of sulfur in cellulose derivatives, based on oxidation with nitric acid and fusion with potassium nitrate.

Samples of cellulose acetate have been prepared containing both large amounts of salt sulfates and combined sulfate and the application of dilute hydrochloric acid rinses to these esters showed that salt sulfate is readily washed out, while combined sulfate is retained. It is thus possible to differentiate between combined and noncombined sulfate, since two short rinses of the finely ground cellulose acetate with 0.1 per cent hydrochloric acid have been found sufficient to remove the latter.

Hydrochloric acid rinses of a distilled water processed cellulose acetate reduced the sulfur content only when the sample was dried before acid rinsing, indicating that some of the combined sulfate was split off during the drying.

SULFURIC acid is widely used as a catalyst in the commercial production of cellulose esters and small amounts of sulfur remain in such esters after precipitation, washing, and stabilization. The amount of sulfur in a cellulose ester is of considerable importance because it exerts an effect on the stability of the ester towards heat and on the physical properties after aging.

Sulfur may be present in a cellulose ester as combined sulfate resulting from the reaction between sulfuric acid and the cellulose, and as noncombined sulfate such as sulfate salts from the wash water. Another source of sulfate in the latter

form may be the cleavage of originally combined sulfate. The determination of the sulfur content of a cellulose ester naturally gives the total sulfur content from both these sources. However, the amount chemically combined is really the significant value, since small amounts of sulfate salts from the wash water are not harmful to the stability of the cellulose ester. A method by which the amount of chemically combined sulfate may be determined is outlined below.

To carry out this work a reliable method for the determination of small amounts of sulfur in cellulose esters was necessary. The methods available from the literature (*1*, *3*) for the determination of sulfur were not satisfactory without modification because of the minute amounts involved. Upon increasing the size of sample, the main problem was the removal or destruction of the organic matter. Simple ashing resulted in considerable loss of sulfur.

The following method is a modification of the procedure described by Zahnd and Clarke (*5*) and by Klingstedt (*2*). The oxidation of sulfur to sulfate and the destruction of the organic matter are accomplished by digestion with concentrated nitric acid, followed by fusion of the residue with potassium nitrate. The nitrate is removed by evaporation with hydrochloric acid and the sulfur precipitated as barium sulfate in the usual way.

Determination of Sulfur

A sample of 13.7 grams of cellulose ester is placed in a 500-ml. round-bottomed flask with a standard-taper ground-glass joint. After adding 0.5 gram of potassium nitrate, a small crystal of Carborundum to prevent bumping, and 60 ml. of concentrated nitric acid, the flask is heated under a reflux condenser on a hot plate in the hood. The ester dissolves readily in the hot nitric acid and a copious quantity of nitrogen dioxide is liberated during the first few minutes of heating.

After refluxing overnight the spent nitric acid is distilled off over a Bunsen flame, care being taken to keep the flask constantly in motion and to interrupt the distillation when 2 or 3 ml. of liquid remain.

The oxidation is continued by further heating to reflux on the hot plate for 4 hours after adding 5 grams of potassium nitrate and 10 ml. of concentrated nitric acid. The solution is then carefully evaporated to dryness over a low flame. After all the liquid has been boiled out, a hotter flame is applied to fuse the potassium nitrate. At this point the residue should be light colored or have only local dark areas; if the residue begins to blacken the oxidation is incomplete and the fusion should not be attempted. In such a case, an additional 10 ml. of concentrated nitric acid should be added and the sample boiled another hour. As the molten potassium nitrate cools, the flask is kept in motion to distribute the crystals.

The major portion of the nitrate is displaced by evaporation to dryness with 20 ml. of concentrated hydrochloric acid. The residue of potassium chloride is heated strongly to drive out all the liquid, but not to the point of fusion.

After allowing to cool, the residue is dissolved in 100 ml. of distilled water and the solution filtered to remove any suspended matter. The volume of filtrate and washings should be about 200 to 250 ml. The solution is acidified with 0.5 ml. of concentrated hydrochloric acid and after heating on the steam bath, the sulfate is precipitated by adding 5 ml. of 5 per cent barium chloride solution. The sample is allowed to digest on the steam bath overnight and the barium sulfate filtered off on a porous-bottomed crucible. The percentage of sulfur in the sample is numerically equal to the weight of barium sulfate in grams, after correction for the blank.

Discussion of Method

Certain details in the procedure have been incorporated to eliminate the danger of explosions during the final stages of the evaporations with nitric acid. There is danger of explosion if the first evaporation is carried completely to dryness, especially if the flask is not kept in motion. The interruption of this evaporation at a volume of 2 or 3 ml. overcomes any danger of explosion at this point.

During the fusion of the potassium nitrate at the end of second evaporation an explosion may result if too much organic matter remains. If the residue is black at this stage further oxidation with nitric acid is necessary.

A portion of the potassium nitrate is added at the start to prevent loss of sulfate due to local overheating during the first evaporation. It is not all added at the start, to avoid the danger of too much potassium nitrate and organic matter towards the end of the first evaporation.

The length of time of the initial heating period may be shortened from overnight to 2 hours, but this leaves more organic matter and does not provide as great a margin of safety during the subsequent evaporations.

The amount of barium sulfate obtained from samples of cellulose acetate is usually from 10 to 30 mg.; so special care must be taken that none adheres to the beaker during filtration and washing. On this account it is advisable to use beakers free from scratches for the precipitations. If the solution is stirred during the addition of the barium chloride, care should be taken not to scratch the glass with the stirring rod. Because of this effect and because the amount of barium sulfate is so small that no visible precipitation occurs for several minutes, the procedure was adopted of adding the barium chloride solution rapidly and without stirring.

A blank must be run on the reagents used and values of 1 to 2 mg. of barium sulfate have been commonly obtained. To promote uniformity of the blank it has been found advantageous to prepare a composite of several bottles of nitric acid and also a stock solution of potassium nitrate in nitric acid (500 grams of potassium nitrate plus 1000 ml. of nitric acid). Approximately 12 ml. of this solution contain 5 grams of potassium nitrate and 10 ml. of nitric acid.

Experiments were carried out to determine whether the complete oxidation by fusion with potassium nitrate was necessary. Samples were diluted with water after the first evaporation to dryness and the sulfate was precipitated at this point. Other samples were precipitated without displacing the nitrate by chloride. In both instances slightly lower results were obtained. The precipitates filtered more slowly and were sometimes black on ignition.

An attempt was made to reduce the elapsed time by precipitation in the presence of picric acid (*4*), but this method does not seem to be applicable where such small amounts of precipitate are involved. Filtration of the barium sulfate after boiling for 10 minutes after adding the barium chloride gave only 40 per cent of the amount of precipitate obtained after digestion overnight. Filtration after digestion for one hour on the steam bath gave 75 per cent of the total sulfate.

The determination of sulfur has been applied to sufficient variety of cellulose derivatives to permit some generalizations regarding its applicability. Samples of cellulose acetate propionate and cellulose acetate butyrate are more difficult to decompose than cellulose acetate, but the conditions outlined are sufficiently severe to oxidize these materials.

The method is applicable to cellulose itself, but occasionally more drastic conditions must be imposed. If the initial evaporation cannot be carried to a volume of 2 to 3 ml. without the deposition of sludge, as much as possible of the nitric acid is boiled off and replaced by fresh nitric acid. The mixture is then boiled for an additional 2 hours and evaporated again. Cellulose ethers can be handled in the same manner as cellulose.

Cellulose esters containing a large amount of higher acyl, such as stearyl, resist complete oxidation by this method. Neither is the method applicable to aromatic sulfonic esters of cellulose.

Removal of Noncombined Sulfur

During the course of this work it was found that treatment with simple reagents under very mild conditions reduced the

Table I. Sulfur Content after Washing Treatments

Time *Hours*	0.1% HCl	0.05% NaOH	Boiling Distilled Water	0.1% Detergent
		Commercial Cellulose Acetate		
0	0.0141	0.0141	0.0141	0.0141
0.5	0.0075	0.0065	0.0089	0.0080
1	0.0080	0.0062	0.0073	0.0062
2	0.0082	0.0062	0.0075	0.0086
4	0.0077	0.0069	...	0.0077
7	0.0079	0.0072	0.0071	0.0074
		Cellulose Acetate High in Salt Sulfate		
0	0.150	0.150	0.150	0.150
0.5	0.0182	0.0207	0.0396	0.0268
1	0.0165	0.0206	0.0243	0.0280
2	0.0160	0.0199	0.0212	0.0323
4	0.0152	0.0182	0.0186	0.0284
7	0.0137	0.0153	0.0154	0.0301
		Cellulose Acetate High in Combined Sulfate		
0	0.392	0.392	0.392	0.392
0.5	0.356	0.350	0.170	0.324
1	0.349	0.344	0.119	0.322
2	0.353	0.350	0.087	0.329
4	0.356	0.366	0.077	0.314
7	0.371	0.360	0.073	0.298

sulfur content and that repeated treatments with the same reagent in many cases soon yielded limiting values for the sulfur content. When these treatments were applied to esters high in salt sulfate and combined sulfate, respectively, it was found that the former is readily removed whereas the latter is retained.

A sample of cellulose acetate high in salt sulfate was prepared by hydrolyzing a cellulose acetate esterification dope to acetone solubility. To each gallon of water used for precipitation and washing were added 30 ml. each of 15 per cent magnesium sulfate and calcium chloride solutions.

The cellulose acetate high in combined sulfate was prepared by esterifying acetone-soluble cellulose acetate with a limited amount of chlorosulfonic acid in pyridine solution. The amount of sulfate introduced depends on the amount of chlorosulfonic acid used and on the amount of water in the system. In one experiment 200 grams of cellulose acetate were dissolved in 1500 ml. of pyridine and a solution of 10 ml. of chlorosulfonic acid in 50 ml. of dioxane was added slowly with stirring. After dilution with acetone the product was precipitated and washed in distilled water and contained 0.392 per cent of sulfur.

The reagents selected for the washing treatments were 0.1 per cent hydrochloric acid, 0.05 per cent sodium hydroxide, boiling distilled water, and a 0.1 per cent detergent solution (diglycol oleate). The details of a typical washing series are as follows: A 250-gram portion of the cellulose ester was finely ground and stirred for half an hour at room temperature with 20 parts of 0.1 per cent hydrochloric acid. The ester was then pressed dry on a Büchner funnel and one fifth of the wet cake was taken as the first sample, given two distilled water rinses, and dried. The remainder was given further rinses in 20 parts of 0.1 per cent hydrochloric acid. In this way samples were obtained after 0.5, 1, 2, 4, and 7 hours. Fresh acid wash solution was supplied after 0.5 and 1 hour and at hourly intervals thereafter.

Table I summarizes the results obtained in the removal of sulfur by these rinses from a commercial cellulose acetate, a cellulose acetate high in salt sulfate, and a cellulose acetate high in combined sulfate.

All these treatments rapidly remove about half of the sulfur from the commercial cellulose acetate, but the dilute hydrochloric acid rinse most effectively reduces the sulfur from the cellulose acetate high in salt sulfate. Combined sulfate is not removed by the hydrochloric acid rinse. The cellulose acetate high in combined sulfate lost a small amount of sulfur during the first hydrochloric acid rinse, but this represents originally combined sulfate split off during the drying treatment, since washing the dried sample with distilled water alone caused a comparable reduction in sulfur content. Furthermore, repeated rinses with dilute hydrochloric acid without intermittent drying did not cause further reduction in sulfur content.

Determination of Combined Sulfur

On the basis of these results, a recommended procedure for the removal of noncombined sulfate from cellulose acetate is to give the finely ground sample two half-hour rinses with 20 parts of 0.1 per cent hydrochloric acid, followed by distilled water rinses to neutrality. The sulfur remaining is combined and is determined as described above.

However, in the case of more water-resistant cellulose esters such as cellulose acetate propionates and cellulose acetate butyrates which are almost fully esterified, a reprecipitation for dilute acetone solution into distilled water containing 0.1 per cent of hydrochloric acid was found to be a more efficient way of removing noncombined sulfate.

The reprecipitation of samples of cellulose acetate from dilute acetone solutions into distilled water containing 0.1 per cent of hydrochloric acid was found to reduce the sulfur content to the same level as the series of hydrochloric acid rinses. For instance, the commercial sample of cellulose acetate used in Table I contained 0.0078 per cent of sulfur after reprecipitation in this manner.

Rinses with dilute hydrochloric acid, applied to dried samples of cellulose acetate which had been precipitated and washed in distilled water, invariably caused reductions in the sulfur content.

A commercial grade of acetone-soluble cellulose acetate was precipitated from the reaction mixture and washed to neutrality in distilled water. Half of the product, without drying, was given repeated half-hour rinses in dilute hydrochloric acid. Samples were withdrawn after each rinse, washed to neutrality, and dried.

The remaining half was dried for 48 hours in a current of air at 70° C., then given a half-hour rinse in dilute hydrochloric acid, and washed to neutrality. This process was repeated several times to obtain a series of samples having had hydrochloric acid rinses with intermittent drying.

The data in Table II show that the hydrochloric acid rinses after drying removed sulfur from distilled water—processed cellulose acetate. When the rinses were applied without drying, no such reduction of sulfur content was observed.

Table II. Reduction in Sulfur Content of Cellulose Acetate by Hydrochloric Acid Rinses without and with Intermittent Drying

No. of HCl Rinses	Sulfur Content Without drying %	With drying %
0	0.0170	0.0170
1	0.0161	0.0108
2	0.0166	0.0083
3	0.0177	0.0063

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A Slide Rule for Carbonate Equilibria and Alkalinity in Water Supplies

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COINCIDENT with the application of mass action relations to the determination of carbonate in water supplies came the realization that the various ionic forms may be present in ordinarily unsuspected pH zones and more than two species of carbonate or alkalinity may coexist in the same water sample. Even from the standpoint of conventional stoichiometry the determination of carbon dioxide by titration is open to errors (*1*) and the phenolphthalein–methyl orange method has been shown to be imperfect (*4*). It is well known that hydroxide alkalinity by titration does not necessarily agree with the calculated value based on the pH of the water. True evaluation and differentiation of the various types of carbonate ions in solution are important wherever carbonate equilibria play a role, as in an accurate statement of ionic content, softening calculations, corrosion control, and boiler scale conditioning at low pressures.

Distribution of the various carbonate forms as dependent on pH value was first calculated from ionization constants and the principle of electroneutrality by McKinney (*2*), who formulated the fraction each form represented of the total carbonate determined by evolution or by titration through the pH interval from 8.0 to 4.5. By somewhat similar reasoning but adhering to the A. P. H. A. definition of total alkalinity, Langelier and DeMartini (*1*) developed equilibrium equations to give the molar concentration of each carbonate and alkalinity ion as a function of pH and total alkalinity.

Since most water plant laboratories employ the usual A. P. H. A. titration for total alkalinity rather than determine total carbonates by evolution, the latter set of equations is more applicable to treatment plant data and hence was selected for the basis of the slide rule described below. This total alkalinity equation assumes absence of salts of other weak acids; this assumption is usually valid except in the case of phosphate-treated boiler waters. Moore (*3*) modified Langelier and DeMartini's equations, substituting routine plant units instead of fundamental molal concentrations, and drew a series of graphs showing separately each form of carbonate and alkalinity ion plotted against total alkalinity. Deviation between phenolphthalein–methyl orange titration results and the calculated alkalinity pattern by means of equilibrium methods is especially marked at high pH values.

Moore's versions of Langelier and DeMartini's equations, with substituted ionization constant values for carbonic acid $K_1 = 4.54 \times 10^{-7}$, $K_2 = 5.61 \times 10^{-11}$, and for water, $K_w = 1 \times 10^{-14}$, at 25° C. are as follows:

$$\text{CO}_2\text{, as p. p. m. of CO}_2 = 9.70 \times 10^{10}\,(\text{H}^+)\,\frac{\left[\frac{Alk}{50{,}000} + (\text{H}^+) - \frac{10^{-14}}{(\text{H}^+)}\right]}{1 + \frac{11.22 \times 10^{-11}}{(\text{H}^+)}} \quad (1)$$

$$(\text{HCO}_3^-)\text{, as p. p. m. of CaCO}_3 = 50{,}000 \times \frac{\left[\frac{Alk}{50{,}000} + (\text{H}^+) - \frac{10^{-14}}{(\text{H}^+)}\right]}{1 + \frac{11.22 \times 10^{-11}}{(\text{H}^+)}} \quad (2)$$

$$(\text{CO}_3^{--})\text{, as p. p. m. of CaCO}_3 = \frac{5.61 \times 10^{-6}}{(\text{H}^+)} \times \frac{\left[\frac{Alk}{50{,}000} + (\text{H}^+) - \frac{10^{-14}}{(\text{H}^+)}\right]}{1 + \frac{11.22 \times 10^{-11}}{(\text{H}^+)}} \quad (3)$$

$$(\text{OH}^-)\text{, as p. p. m. of CaCO}_3 = \frac{5 \times 10^{-10}}{(\text{H}^+)} \quad (4)$$

Alk = total alkalinity, as p. p. m. of $CaCO_3$
(H^+) = hydrogen-ion concentration, moles per liter

In considering the effects of temperature and ionic strength on the validity of the above equations Moore noted that errors due to usual water temperatures lower than 25° C. and presence of other salts were compensatory in direction; he concluded that for the temperature range 15° to 25° C., pH below 10.0, and dissolved solids less than 500 p. p. m., all corrections might be neglected and the equations and his graphs used in the form given. Accordingly, the slide rule based on these equations will likewise be subject to the same limitations. For use beyond this range the underlying equations should be suitably corrected, as explained by Moore, for variations in the values of the ionization constants with temperature and dissolved salts.

In the first three equations the recurring function

$$\frac{\left[\frac{Alk}{50{,}000} + (\text{H}^+) - \frac{10^{-14}}{(\text{H}^+)}\right]}{1 + \frac{11.22 \times 10^{-11}}{(\text{H}^+)}}$$

represents the total alkalinity titer corrected for the ions of water and the second dissociation constant of carbonic acid; these corrections are most important at low alkalinities and at pH values differing from neutrality. For convenience let

$$\frac{\left[\frac{Alk}{50{,}000} + (\text{H}^+) - \frac{10^{-14}}{(\text{H}^+)}\right]}{1 + \frac{11.22 \times 10^{-11}}{(\text{H}^+)}} = F(Alk, \text{pH}) \quad (5)$$

Then Equations 1, 2, and 3 may be concisely rewritten:

$$\text{CO}_2\text{, as p. p. m. of CO}_2 = 9.70 \times 10^{10}\,(\text{H}^+) \times F\,(Alk, \text{pH}) \quad (6)$$

$$(\text{HCO}_3^-)\text{, as p. p. m. of CaCO}_3 = 50{,}000 \times F\,(Alk, \text{pH}) \quad (7)$$

$$(\text{CO}_3^{--})\text{, as p. p. m. of CaCO}_3 = \frac{5.61 \times 10^{-6}}{(\text{H}^+)} \times F\,(Alk, \text{pH}) \quad (8)$$

Since the individual carbonate concentrations involve only (H^+) and $F\,(Alk, \text{pH})$, this set of equations is well adapted for special slide rule design. The object of the slide rule described below is to provide a mechanical solution to Equations 4, 6, 7, and 8, so that the concentration of various carbonate and alkalinity species can be calculated expeditiously from the total alkalinity and pH value. In most cases a single setting of the rule suffices to define the carbonate and alkalinity distribution in a water sample.

Description

In the carbonate equilibria slide rule (Figure 1) the stock contains a logarithmic graph of $F\,(Alk, \text{pH})$ and a scale for bicarbonate alkalinity. Incidentally, all carbonate forms are read off from this particular scale. The slide consists of two pH scales, the upper for determining carbon dioxide and the lower for evaluating

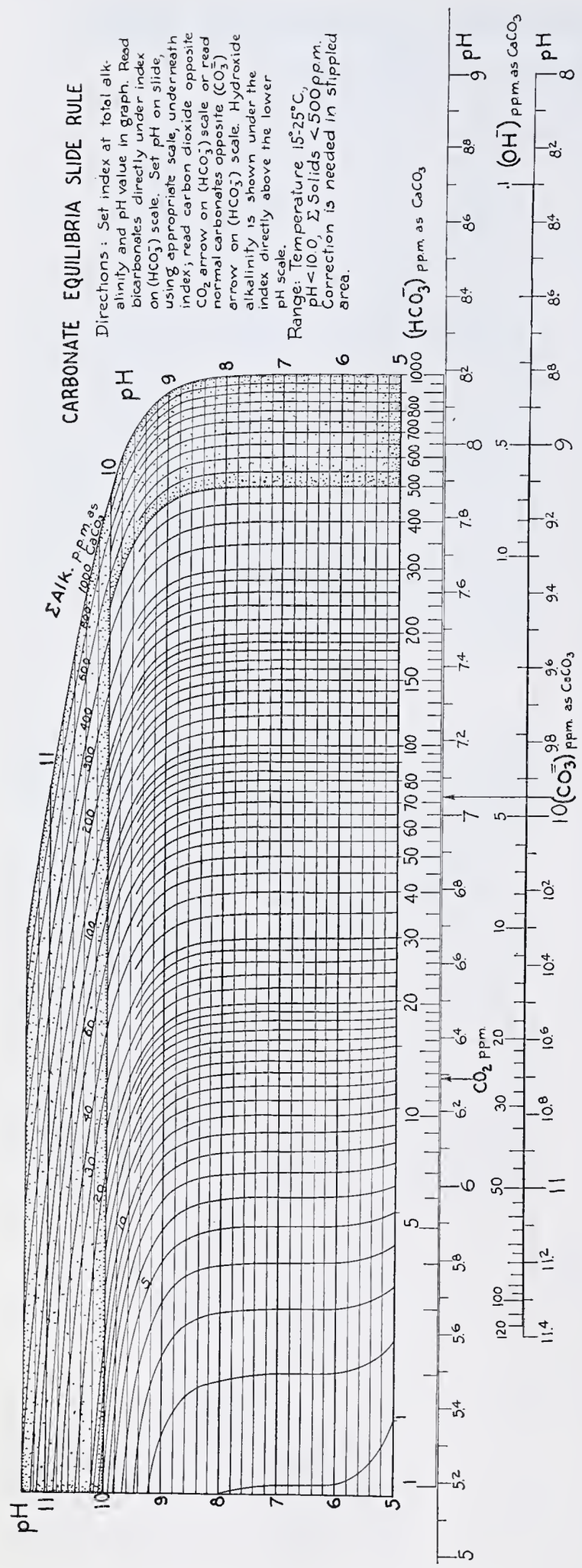
CARBONATE EQUILIBRIA SLIDE RULE
Directions: Set index at total alkalinity and pH value in graph. Read bicarbonates directly under index on (HCO_3^-) scale. Set pH on slide, using appropriate scale, underneath index; read carbon dioxide opposite CO_2 arrow on (HCO_3^-) scale or read normal carbonates opposite $(CO_3^=)$ arrow on (HCO_3^-) scale. Hydroxide alkalinity is shown under the index directly above the lower pH scale.
Range: Temperature 15°-25°C, pH<10.0, Σ Solids <5000 ppm. Correction is needed in stippled area.
Alk. p.p.m. as $CaCO_3$
pH
(HCO_3^-) ppm. as $CaCO_3$
(OH^-) ppm. as $CaCO_3$
CO_2 ppm.
$(CO_3^=)$ ppm. as $CaCO_3$

FIGURE 1. CARBONATE EQUILIBRIA SLIDE RULE

normal carbonate alkalinity. Along the lower pH scale is also drawn the scale for hydroxide alkalinity, as this latter quantity is dependent solely on the pH value.

In the *F* (*Alk*, pH) graph, the alkalinity parameters at very low alkalinities exhibit an ogive type of curvature; at total alkalinities above 50 p. p. m. as calcium carbonate the curvature at low pH due to hydrogen ions becomes masked by the comparatively larger alkalinity. This curl at low pH and low total alkalinity indicates that true bicarbonate alkalinity is somewhat greater than the apparent bicarbonate value by titration, as at high (H^+) a head start is already covered toward the end point. At high pH these curves sweep decidedly toward the left, since the value of *F* (*Alk*, pH) rapidly decreases with increasing hydroxyl concentration.

A convenient way to construct this rule for personal use is to photostat Figure 1 to desired size, cut out the scales, and mount them on stiff material like 6-mm. (0.25-inch) plywood. A guide strip is fastened to the top of the *F* (*Alk*, pH) plot along which a triangle, used as an index or hairline, can be moved in alignment as with a tee square.

Method of Use

BICARBONATE ALKALINITY. To find bicarbonate alkalinity, simply set the index at a point in the *F* (*Alk*, pH) plot corresponding to the given pH and total alkalinity; read the bicarbonate alkalinity, as p. p. m. of calcium carbonate, directly below on the (HCO_3^-) scale. Using Equation 7 the numerical value of *F* (*Alk*, pH) may be readily calculated by dividing the bicarbonate alkalinity by 50,000.

CARBON DIOXIDE. With the index still in position in the *F* (*Alk*, pH) plot, shift the slide so the given pH figure on the upper scale of the slide comes under the index. Opposite the CO_2 arrow is read the p. p. m. of carbon dioxide on the (HCO_3^-) scale.

NORMAL CARBONATE. In a manner analogous to the above, carbonate alkalinity is found with the index still fixed at the appropriate point in the *F* (*Alk*, pH) graph; in this case shift the slide so that the pH figure in the lower sliding scale falls under the hairline. Normal carbonate alkalinity, as p. p. m. of calcium carbonate, is read along the (HCO_3^-) scale opposite the $(CO_3^{=})$ arrow.

HYDROXIDE ALKALINITY. Simply note the hydroxide alkalinity, as p. p. m. of calcium carbonate, on the superposed scale above the prevailing pH on the $(CO_3^{=})$ scale.

Example 1. Given a well water with pH 6.6 and total alkalinity of 300 p. p. m., find carbon dioxide, bicarbonate alkalinity, and normal carbonate alkalinity. In the *F* (*Alk*, pH) plot set the index where the 300 p. p. m. total alkalinity line crosses the pH 6.6 ordinate. Under the hairline read (HCO_3^-) equals 300 p. p. m. as $CaCO_3$. Above 50 p. p. m. total alkalinity (H^+) as high as 10^{-5} molar is without effect on the value of *F* (*Alk*, pH); hence above this total alkalinity all alkalinity is in the bicarbonate form in slightly acid waters. Set the upper pH sliding scale so the pH value 6.6 comes under the hairline; read where the CO_2 arrow points on the (HCO_3^-) scale that carbon dioxide equals 147 p. p. m. of CO_2.

Example 2. Given a lime-treated water having a pH of 9.8 and total alkalinity equal to 40 p. p. m. of $CaCO_3$, find bicarbonate alkalinity, normal carbonate alkalinity, and hydroxide alkalinity. In the *F* (*Alk*, pH) graph set the index at pH 9.8 and total alkalinity 40; immediately beneath, on the (HCO_3^-) scale, read bicarbonate alkalinity

Table 1. Formulas for Ratios of Concentrations of Various Carbonate and Alkalinity Species

Bases	Numerators: CO_2, as p. p. m. of CO_2	Numerators: (HCO_3^-), as p. p. m. of $CaCO_3$	Numerators: (CO_3^{--}), as p. p. m. of $CaCO_3$
(H^+), moles per liter	$9.70 \times 10^{10} \times F\ (Alk, pH)$	$\frac{50{,}000}{(H^+)} \times F\ (Alk, pH)$	$\frac{5.61 \times 10^{-6}}{(H^+)^2} \times F\ (Alk, pH)$
CO_2, as p. p. m. of CO_2	.....	$\frac{5.16 \times 10^{-7}}{(H^+)}$ or $10^{pH-6.288}$	$\frac{5.78 \times 10^{-17}}{(H^+)^2}$ or $10^{2pH-16.237}$
(HCO_3^-), as p. p. m. of $CaCO_3$	$1.94 \times 10^6 (H^+)$ or $10^{6.288-pH}$	.....	$\frac{1.122 \times 10^{-10}}{(H^+)}$ or $10^{pH-9.95}$
(CO_3^{--}), as p. p. m. of $CaCO_3$	$1.728 \times 10^{16} (H^+)^2$ or $10^{16.237-2pH}$	$8.91 \times 10^9 (H^+)$ or $10^{9.95-pH}$	.....
(OH^-), as p. p. m. of $CaCO_3$	$1.94 \times 10^{20} (H^+)^2 \times F\ (Alk, pH)$	$1 \times 10^{14} (H^+) \times F\ (Alk, pH)$	$1.122 \times 10^4 \times F\ (Alk, pH)$

Alk = total alkalinity, as p. p. m. of $CaCO_3$.

$$F\ (Alk, pH) = \left[\frac{\frac{Alk}{50{,}000} + (H^+) - \frac{10^{-14}}{(H^+)}}{1 + \frac{11.22 \times 10^{-11}}{(H^+)}}\right]$$

equals 21.7 p. p. m. as $CaCO_3$. Move the slide so pH 9.8 on the (CO_3^{--}) scale falls under the index; opposite the (CO_3^{--}) arrow read, along the (HCO_3^-) scale, normal carbonate alkalinity equals 15.1 p. p. m. as $CaCO_3$. Directly under the hairline, reading along the (OH^-) scale, is found hydroxide alkalinity equals 3.2 p. p. m. as $CaCO_3$.

Usually pH values fall decidedly below 8.0 in the cases of streams and ground waters, so that there is generally no need to determine normal carbonates. Treated or adjusted waters, on the contrary, are often considerably above pH 8.0, so carbon dioxide is vanishingly small. Hence, a single setting of the index and the slide will ordinarily furnish all the necessary distribution data. A notable exception is in the case of waters in the pH range 8.0 to 9.0 and having a very high total alkalinity; here carbon dioxide and normal carbonate alkalinity may be present together in appreciable amounts and use of the rule requires two settings of the slide, one on each pH scale.

All scales may readily be used for determining various forms beyond the pH ranges provided on the slide merely by using a pH value one or two integral units above or below as needed to obtain a readable scale figure which is then multiplied or divided by 10 or 100 as the case may require. Thus pH 7.9 is slightly outside the range of the (CO_3^{--}) scale, but a pH 8.9 setting may be used if the indicated normal carbonate alkalinity is divided by 10. In this manner at pH 7.9 and total alkalinity of 1000 p. p. m., the normal carbonates are found to be 8.9 p. p. m. as calcium carbonate. A similar method may be employed for extending the pH range in calculating carbon dioxide.

Ratio and Partition of Species

Formulas for the ratio of concentrations of dual species, found from the set of Equations 4, 6, 7, and 8, are tabulated in Table I. Ratios between the concentrations of carbonate forms are dependent solely on the pH of the solution; ratios involving (H^+) and hydroxide alkalinity together with a carbonate form depend on total alkalinity, as $F\ (Alk, pH)$, as well as on pH. A semilogarithmic plot of some important concentration ratios against pH is given in Figure 2. Logarithms of ratios between carbonate species are linear with pH but the ratio $(OH^-)/(CO_3^{--})$ depends also on total alkalinity, and is given for three total alkalinity levels, 10, 50, and 250 p. p. m. of calcium carbonate, respectively. At a given pH value high alkalinities lower the ratio of hydroxide to normal carbonate alkalinities.

Particular pH values, at which any two species may be present in numerically equal amounts, may be found either directly from Figure 2 or by solving the equations in Table I set to unity. These special pH values are shown in Table II.

Table II. Special pH Values for Equality between Various Carbonate and Alkalinity Species

Equality	Σ *Alk*	pH
CO_2, as p. p. m. of CO_2 = (HCO_3^-), as p. p. m. of $CaCO_3$		6.288
CO_2, as p. p. m. of CO_2 = (CO_3^{--}), as p. p. m. of $CaCO_3$		8.119
CO_2, as p. p. m. of CO_2 = (OH^-), as p. p. m. of $CaCO_3$		
From Figure 2	10	8.29
	50	8.63
	250	8.97
(HCO_3^-), as p. p. m. of $CaCO_3$ = (CO_3^{--}), as p. p. m. of $CaCO_3$ = (OH^-), as p. p. m. of $CaCO_3$		9.95
From Figure 2	10	9.79
	50	10.31
	250	10.72
(CO_3^{--}), as p. p. m. of $CaCO_3$ = (OH^-), as p. p. m. of $CaCO_3$		
From Figure 2	10	9.71
	50	10.67
	250	11.36

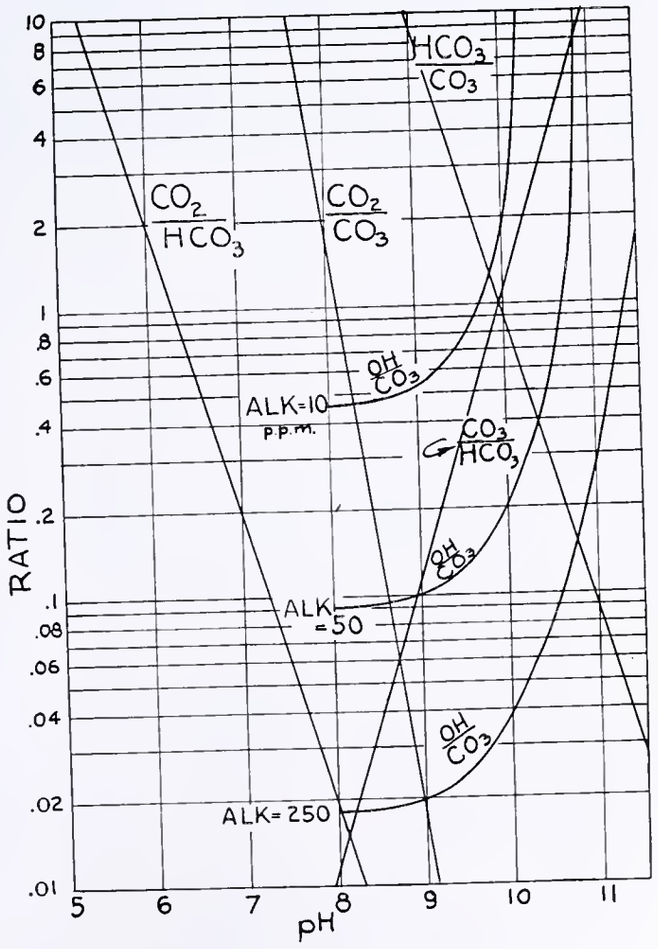

Figure 2. Ratios of Various Carbonate and Alkalinity Species

CO_2 as p. p. m. CO_2, all other forms as p. p. m. $CaCO_3$. Temperature 25° C.

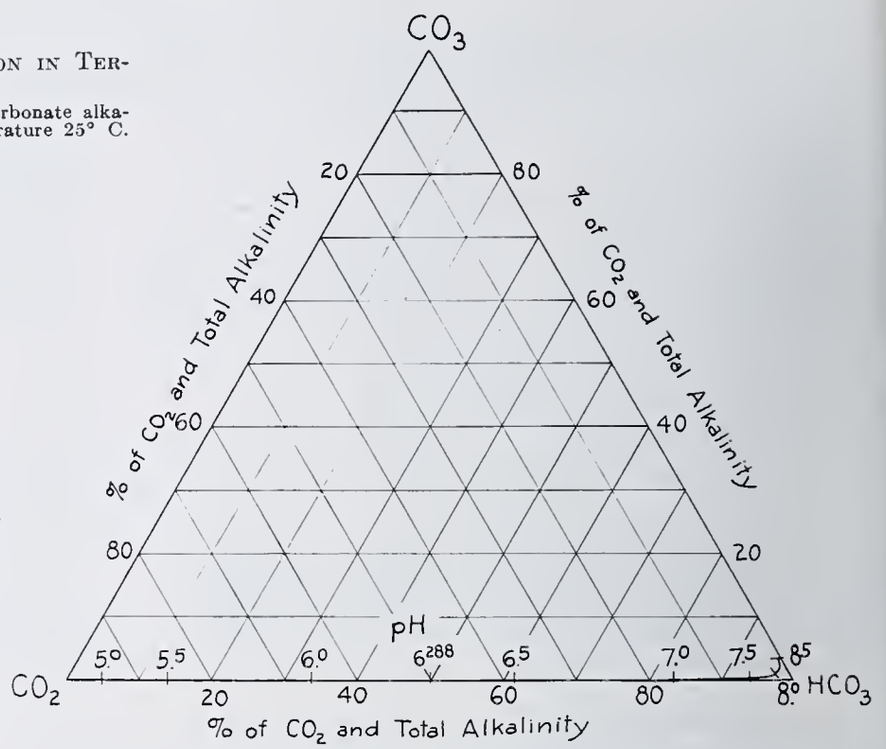

FIGURE 3 (*Right*). DISTRIBUTION IN TERNARY SYSTEM
Carbon dioxide, p. p. m. CO_2, and carbonate alkalinities, as p. p. m. $CaCO_3$. Temperature 25° C.

Such relations are useful in judging by inspection the accuracy of carbon dioxide and alkalinity figures in a water analysis. Thus at pH about 6.3 the reported carbon dioxide should equal the total alkalinity as calcium carbonate; below pH 6.3 the carbon dioxide will exceed the alkalinity; at pH near 7.3 the carbon dioxide should be one tenth the total alkalinity. Similarly, bicarbonate and normal carbonate alkalinities as calcium carbonate are equal at pH 9.95 and analogous relation again hold within this set of alkalinities; however, the usual phenolphthalein–methyl orange titration at this pH will show a bicarbonate content considerably smaller than the normal carbonates.

From a consideration of the underlying equilibria it has been evident since McKinney's (*2*) theoretical development that three alkalinity forms such as CO_2—(HCO_3^-)—(CO_3^{--}) or (HCO_3^-)—(CO_3^{--})—(OH^-) may be present simultaneously in the same water. Distribution of carbonates in the system CO_2—(HCO_3^-)—(CO_3^{--}) as affected by pH only is indicated in Figure 3. Normal carbonates do not appear in significant amounts below pH near 7.5. On the other hand partition within the system (HCO_3^-)—(CO_3^{--})—(OH^-) depends on total alkalinity as well as on pH. By using the special slide rule to determine concentrations of various species at total alkalinity levels of 10, 50, and 250 p. p. m. of calcium carbonate, data for the triangular plot (Figure 4) was obtained. The higher the total alkalinity the greater is the fraction of normal carbonate possible. Tie lines between points of equal pH converge at the (OH^-) vertex because a value for total alkalinity completely as hydroxide exists at any pH as defined in Equation 4. A similar diagram has been developed by the Public Health Service Committee for revision of drinking water standards to replace the present limit on caustic alkalinity (*5*).

As a matter of theoretical interest the possibility of having all four carbonate and alkalinity forms present simultaneously may be investigated with the use of either fundamental Equations 1, 2, 3, and 4 or the various ratios of Table I.

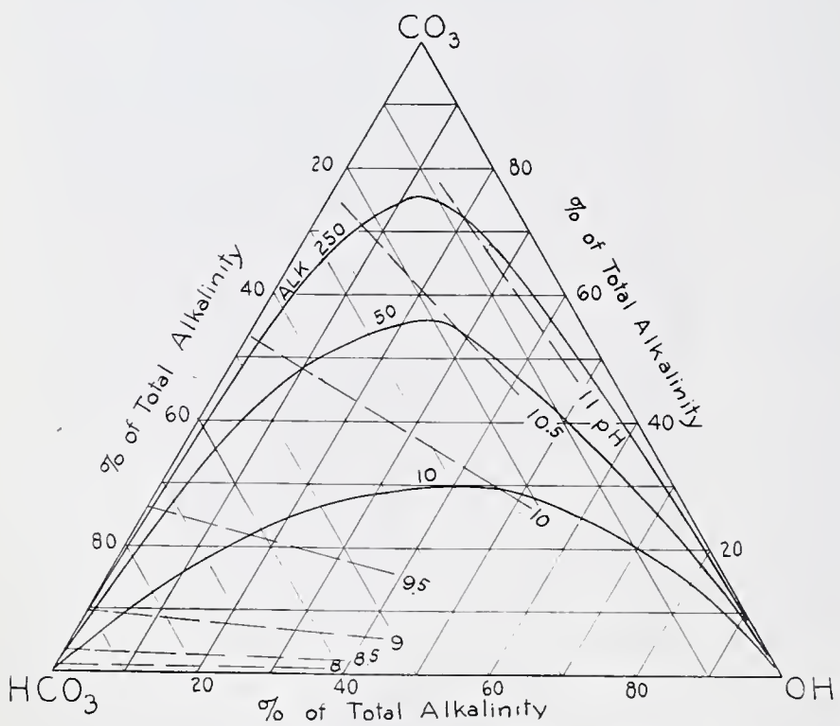

FIGURE 4. DISTRIBUTION IN TERNARY SYSTEM
HCO_3–CO_3–OH alkalinities as p. p. m. $CaCO_3$ at various total alkalinities. Temperature 25° C.

A triple point may be found where carbon dioxide, as p. p. m. of carbon dioxide, normal carbonates, and hydroxide alkalinities, as p. p. m. of calcium carbonate are numerically equal. By equating CO_2 equal to (CO_3^{--}), in Equations 1 and 3 the required pH is found; the total alkalinity is then calculated by equating 3 and 4, substituting the value of (H^+) just found. The calculated values are: pH 8.119 and total alkalinity 4.54 p. p. m. as calcium carbonate; the individual forms are each: $CO_2 = (CO_3^{--}) = (OH^-) = 0.06$ p. p. m. and $(HCO_3^-) = 4.43$ p. p. m. The first three species are below the limit of accurate detection. The same combination of values might also be found by trial and error manipulation of the special slide rule.

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Determination of Inorganic Salts in Crude Oils

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VARIOUS methods have been advocated for determining the salt contents of crude oils such as those described by the U. S. Bureau of Mines (*3*), Universal Oil Products Company (*5*), and Blair (*2*). Recently a cooperative program was initiated among the laboratories of this company to determine the most accurate and reproducible method concomitant with reasonable speed and ease of manipulation. This led to the development of a new method rather than to a recommendation of any of those previously used. This method has been in use in routine work, for over a year, with satisfactory results.

Practically all previous methods agree on one step at least, wherein the sample, diluted with a hydrocarbon solvent, is shaken by hand with boiling distilled water. This involves the obvious difficulties of handling hot glass apparatus, and development of high vapor pressures in a closed container. A second step common to all methods is the separation of the aqueous and hydrocarbon phases after extraction, though the means by which this is accomplished vary.

The tetraethyllead extraction apparatus (*1*) consists of a separatory funnel in which aqueous and hydrocarbon layers can be mixed intimately by boiling and refluxing. The application of this apparatus to the subject determination is readily apparent.

Recommended Procedure

APPARATUS. Hydrochloric acid reflux apparatus (A. S. T. M. D526-41T), 300-ml. Erlenmeyer flask, 50-ml. burets, and a 100-ml. graduate.

CHEMICALS (C. P.). *n*-Butyl alcohol, potassium chloride, potassium chromate, silver nitrate, and xylene (20° or better).

SOLUTIONS. Potassium chloride, 0.05 *N*, 3.7280 grams of pure, dry potassium chloride per liter.

Silver nitrate, 0.05 *N*, about 8.5 grams of silver nitrate per liter.

Chromate indicator, about 65 grams of potassium chromate dissolved in 100 ml. of distilled water, and sensitized by the addition of a few drops of the silver nitrate solution, followed by filtration.

STANDARDIZATION. The determinations involve the titration of about 50 ml. of sample in a 300-ml. Erlenmeyer flask. Consequently, the blank and the standardization of the silver nitrate solution against the standard potassium chloride solution, should be determined under the same conditions. Three drops of chromate indicator are used.

ANALYSIS. *Extraction.* The sample is shaken thoroughly, 50.0 ml. are measured into a graduate and poured into the extractor, and graduate and thistle tube are rinsed with xylene until about 100 ml. of xylene have been used. Fifty milliliters of distilled water are then introduced into the extractor, the heat is turned on, and refluxing is continued for 15 minutes. (The use of water already heated to boiling saves considerable time and minimizes bumping.) At the end of this time the heat is turned off, and the layers are allowed to separate. If the separation is slow, or an emulsion has formed at the interface, the addition of about 5 ml. of butyl alcohol and the judicious application of heat will cause a sharp separation.

The water extract is drained into a 300-ml. Erlenmeyer flask, and cooled to room temperature. Another 50-ml. portion of distilled water is added to the apparatus, and the extraction is repeated. Each 50-ml. extract is titrated separately. If a precision of 1.8 kg. (4 pounds) of sodium chloride per 1000 barrels is desired, a titration of 0.2 ml. of 0.05 *N* silver nitrate may be considered as indicating complete extraction. Generally, the second extractions will require only 0.2 ml. of silver nitrate.

Titration. Each 50-ml. extract should be tested with litmus paper. If alkaline, it should be made slightly acid by the dropwise addition of approximately 1.6 *N* nitric acid. If acid, it should be titrated with 0.2 *N* potassium hydroxide until neutral to litmus paper and the volume recorded. It is then made acid by the dropwise addition of the nitric acid, and the extract is titrated with the silver nitrate solution, using 3 drops of the chromate indicator. If sulfides or mercaptans are present, 5 ml. of 3 per cent hydrogen peroxide should be added, the solution boiled for 5 minutes and cooled, prior to neutralization.

Calculation of Results. The salt content of the crude is usually reported as pounds of sodium chloride per thousand barrels of crude. Correction must be made for any acid present. If desired, the extracts may be analyzed for calcium, magnesium, or other chlorides, and the salt content reported in other terms. In terms of sodium chloride, however,

$$\text{Salt content} = 410\ (V_A N_A - V_K N_K)$$

where V_A is total volume in milliliters of silver nitrate used, corrected for the blank
V_K is total volume in milliliters of potassium hydroxide used
N_A is the normality of the silver nitrate
N_K is the normality of the potassium hydroxide

Discussion

EMULSIFICATION. Butyl alcohol has broken all emulsions encountered to date. If this were not effective in some cases, it is expected that one of the Tretolites (*3*) could be used instead, being added at the same point as was the alcohol. If added before refluxing, the Tretolite so prevents emulsification that quantitative extraction of the chlorides is much more difficult.

BUMPING. With some samples, bumping may be serious. The addition of boiling water and regulation of heat input help to minimize this difficulty. One laboratory has installed an apparatus which is twice the size of the standard tetraethyllead apparatus, so that bumping, even when serious, does not result in loss of sample.

ACIDITY ADJUSTMENT. The Mohr titration of chlorides (*4*) must be conducted in a neutral solution. The Bureau of Mines method (*3*) uses phenolphthalein as the indicator for this adjustment. When the titration medium was distilled water, the ratio of the strength of the silver nitrate solution to that of the potassium chloride solution used was 0.999 to 1. If the acidity was adjusted with sodium carbonate and acetic acid to the phenolphthalein end point, the ratio was 0.964 to 1. The error caused by the use of this indicator is thus 3.5 per cent. Adjusting with carbonate and nitric acid to this end point gave a ratio of 0.999 to 1. However, if the salt of a weak acid were present, the use of nitric acid would give the same error as that obtained with acetic acid. When adjusted to the litmus end point, both acetic and nitric acids gave the ratio 0.999 to 1. If an acid indicator such as 2,4-dinitrophenol is used, the ratio drops to 0.944 to 1.

If the extract is far from neutral, it is convenient to adjust to the phenolphthalein end point first and then to the litmus end point. The second adjustment requires only a few drops of the nitric acid.

SULFIDES AND MERCAPTANS. The standard potassium chloride solution was saturated with hydrogen sulfide and titrated. A black precipitate formed, as would be expected. One milliliter of glacial acetic acid was added and the solution was boiled for 5 minutes, cooled, adjusted to litmus, and titrated. This procedure is recommended by the Bureau of Mines (*3*). No black precipitate formed, but the ratio obtained was 0.922, an error of 7.7 per cent.

Instead of the acetic acid, 5 ml. of hydrogen peroxide were added to the potassium chloride solution, boiled, cooled, neutralized, and titrated as before. The ratio obtained in this case was 0.999 to 1.

SOAPS. The Bureau of Mines method (*3*) recommends the addition of a few drops of 5 per cent zinc acetate solution to

Table I. Salt Content of Crude

(Pounds of NaCl per 1000 barrels of crude. O, own method. P, proposed method.)

Laboratory	A		B		C		D		E		F		G	
	O	P	O	P	O	P	O	P	O	P	O	P	O	P
1	74	77	73	76	54	59	65	67	118	114	77	79	73	76
2	70	72	65	67	54	53	60	62	109	110	79	77	70	73
3	68	70	74	74	48	53	53	63	116	109	64	78	78	72
4	73	79	72	76	58	51	49	67	107	117	62	76	68	74
5	..	67	..	65	..	51	..	63	...	109	..	..	..	68
Av.	71	73	71	72	54	53	57	64	113	112	71	78	72	73

coagulate the soap and prevent a pink coloration. When the soap was added to the standard potassium chloride solution, followed by the zinc acetate, and adjustment to the litmus end point, titration gave a ratio of 0.999 to 1.

Experimental

The adaption of the tetraethyllead extraction apparatus to the determination of the salt content of crude oil is obvious, and requires no detailed description. The balance of the work consisted of an exchange of samples between five laboratories of this company. Each laboratory was asked to supply samples of crude representative of the general run handled, choosing as troublesome samples as possible. Seven samples were circulated, and were analyzed both by the procedure then in use at each laboratory and by the proposed method. Table I gives the results. The proposed method was new to the operators. The reproducibility may be expected to improve with practice.

The time required for the proposed method is longer, for one sample, than that required for the other methods studied. The other methods, however, require constant activity on the part of the operator, while with the proposed method, the extraction is made without attention. With three sets of apparatus, one operator can analyze three samples every 45 minutes.

Duplicate determinations by one operator check to within 2 pounds of sodium chloride per thousand barrels of crude oil. The variations shown in Table I are considerably larger than this, but inexperience with the method and difficulties in sampling prevent an exact evaluation of the reproducibility.

Acknowledgment

The authors wish to thank the personnel of the other laboratories for their cooperation in the preparation and analysis of the crude oil samples.

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Colorimetric Determination of Vitamin C

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VITAMIN C as a moderately strong reducing agent is capable of producing colored reduction products from a number of the phospho- and silico- molybdates and tungstates. One of these reactions has been adapted by Brand and Kassell to a quantitative determination of the vitamin (*1*). Another, which offers certain advantages under suitable conditions, is the reaction between vitamin C and silicomolybdic acid produced by the reaction of ammonium molybdate and sodium silicate in acid solution. The reaction is sensitive, 0.01 mg. of the vitamin in 50 ml. of solution producing a discernible color; the reagent is stable and can be kept for long periods; and the blue reduction product is not reoxidized by exposure to air. In the procedure described below, using the photoelectric colorimeter, natural coloring matter in the yellow and red part of the spectrum does not interfere with the test.

The method described is suitable for determining the vitamin C content of fruits, vegetables, and other materials in which the content of other reducing substances is low—e. g., bananas and lemon juice. In other materials it gives results more nearly comparable to the iodine titration and therefore tends to run higher than the indophenol method. The molybdate reagent is easily reduced by ferrous and stannous ions and more slowly by cysteine, sulfites, and thiosulfates.

Preparation of Reagent. Two grams of ammonium molybdate are dissolved in 50 ml. of water at about 55° C., and 10 ml. of 1 per cent sodium silicate solution ($Na_2SiO_3.9H_2O$) freshly prepared are added, followed by 5 ml. of glacial acetic acid. The volume of the solution is then made up to 100 ml. After standing overnight the reagent is ready for use.

The reagent keeps indefinitely. After several weeks a white precipitate forms (probably molybdic acid) but the loss of this material does not interfere with the use of the reagent.

Procedure. An extract of the material to be examined is prepared by grinding a weighed quantity with 5 per cent acetic acid and sand in a mortar. After filtration and washing of the residue with fresh 5 per cent acetic acid, the filtrate is made up to a suitable volume. An extract of this type is stable for approximately 3 or 4 hours. If kept overnight, even in a refrigerator, loss of vitamin may run as high as 10 per cent. With some materials, such as bananas, extraction may be made directly with a measured quantity of diluted reagent and sand.

In making a determination, a suitable quantity of filtrate containing up to 1 mg. of vitamin C is placed in a 50-ml. volumetric flask with approximately 25 ml. of water; 5 ml. of reagent are added, followed by distilled water up to the graduation mark. One milliliter of reagent is sufficient in the range of 0.01 to 0.1 mg. The contents of the flask are thoroughly mixed and allowed to stand for 15 minutes before making a reading. If the solution is turbid it may be filtered. The 50-ml. volume was chosen for convenience in measuring reagents; any smaller volume, however, may be used with proportionate decreases of reagent and dilution water.

The depth of color may be estimated in two ways, using standards in Nessler tubes or a photoelectric colorimeter, the latter method being preferred. The Duboscq colorimeter is not suitable in this procedure because of the presence in the final product of varying amounts of excess of the yellow reagent.

For the preparation of standards in Nessler tubes, solutions of pure ascorbic acid may be used. The convenient range contains 0.01 to 1 mg. per 50-ml. final volume, in steps ranging from 0.01 or 0.02 to 0.2 mg. In this higher range the colors may be matched from the side. The fact that the final color results from the partial disappearance of yellow and the formation of a blue color accentuates the difference between the standards. During the course of 2 days after preparation, the standards tend to increase in color. If at the end of this time they are readjusted with diluted reagent, they remain unchanged for several weeks. Further storage results in a gradual loss of the blue color.

For more precise readings a photoelectric colorimeter can be used to great advantage. As in other methods with this type of instrument, a curve of instrument readings against concentration is plotted with known standards and subsequent readings obtained with unknowns are translated into concentration by reference to the curve. The most important problem in the use of the colorimeter is the choice of a proper light filter. The reagent, although yellow in color, transmits practically all of the red end of the spectrum. If, therefore, a red filter is used, the reagent itself gives no reading, while the blue compound, which absorbs red light, is effectively measured by the photoelectric cells. A number of filters, such as the Pyrex No. 241 pyrometer red glass and the Klett Summerson 66, have proved satisfactory. Use of red light for absorption has a number of advantages: (1) Certain turbidities such as result when fresh lemon juice is tested, and which cannot be removed by filtration, do not affect the readings, and (2), a variety of natural colors are in the yellow or red part of the spectrum and therefore have no appreciable effect on the absorption of the beam of light.

The method should prove useful for routine comparative tests in materials free from interfering substances or where such substances are present in quantities that can be ignored.

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The Distribution of Cut Fiber Lengths

In Wool Sampled by Cylindrical Tube Borings

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THE sampling of raw wool by the method described by Wollner and Tanner (*4*) results in the cutting of some fibers of the wool. The distribution of the various resulting lengths of cut fibers is of interest both to the laboratory testing the wool samples and to the manufacturer using the wool. The presence of considerable quantities of fiber of very short length in the sample may result in error, since these may be lost in the subsequent analysis. On the other hand, the presence of considerable quantities of short fiber in the bulk of the raw wool may introduce difficulties in certain manufacturing operations.

The distribution of cut fiber lengths by physical measurements of staple lengths can be determined by the usual equipment used for such purpose, provided, of course, that the precision of the method is sufficient to reveal the increase in short fibers resulting from sampling. This problem may also be subjected to mathematical analysis. The mathematical treatment presented herein attempts to determine the average distribution of cut fiber lengths when the fiber cutting incident to sampling is at a maximum. While this calculation of cut fiber lengths is made with particular reference to the sampling method described (*4*), it may also be useful in other sampling or cutting problems of similar nature.

The sampling method consists of drawing a cylindrical core of wool from a bale by means of a rotating sharp-edged circular tube. Figure 1 shows such a tube partly thrust into a bale, *ABDC* outlining the core which would be withdrawn. The fibers which are cut are those lying in the path of the cutting edge. Part of the cut fibers is in the core of wool in the sampling tube; the balance is left in the bale. Formulas for determining the quantities of cut fibers both inside and outside the tube, and tables of weights and percentages calculated from these formulas, are given below. The derivations of the equations are presented following the tables. The formulas deal only with the short fibers resulting from the sample cutting operation, and not with such quantities of short fibers as may already be present through double cutting in the original shearing of the wool.

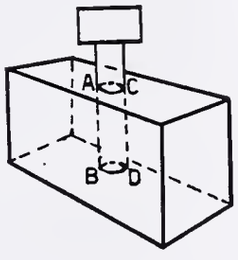

FIGURE 1

Equations 1 and 2 express the bulk volume of cut fibers of length k or less in terms of practical physical factors which can be determined easily. These factors are the average original fiber length, the sampling tube diameter, and the depth of penetration of the tube into the bale. These equations represent the average effect of maximum cutting.

Remaining in the bale (outside the sampling tube):

$$V = \frac{dpk^2}{f} \tag{1}$$

In the core sample (inside the sampling tube):

$$V = kp\sqrt{d^2 - k^2}\,(k/f - 1/2) + \frac{\pi d^2 p}{360}\,\text{arc sin}\,(k/d) \tag{2}$$

where V = volume of cut fibers of length k or less
k = any given cut fiber length
d = diameter of sampling tube
p = depth of penetration of tube into bale
f = average original fiber length
Arc sin (k/d) is expressed in degrees.

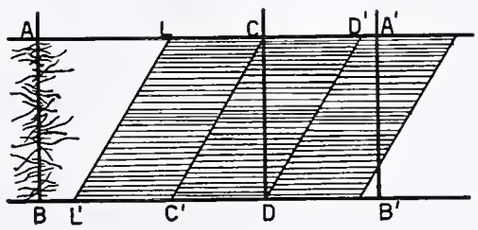

FIGURE 2

A formula for the weight of cut fibers of length k or less remaining in the bale is obtained by multiplying Equation 1 by the density of the bale, D.

$$\text{Weight} = \frac{dpk^2D}{f} \tag{3}$$

A formula for the percentage remaining in the bale is obtained by multiplying Equation 1 by $100/V_B$, where V_B is the volume of a bale:

$$\% = \frac{100dpk^2}{fV_B} \qquad (4)$$

Average densities and volumes of bales may be obtained from the data in the American Wool Handbook (*1*), or may be measured directly.

Equation 2 will express the percentage of cut fibers in the sample by multiplying by $400/\pi d^2 p$, where $\pi d^2 p/4$ is the volume of the core sample:

$$\% = \frac{400k\sqrt{k^2 - d^2}(k/f - 1/2)}{\pi d^2} + \frac{10}{9}\arcsin(k/d) \qquad (5)$$

It may be seen by inspection that the percentage of cut fibers in the sample, not being a function of p, will be constant for any depth of penetration of the sampling tube into the bale. It is also independent of the size or density of the bale.

Table I is computed from the formulas derived from Equation 1. Table II is computed from the percentage formula derived from Equation 2.

Derivation of Formulas

Arrangement of Fibers. A vertical section through the sampling tube will show the two parallel vertical lines along which fiber cutting occurs in this cross section during sampling and which are elements of the generated cylinder.

Consider such an element as line AB in Figure 2, which is a vertical section through the bale and tube shown in Figure 1, to the depth of penetration of the tube. AL is the top of the bale, BB' is the level of greatest depth of penetration of the sampling tube.

AB cuts all fibers in the path. The fibers lie in a multitude of random positions and angles with respect to AB. The number of fibers cut by AB is large and the point along the length of each fiber at which cutting occurs is purely random. By the laws of chance, then, every point along the original fiber length is equally likely to be cut, so that every possible size of segment is produced. Furthermore, an approximately equal number of fibers will be cut at any given point along the original fiber length as will be cut at any other point. Therefore, line AB will produce an equal number of all possible lengths of cut fiber.

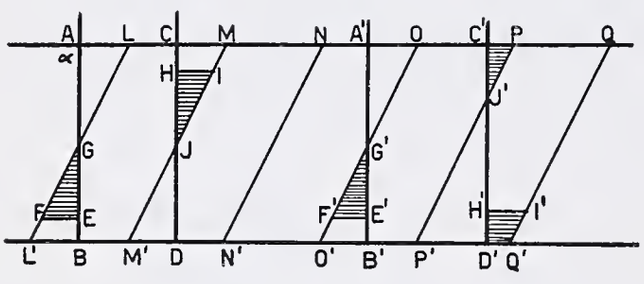

Figure 3

This condition may be reproduced for purposes of mathematical investigation by a suitable hypothetical rearrangement of the original fiber positions, as shown in Figure 2. (All original fiber lengths used herein are the average original fiber lengths. Calculations by means of the formulas, using normal variations in fiber length, *3*, show no significant differences from calculations using average fiber lengths.)

Let LC be the original fiber length, and let $L'C'$ also be the original fiber length, where C' is vertically below L. Similarly $CD' = C'D = LC$.

Now rearrange all fibers into parallel horizontal positions such that their ends fall along the diagonals LL', CC', DD', etc.

An equal number of all possible points along the original fiber lengths will now be cut by any vertical line of length AB—for example, line CD produces an equal number of all possible cut fiber lengths.

The essential conditions of the original problem are thus reproduced, for the maximum cutting effect, in a form which makes it possible to derive formulas for the distribution of cut fiber lengths.

Table I. Cumulative Distribution of Cut Fiber Lengths Remaining in Bale

(Wool cut to fiber length k or less by a single 10-inch boring with a 2-inch diameter tube; Australian type bale)

Cut Fiber Length k	Original Fiber Length f (Inches) 1.5	2.0	2.5	3.0	3.5	4.0	5.0	6.0
Inches	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lbs.*	*Lbs.*
0.25	0.008[a]	0.006	0.005	0.004	0.004	0.003	0.002	0.002
0.50	0.034	0.025	0.020	0.017	0.014	0.013	0.010	0.008
0.75	0.075	0.056	0.045	0.038	0.032	0.028	0.023	0.019
1.0	0.134	0.101	0.081	0.067	0.057	0.050	0.040	0.034
1.5	0.302	0.226	0.181	0.151	0.129	0.113	0.091	0.076
2.0	...	0.403	0.322	0.268	0.230	0.201	0.161	0.134
2.5	...	...	0.504	0.420	0.359	0.314	0.252	0.210
3.0	...	...	...	0.604	0.517	0.453	0.363	0.302
3.5	...	...	...	...	0.704	0.616	0.494	0.412
4.0	...	...	...	...	...	0.805	0.645	0.538
5.0	...	...	...	...	...	...	1.007	0.840
6.0	...	...	...	...	...	...	...	1.210
	%	%	%	%	%	%	%	%
0.25	0.002[b]	0.002	0.001	0.001	0.001	0.001	0.001	0.001
0.50	0.010	0.008	0.006	0.005	0.004	0.004	0.003	0.002
0.75	0.023	0.017	0.014	0.011	0.010	0.009	0.007	0.006
1.0	0.041	0.030	0.024	0.020	0.017	0.015	0.012	0.010
1.5	0.091	0.069	0.055	0.046	0.039	0.034	0.027	0.023
2.0	...	0.122	0.098	0.081	0.070	0.061	0.049	0.041
2.5	...	...	0.152	0.127	0.109	0.094	0.076	0.064
3.0	...	...	...	0.183	0.157	0.137	0.110	0.092
3.5	...	...	...	...	0.213	0.187	0.149	0.125
4.0	...	...	...	...	...	0.243	0.195	0.163
5.0	...	...	...	...	...	...	0.305	0.254
6.0	...	...	...	...	...	...	...	0.366

[a] To convert these weights to those applicable to Cape, New Zealand, or Scotch bales, multiply by 0.775; to B. A. or Monte type bales, multiply by 1.55.

[b] To convert these percentages to those applicable to Cape or New Zealand type bales, multiply by 0.734; for B. A. or Monte type bales, multiply by 0.506.

This chosen rearrangement will yield formulas which give the maximum effects of cutting because all the fibers lie parallel to each other and perpendicular to the path of the cutting edge. Rearrangement at other angles is also possible; the more general formulas derived therefrom would have the factor $\sin\alpha$, where α is the angle the fibers form with the path of the cutting edge (Figure 3). Theoretically, the average effect for parallel fibers of random angles to the path of the cutting edge positions would be to lower the maxima computed below by the factor 0.707 ($\sin 45°$). In practice, however, the method of sampling requires penetration of the bale in the direction of baling compression. This compression, being in one direction only, tends to force the fibers into positions perpendicular to the direction of compression, and the cutting effect tends to be the maximum rather than the theoretical average. Hence the rearrangement given above is chosen for deriving the formulas.

Several other factors tend to lower the quantities below the maxima given by the formulas and tables. The thickness of the fibers causes longer lengths to be produced when the fibers are sliced at angles less than 90°. However, this effect is negligible because of the very small fiber diameters. The crimp in the fibers affects the results in the core samples; this factor, too, tends to produce lower quantities than the maxima given. These factors are not taken into account in the derivations, which seek to evaluate the effect of maximum cutting.

Distribution of Cut Fiber Lengths Remaining in the Bale. Figure 3 represents a vertical section of a bale from the top down to the depth of penetration of the sampling tube,

in which all the fibers have been rearranged according to the method given above.

ABDC represents a section of a core sample, *A'B'D'C'* a second core sample in another position.

Let f = original fiber length = $LM = MN = \ldots = PQ = L'M' = \ldots = P'Q'$
p = depth of penetration of tube = $AB = CD = A'B' = C'D'$
k = any given cut fiber length, such as EF

The quantity of cut fibers of length k or less is the quantity to be determined. To do so, the bulk volume occupied by all these fibers must be found, in terms of f, p, and k.

In any vertical section through a bale and sampling tube parallel to the fibers, such as in Figure 3, the total profile area of fibers cut to length k or less by sampling tube *ABDC* and remaining in the bale is contained in the triangles *EFG* and *HIJ*, where $EF = HI = k$.

The area of ΔEFG is equal to the product of its base EF and half its altitude EG, or $k \times 1/2 \times pk/f$. Since $\Delta EFG = \Delta HIJ$, the total area is equal to pk^2/f. This area, a function of p, k, and f alone, holds for all parallel vertical sections through the sampling tube.

These parallel vertical sections extend through a distance equal to the diameter of the tube, d, as shown in Figure 4, which is a view from the top, where EF, $E'F'$, etc., are the edge views of parallel vertical sections. Since for any given p, k, and f there is a constant area, pk^2/f, in each possible parallel vertical section, then the total volume of cut fibers of length k or less is equal to the product of this area and the tube diameter, d. Thus, for each core sample taken, this volume is as given in Equation 1.

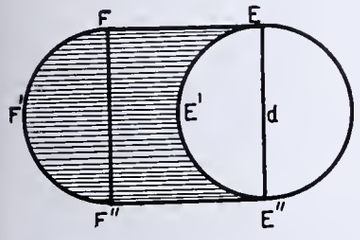

Figure 4

Distribution of Cut Fiber Lengths in the Core Sample. There remains for consideration the distribution of cut fiber lengths in the core of wool withdrawn from the bale by the sampling tube.

Figure 5 represents a vertical section of a bale of wool, similar to Figure 3. *ABDC* is a narrow sampling tube in section, or a section through a narrow part of a tube. *A'B'D'C'* is a section nearer to the axis of the tube, and therefore wider. *AC* and *A'C'* are parallel chords of two circles made by taking sections of the sampling tubes; the lengths of these chords will depend on the distance of the vertical plane section from the axis of the tube.

In each vertical section the areas of fibers cut to length k or less are triangles *EFG* and *HIJ*, provided chord *AC* is greater than k. Where chord *AC* is less than k, the entire vertical section of the core contains only fibers of length less than k. The total volume of cut fibers of length k or less is therefore composed of three parts: two solid segments, one at each side of the sampling tube, in which all the fibers are of length k or less, and the volume formed by the triangles in the vertical sections between the solid segments.

Table II. Cumulative Distribution of Cut Fiber Lengths within Core Samples

(Percentages of wool cut to fiber length k or less within core samples taken by 2-inch diameter tubes)

Cut Fiber Length k	Original Fiber Length f (Inches) 1.5	2.0	2.5	3.0	3.5	4.0
Inches	%	%	%	%	%	%
0.0625	0.178	0.136	0.111	0.091	0.082	0.073
0.125	0.684	0.518	0.418	0.352	0.304	0.269
0.25	2.73	2.07	1.68	1.41	1.22	1.08
0.50	11.0	8.4	6.8	5.8	5.1	4.5
0.75	24.5	18.9	15.6	13.4	11.8	10.6
1.0	42.5	33.3	27.8	24.2	21.5	19.6
1.5	....	69.7	60.3	54.0	49.5	46.1

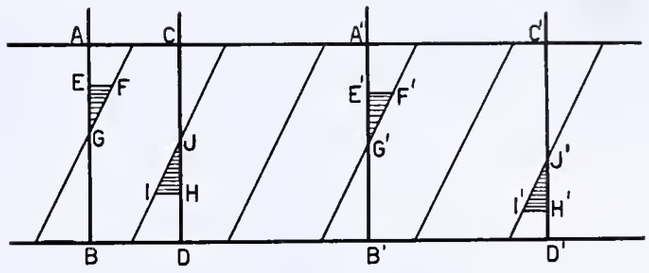

Figure 5. Diagram of Vertical Section in Bale of Wool

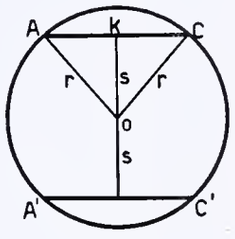

Figure 6

Figure 6 is a view of the core sample from above. Chords *AC* and *A'C'* are equal to the selected k. The area of each segment bounded by these chords is the area of sector *OAC* minus the area of triangle *OAC*. The volume of both solid segments is $2p$ times the area of a segment, or

$$\frac{4p\,\pi\,r^2 \arcsin (k/d)}{360} - \frac{pk}{2}\sqrt{d^2 - k^2}.$$

The volume formed by the triangles in all the vertical sections where the chord is greater than k is $2spk^2/f$, or $\frac{pk^2\sqrt{d^2 - k^2}}{f}$.

The total volume of cut fibers is the sum of these several parts (Equation 2), where arc sin (k/d) is expressed in degrees.

Conclusion

Examination of the tables shows that the increase of short fiber lengths in a bale of wool caused by the core-boring sampling method is negligible. Raw wool normally contains a quantity of short fibers due to natural variations in fiber length and to accidental double cutting in shearing; these are usually removed as noils. The maximum possible increase in quantity of noils due to sampling is indicated by Table I, k = 0.5 inch or k = 0.75 inch, depending on the mill machinery. For example, a good Australian combing wool of average fiber length 3.5 inches will suffer an increase in noils of 0.004 per cent in each bale from which a core is taken, in a mill whose machines can utilize fibers longer than 0.5 inch; whereas a mill whose machines do not utilize fibers shorter than 0.75 inch will have an increase in noils of 0.010 per cent for the same wool. There is no known testing technique by which such small increases could be discovered. The general average quantity of noils removed in combing is 9 per cent, ranging from 4 to 12 per cent (*2*).

The percentages of short fibers (produced by the sampling operation) in the cores, primarily because they are based on the volume of the core instead of the bale, are considerably greater, and are of the same order of magnitude as the percentage of short fibers originally present—for instance, in a sample of the type of wool mentioned above, 0.30 per cent of the fibers will be cut shorter than 0.125 inch. This information may be of value in the design of equipment for the processing of such wool.

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Quantitative Field Test for Estimation of Peroxidase

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BLANCHING is now regarded as an essential operation in the dehydration of many vegetables. One of its objects is to inactivate enzymes that might otherwise cause deterioration of the product. The degree of blanching to which a given product is subjected is ordinarily specified by stating the time and temperature of the heat treatment. It is difficult to describe the blanching effect accurately in these terms, however, because of the difference in types of blanchers used and variation in the heat required by vegetables from different sources.

Attempts have therefore been made to define the heating which a product has undergone by observing the extent to which certain easily measured enzymes have been destroyed thereby. Tests for oxidizing enzymes, such as the well-known tests with guaiacol and benzidine, have been tried as indicators of the proper degree of blanching, but the results have been rather unsatisfactory, probably because such tests were not sufficiently quantitative to permit a graded measurement of the heat effect. To be satisfactory, such a test must be not only quantitative, but applicable to vegetables possessing color, and so simple and rapid as to be useful in the field. The method presented here is thought to meet these requirements.

Table I. Peroxidase Destruction by Heat

(Water extract of cabbage)

Immersed in Boiling Water	To Reach End Point
Sec.	*Sec.*
0	12
15	11
30	13
60	90
60[a]	46
120	118
120[a]	57
180	115

[a] 20 hours after cooling.

In selecting an enzyme suitable for the test it is necessary to avoid certain enzymes, such as catalase, which are so unstable as to be completely inactivated by a little heat, as well as to avoid those seriously affected thereby. Peroxidase (*2*), which has a certain apparent thermostability (Table I), was selected for the test.

Bach and Chodat (*1*) found that peroxidase in a weak acetic acid medium catalyzed the oxidation of potassium iodide by hydrogen peroxide, releasing iodine. The iodine released in a given time was titrated with sodium thiosulfate solution. More recently Jayle (*3*) modified the method by adding a known amount of ascorbic acid to the system which also contained starch. The time necessary to free sufficient iodine in the buffered mixture to oxidize all the ascorbic acid was indicated by the sudden appearance of a blue-black starch-iodine color. This time is a measure of the peroxidase activity. Sodium thiosulfate, which is more stable and more easily obtained than ascorbic acid, may be used as the reducing substance. It is also more convenient to prepare a solution containing the potassium iodide, starch, sodium thiosulfate, and buffer mixture for use as the extraction medium. Only the peroxide needs to be added to such an extract, after which a record is made of the time required to form the blue-black color. In most cases, the strength of the iodide and the thiosulfate solutions may be adjusted, so that the end point can be observed in a conveniently short time.

When other constituents are kept constant, the speed of the reaction is determined by the reducing substances, both those present in the tissue and that which is added, and by the quantity of peroxidase. Ascorbic acid is the most important of the reducing substances present in the tissues of vegetables. A decrease in the quantity of ascorbic acid in vegetables caused by blanching or dehydration would decrease the reaction time. A relatively large quantity of thiosulfate must therefore be used, so that changes in the amount of ascorbic acid do not introduce large errors. In the few cases where this is impracticable, the decrease in naturally occurring reducing substances may be found by iodine titration, or the reaction time may be found without the addition of thiosulfate and a correction thus made for the natural reducing substances.

A representative sample of the tissues weighing 25 grams is reduced to a fine state of subdivision in the Waring Blendor, using 140 ml. of buffer mixture as the extraction medium. The liquid is filtered through four thicknesses of cheesecloth. The cloth is squeezed practically dry by hand after the main bulk of the liquid flows through rapidly. Sufficient buffer mixture is added to the filtrate to make 200 ml. To a 50-ml. aliquot of the well-mixed extract, measured in a graduated cylinder, is added 1 ml. of 0.9 per cent hydrogen peroxide solution (prepared by diluting a pure 30 per cent solution containing no stabilizer) from a quick-flowing blow-out type of graduated pipet. The stop watch is started when the pipet is half empty. An ordinary watch or clock with second hand may be used instead of the stop watch. The time required for the appearance of the blue-black color, or the first appearance of change in color, is recorded as the reaction time. The reciprocal of this time is a measure of the enzyme activity.

The buffer solution used in these investigations consisted of a mixture of 20 ml. of 2 per cent soluble starch, 10 ml. of 0.1 *N* sodium thiosulfate, 4.5 grams of potassium iodide, and sufficient 0.2 *N* acetate buffer (pH 4.7) to make 1 liter. This mixture will keep for 24 hours.

In Table II are given some representative results by this method. Two-gram samples of dried, blanched cabbage and mustard greens and 3-gram samples of dried, blanched beets and carrots, and 25-gram samples of the same blanched vegetables undried were used. All the extracts were colored, the beet extract more than the others, but in none was the end point indefinite; in fact, the quick "flash" of the end point is very striking. However, the method is capable of modification to give clear extracts if ever found necessary.

Table II. Application of Field Test for Peroxidase Destruction in Vegetables

Vegetable	Blanched	To Reach End Point			
		Before drying[a]		After drying[a]	
	Min.	*Sec.*	*Sec.*	*Sec.*	*Sec.*
Purple cabbage	0	4.0	4.5	6.5	7.5
	1	29	25	62	97
	2	68	72	145	173
	5	480	655	290	300
Mustard greens	0	7.0	7.5	18	20
	0.5	209	255	285	306
	1	330	382	335	352
	4	550	655	452	453
Beets	0	33	48	67	62
	10	215	215	255	250
	20	265	315	295	322
Carrots	0	225	230	223	217
	1	230		222	235
	6	180	180	216	251

Duplicate samples run.

The data in Table II show that the destruction of peroxidase is progressive and changes over a wide range with the blanching. The extent of blanching may therefore be easily defined, even when the blanching has been greatly overdone, as was the case with cabbage heated 5 minutes.

It is sometimes observed that the reaction time for dehydrated material is less than that for the freshly blanched material. This may be due to loss of ascorbic acid or to regeneration of the enzyme. Table I gives evidence of such regeneration in cabbage extract.

This test is not supposed to fix the proper amount of blanching, but only to show when that amount has been obtained. The proper amount of heating naturally varies with each kind of vegetable, but when it has once been determined there should be no difficulty in specifying this amount in terms of the peroxidase value, and in using the peroxidase value as a means of factory control thereafter.

Summary

The method described appears to meet the requirements for a simple, swift test for the blanching of vegetables, even if extracts of them are highly colored. It is particularly applicable to control of the blanching time of cabbage, which has been a problem in the dehydration field. No doubt the test may be used in other fields, such as blanching before quick-freezing. As a result of this work a new method for the determination of ascorbic acid is suggested, the details of which will be presented in another paper.

Acknowledgment

The aid of A. K. Balls, of the Enzyme Research Laboratory of this bureau, is gratefully acknowledged.

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Agricultural Chemical Research Division Contribution No. 87.

Preparation of Diphenylthiocarbazide and Diphenylthiocarbazone (Dithizone)

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ALTHOUGH in recent years diphenylthiocarbazide and especially diphenylthiocarbazone (*2*, *5*) have become important organic reagents for the analysis of certain metals, the only directions for the preparation of these compounds in the literature are essentially those of Fischer (*3*, *4*, *6*), who prepared them for the first time. Fischer's method is limited to the preparation of very small quantities in low yields.

The following method is based on Fischer's procedure, but with modifications that permit a substantial quantity of product to be made at one time. The reactions involved are:

$$2\ C_6H_5NHNH_2 + CS_2 \longrightarrow C_6H_5NHNHCSNH_3NHC_6H_5 \quad \text{(I)}$$

$$(C_6H_5NHNH)_2CS + H_2S \quad \text{(II)}$$

$$2\ (C_6H_5NHNH)_2CS \xrightarrow{NaOH} C_6H_5N{=}NCNHNHC_6H_5 + C_5H_5NHNHCNH_2 + C_6H_5NH_2 \quad \text{(III)}$$

The condensation of phenylhydrazine and carbon disulfide yields I, the phenylhydrazine salt of β-phenyldithiocarbazic acid, which on heating forms diphenylthiocarbazide (II). This substance in the presence of alcoholic sodium hydroxide undergoes mutual oxidation-reduction, yielding diphenylthiocarbazone (III).

Experimental

Fifty-four grams (50 cc., 0.5 mole) of freshly distilled phenylhydrazine and 200 cc. of benzene are mixed in a 500-cc. three-necked round-bottomed flask fitted with an efficient Nichrome wire stirrer (*7*), reflux condenser, and dropping funnel. From the funnel 20 grams (16 cc., 0.27 mole) of carbon disulfide are added with vigorous stirring in the course of 15 minutes. The mixture of the salt (I) and benzene is then heated on the steam bath with stirring at gentle reflux either under the hood or with the condenser connected to a gas trap (*8*) until the evolution of hydrogen sulfide practically ceases (about 3.5 hours). Near the end of this heating period the evolved gas should be tested frequently with moist lead acetate paper. The reaction may be considered complete when the test paper does not turn dark until exposed for several seconds. (Overheating is indicated by the formation of a very dark color and the rapid evolution of ammonia must be avoided. Ammonia is slowly evolved during heating and this side reaction reduces the yield somewhat.) At this point the benzene layer is usually green in color and there is a heavy crystalline precipitate. The mixture is thoroughly cooled at 0° to 10° C. and suction-filtered, and the solid is washed with two 15-cc. portions of benzene. The yield of crude diphenylthiocarbazide is 48 to 50 grams, 80 to 83 per cent of the theoretical.

If diphenylthiocarbazone is the desired product, the crude diphenylthiocarbazide may be used without further purification. However, if the diphenylthiocarbazide is to be used as a reagent, it must be crystallized from 900 cc. of 95 per cent ethanol. As the alcoholic solution decomposes slowly at its boiling point, it should be boiled no longer than necessary to dissolve practically all of the solid. The solution is filtered hot, thoroughly cooled at 0° to 10°, and suction-filtered, and the fine needlelike crystals of diphenylthiocarbazide are washed with three 20-cc. portions of benzene. The yield of almost colorless product is 28 to 30 grams, 47 to 50 per cent of the theoretical; melting point about 145–150° C. with decomposition. No additional product of suitable purity can be obtained from the filtrate from this crystallization.

To prepare diphenylthiocarbazone the crude diphenylthiocarbazide is dissolved in a solution of 200 cc. of methanol and 80 cc. of 25 per cent sodium hydroxide. [In the mutual oxidation-reduction of diphenylcarbazide to diphenylcarbazone Slotta and Jacobi, (*9*), obtained improved yields by adding a small amount of dilute hydrogen peroxide to the alcoholic alkaline solution. In the case of diphenylthiocarbazone added hydrogen peroxide has no effect.] This solution is refluxed on the steam bath for 5 minutes (longer heating will materially reduce the yield), poured into 1 liter of water, and filtered. The filtrate is acidified with dilute sulfuric acid (1 volume of concentrated acid to 4 volumes of water); approximately 120 cc. of acid are required. The black precipitate of crude diphenylthiocarbazone is removed by suction filtration and then dissolved in an ammonia solution made from 100 cc. of concentrated ammonium hydroxide and 250 cc. of water. This dark red solution of the ammonium

salt of diphenylthiocarbazone is allowed to stand in a stoppered flask overnight. (If the solution is not allowed to stand 8 to 10 hours, the diphenylthiocarbazone will not be pure.) This solution is filtered, diluted with 500 cc. of water, and then acidified with dilute sulfuric acid (same concentration as before). The blue-black precipitate of diphenylthiocarbazone is removed by suction filtration, washed with warm water until the filtrate is substantially free of sulfate ions, and dried in a vacuum desiccator over concentrated sulfuric acid. The yield is about 15 grams, 5 per cent of the theoretical based on the phenylhydrazine taken.

On standing, diphenylthiocarbazone undergoes some oxidative deterioration which may interfere with its use in analysis. A satisfactory purification method is described by the Association of Official Agricultural Chemists (*1*).

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Automatic Leveling Bulb Lifter for Butadiene Analysis

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IN THE gasometric determination of butadiene in gas mixtures by the method of Tropsch and Mattox (*2*), the sample is passed back and forth through a U-tube maleic anhydride absorber, from a gas buret into a gas reservoir. The routine analysis of a large number of samples is tedious and time-consuming, unless an automatic device for raising and lowering the leveling bulb is used. A semiautomatic hydraulic lift has been suggested (*1*).

The automatic device consists of a wheel mounted on a support, designed to translate circular motion into linear simple harmonic motion. The driving power is supplied by a 1725 r. p. m. motor, which is geared and belted down to a speed of 0.92 r. p. m. If mercury is used as confining fluid, its weight must be supported by a steel chain, which is fastened to a ring, that is free to rotate on a pin, near the rim of the wheel and run over a pulley, and attached to the top of the leveling bulb by a brace. As the wheel revolves, the bulb is raised to its highest position when the pin is at the bottom of the wheel, and to its lowest position when the pin is at the top of the wheel.

The diameter of the wheel is equal to the lift, which is the linear distance the leveling bulb travels to move the unabsorbed gases out of the buret. For a 100-cc. gas buret and a 50-cc. sample containing approximately 15 cc. of butadiene, a wheel with a diameter of 45 cm. (18 inches) is used. To prevent drawing mercury into the absorber, an iron ring is placed under the leveling bulb to act as a safety stop. The above sample requires the ring to be set at the 35-cc. buret graduation.

Samples up to 30 per cent butadiene require only filling the buret with a gas sample, recording initial volume, starting the device, and recording final volume after 20 minutes. Samples rich in butadiene can be handled by dilution of a 50-cc. sample with air to leave a 35-cc. residue. Samples absorbing more than anticipated can best be handled by raising the iron ring during the run to shorten the lift. No harm results from those absorbing less, except the minor disadvantage of the fact that all the gas is not circulated, and only a few more automatic cycles are required.

One device may run two or more burets, by using a longer pin, with parallel chains. For compactness, two burets are placed at opposite ends of a small table, and the wheel is set between them at right angles to each, so as to drive both simultaneously, the intervening space being used for outlets and sampling bottles.

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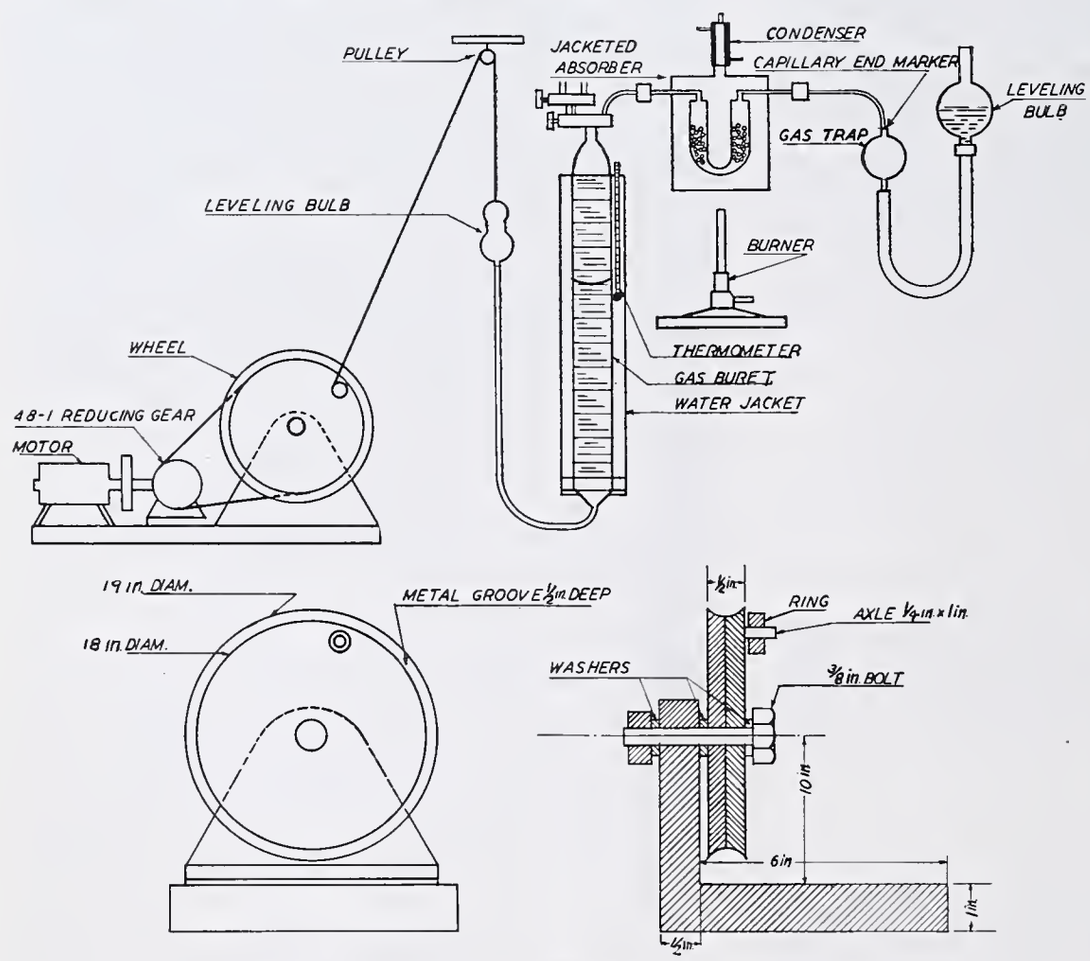

FIGURE 1. APPARATUS FOR DETERMINATION OF BUTADIENE

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A Dichromate–Ferrous Sulfate Method for Antimony

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IN ESTIMATING iron by oxidation with potassium dichromate in hydrochloric acid solution, it is necessary to remove antimony. This suggested the possibility of a useful method for the determination of this metal.

Accordingly, experiments were made on several materials containing known amounts of antimony in combination with copper, iron, tin, lead, zinc, manganese, arsenic, etc., and two general methods of analysis were devised.

Suggested Procedure

FOR TIN-BASE ALLOYS. Treat a 0.5- to 1.0-gram sample in a 300-cc. Erlenmeyer flask with 10 cc. of sulfuric acid (specific gravity 1.84), heat on a hot plate or over a flame until solution or decomposition is complete and cool. Add 40 cc. of water and boil a minute to remove sulfur dioxide. (If arsenic is present it may be removed by volatilization at the point where sulfur dioxide is boiled out by adding 50 cc. of hydrochloric acid, no water, and boiling gently to 20 to 30 cc.) Add 20 cc. of hydrochloric acid (specific gravity 1.18) and when solution is clear dilute to 150 cc. with cold water (cool if necessary to 40° C., as too hot solutions impair the indicator), and titrate as follows:

From a buret add standard dichromate solution with thorough mixing until the orange color of an excess can be seen through the green of the chromous chloride. Now add 2 to 3 drops of diphenylamine sulfonic acid indicator and 5 cc. of phosphoric acid (H_3PO_4, 85 per cent), and back-titrate with ferroussulfate solution, using 0.2 to 0.3 cc. in excess, and finish the titration with dichromate. The dichromate equivalent of the ferrous solution used is best determined at this point by adding to the solution just titrated the number of cubic centimeters used in the determination and titrating this with dichromate. This volume of dichromate is subtracted from the original titration. The indicator color is violet when oxidized, green when reduced.

The dichromate solution may be of any desired strength. Solutions 1 cc. = 0.001 gram of antimony and 1 cc. = 0.005 gram are convenient. It is standardized against pure antimony metal treated the same as the sample.

Ferrous ammonium sulfate solution, 1 cc. = 1 cc. of dichromate solution for convenience.

TABLE II. RESULTS OF OUTLINED PROCEDURES ON VARIOUS TYPES OF ANTIMONIAL MATERIAL

Material	Element Present: Sb %	Pb %	Cu %	As %	Sn %	Fe %	Ni-Co %	Zn %	S %	Sb Found %
Sb-Pb metal	2.70	97.2	0.06	..	..	..	..	...	..	2.70
	..	..	..	..	..	..	..	...	..	2.72
	..	..	..	..	..	..	..	...	..	2.70
Sb-Pb metal	0.95	98.9	..	..	..	..	..	...	..	0.93
	..	..	..	..	..	..	..	...	..	0.95
	..	..	..	..	..	..	..	...	..	0.96
Bearing metal	4.84[a]	0.14	2.45	0.05	92.2	..	..	...	..	4.85
	..	..	..	..	..	..	..	...	..	4.85
	..	..	..	..	..	..	..	...	..	4.90
Bearing metal	12.98[a]	0.10	3.00	0.05	82.8	..	..	...	..	12.95
	..	..	..	..	..	..	..	...	..	13.05
	..	..	..	..	..	..	..	...	..	13.0
Bearing metal	14.27[a]	17.8	1.70	0.05	66.0	..	..	..	..	14.25
	..	..	..	..	..	..	..	...	..	14.2
	..	..	..	..	..	..	..	..	..	14.2
Bearing metal	8.88[a]	90.6	0.10	0.04	..	..	..	...	..	8.9
	..	..	..	..	..	..	..	...	..	8.95
	..	..	..	..	..	..	..	...	..	8.95
Matte	1.53	19.6	17.7	0.77	..	29.0	3.8; .9	9.1	17.5	1.54
	..	..	..	..	..	..	..	...	..	1.52
	..	..	..	..	..	..	..	...	..	1.54

[a] Analysis of bearing metals shown is average of results obtained in laboratories of five different companies.

Indicator, 1 gram of barium diphenylamine sulfonate dissolved in 100 cc. of concentrated sulfuric acid. Make fresh about every 3 months.

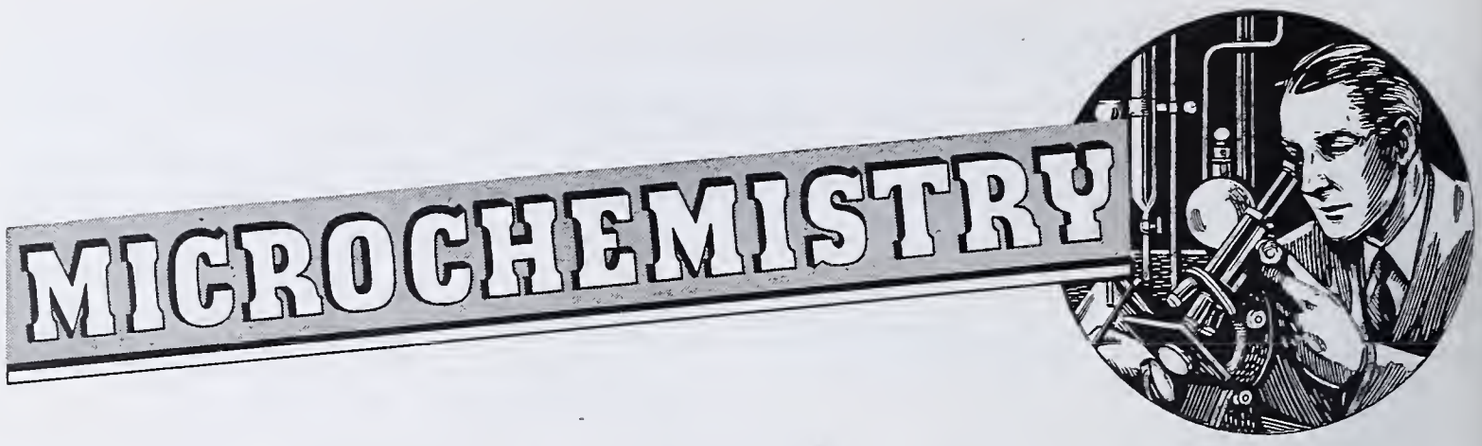
FOR LEAD-BASE ALLOYS, MATTES, AND CONCENTRATES. Many antimony-lead alloys are difficult to decompose with sulfuric acid to produce a white lead sulfate. Dissolve a 0.5- to 1-gram sample in a 300-cc. Erlenmeyer flask with 15 cc. of dilute nitric acid (1 to 1) and 0.5 gram of tartaric acid on a warm plate. When solution is complete, cool, add 10 cc. of concentrated sulfuric acid and 10 grams of potassium sulfate, and take to a melt, starting on the hot plate where the charred tartaric acid may be partially burned off, if it rises too high in the flask, by adding nitric acid dropwise. When sulfur trioxide fumes appear, transfer the flask to a ring stand over a free flame and boil until the charred mass has become a light brown. Now lay the flask on its side and finish boiling out the excess sulfuric acid (catching the drip in a beaker). Cool the melt (add 50 cc. of hydrochloric acid and boil down to 20 cc. if arsenic is present), add 25 cc. of water, 20 cc. of hydrochloric acid, and 10 grams of ammonium chloride, and warm until solution is complete. Dilute with cold water to 40° C. or less and titrate as above.

For the decomposition of mattes and concentrates, use concentrated nitric acid and add about 0.2 gram of tartaric acid or a bit of filter paper with the sulfuric acid. With this type of material (high iron) the phosphoric acid is added before the dichromate in order to decolorize the yellow ferric ions.

TABLE I. ANALYSIS OF EXPERIMENTAL SYNTHETIC SOLUTIONS

(Containing elements commonly associated with antimony, using $FeSO_4$–$K_2Cr_2O_7$)

No. of Determinations	Element Present: Sb *Gram*	Pb *Gram*	Cu *Gram*	As *Gram*	Sn *Gram*	Fe *Gram*	Ni *Gram*	Zn *Gram*	Found Sb *Gram*
3	0.150	...	...	...	0.850	...	..	...	0.149
	...	...	...	....	...	...	..	...	0.150
	...	...	...	...	...	...	..	...	0.150
3	0.150	0.200	...	...	0.650	...	..	...	0.149
	...	...	...	...	...	...	..	...	0.149
	...	...	...	...	...	...	..	...	0.151
3	0.150	0.850	...	...	...	...	..	...	0.150
	...	...	...	...	...	...	..	...	0.149
	...	...	...	...	...	...	..	...	0.149
3	0.0250	0.200	0.200	0.010	...	...	..	...	0.0247
	...	...	...	...	...	...	..	...	0.0250
	...	...	...	...	...	...	..	...	0.0250
3	0.0250	0.700	...	...	...	0.200	0.04	0.100	0.0251
	...	...	...	...	...	...	..	...	0.0252
	...	...	...	...	...	...	..	...	0.0250
3	0.0150	0.200	0.200	0.010	...	0.200	..	0.100	0.0152
	...	...	...	...	...	...	..	...	0.0150
	...	...	...	...	...	...	..	...	0.0150



A Review of Organic Microchemistry

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DURING the past thirty years a great part of the research in the different branches of chemistry has dealt with a study of the role of traces of elements and compounds. To isolate, identify, and determine these small quantities has required the development of a new technique called microchemistry. Microchemistry may be broadly defined as that branch which deals with the apparatus and technique of working with small quantities of substances, usually less than a gram. For analysis, the weight of constituent determined is usually 1 to 10 mg., but it may be only a fraction of a milligram.

Organic microchemistry, with which this paper deals, has had a spectacular development, particularly in the field of biochemistry where working with minute quantities is often a necessity. As is so often true with new advances in science, we have the overenthusiastic microchemist who is apt to lose his perspective, and the older chemist who believes the new methods are not practical. This new branch of chemistry has fortunately matured to the point where the pristine glamor and magical trappings which it early acquired have largely disappeared. This has been due in large part to a careful study of the basically important factors by critical chemists in both academic and applied fields. The resulting improvements in apparatus and operating technique have made the methods far more practical than was at first thought possible.

One purpose of this review is to emphasize these later developments and wider applications. While the analysis of organic material is a large and important branch, some attention will be given to those branches which are not strictly analytical. The microanalyst, because of his special skill and knowledge in dealing with small quantities, is often called upon for methods of isolation, purification, and synthesis.

The obvious advantages of saving time and reagents in decomposing and working with small samples have been pointed out repeatedly. In those macromethods where it is necessary to digest or burn a large sample requiring several hours, the time can, in many cases, be reduced to a few minutes when small samples are used. This applies also to filtering large volumes of solutions.

It is not the purpose of this review to give a complete bibliography of the topics discussed. Such data are readily available and are indicated in the text. There does seem to be a need, however, to summarize the general conclusions of the many published papers, so that modifications of methods can be made which will incorporate those improvements established by several independent workers. The mass of published material on the common determinations, such as those in elementary organic analysis, is becoming so great that it is increasingly difficult to separate the important from the less essential matter, much of which is optional, depending on personal preference.

Organic microchemistry has shown important developments in the design and standardization of apparatus; the use of automatic combustion apparatus in routine analysis, which permits full advantage to be taken of the savings in time which are possible with micromethods; successful extension of the methods to the analysis of microgram quantities; application of physical methods to the analysis of small quantities; the design of apparatus for synthesis on a micro scale; and the adaptation of micromethods to systematic organic qualitative analysis.

There are many factors to be considered when an analytical procedure is critically evaluated. These range from the personal prejudices and skill of the analyst to inherent defects in the method which can only be partially controlled, and largely determine its accuracy and precision. In some cases microprocedures will exaggerate these limitations. An analyst who finds macroprocedures difficult will certainly not possess the qualifications required in microtechniques. Likewise, some factors in precipitation, which are not apparent when dealing with 20 to 50 mg. of a constituent, may cause the method to break down when applied to a milligram or less, which is the usual micro range.

It must be constantly borne in mind that all analytical procedures fail to give satisfactory results with certain types of material. Here, judgment is required and the method is not necessarily condemned. Published methods usually apply to a very limited range of materials which are of interest to the particular analyst, although the author often gives the impression in his enthusiastic account that it has unlimited application. In the hands of another, perhaps less skilled worker, or when applied to other materials, the method may yield poor results. This limited study of new procedures naturally leads to much controversy which can best be settled by detailed study and experience in several laboratories. For many micromethods we have, fortunately, a background of published experience over a period of years. In addition, most of the commonly used micromethods are adaptations of standard macroprocedures. The adaptations manifest themselves mostly as equipment designed to weigh and handle small quantities.

The difficulty of obtaining reagent chemicals of the required purity for microprocedures is no longer a problem. In this country the necessary standards have been worked out through the cooperation of the AMERICAN CHEMICAL SOCIETY Committee on Reagents and the Division of Analytical and

Micro Chemistry. Much needed standard specifications on microapparatus are being drawn up by an American Chemical Society committee, in active cooperation with American manufacturers of scientific apparatus. These specifications, as they are finally approved, are being published in the Analytical Edition of Industrial and Engineering Chemistry. The first report (*280*) covered apparatus for carbon and hydrogen and Dumas nitrogen; that for sulfur and halogen will be ready soon.

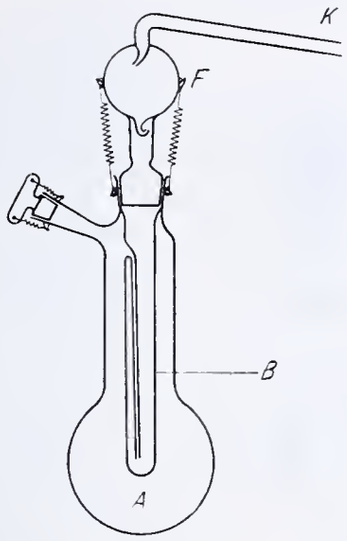


Figure 1. Steam Distillation Apparatus

The purity of the samples analyzed in published analytical work can sometimes be questioned. The National Bureau of Standards has recently undertaken the preparation of standard samples for organic elementary analysis. These samples have been analyzed by both micro- and precision macromethods and should prove invaluable to all analysts in standardizing procedures for their own laboratories, and as a measure of their precision and accuracy in comparison with others. Two standards are now available—acetanilide and benzoic acid.

Reagents of adequate purity, properly designed apparatus, and standards of unquestioned purity are prime prerequisites in developing and using any analytical procedure. That these three factors are receiving careful study indicates that micromethods are being critically examined as to their requirements and limits of precision and accuracy. So far, the results by micromethods can be considered as accurate within their established limits of precision as those obtained by equivalent macroprocedures.

The quantitative branch of microchemistry initiated by Emich, using a quartz fiber balance, was limited in application, owing to the small load capacity of the balance. With the development of balances of 20-gram capacity and high sensitivity by Kuhlmann and others, the design of equipment and the development of methods for gravimetric procedures have been made practical. Progress in instrument design and the application of new principles to analytical procedures in general have been adapted to microanalysis with a resulting improvement in precision and convenience.

It is the usual practice in analytical procedures to select a sample weight sufficiently large so that the weighing error is small. Then the precision and accuracy of the method are determined by its inherent chemical and manipulative limitations. The weighing precision of standard analytical balances has been improved over a period of years, so that, in addition to the usual balance weighing to ±0.2 mg., most laboratories possess one or more balances weighing to ±0.02 mg. Using these balances and weighing samples of 20 to 50 mg., many laboratories have found that they are able to obtain excellent results without the expense of special balances and equipment. Generally, the apparatus is slightly larger than that used in microprocedures. The skill required with these semimicromethods is not so exacting, and for many academic institutions it is becoming the accepted method. The fact that instruments are now made having greater precision and that microchemistry has demonstrated that smaller samples may be used has had a far-reaching effect on all analytical chemistry. Because of this, even macrosamples are now more often in the range of 50 to 100 mg. This gradual but very definite change has made the dividing line between macro- and micromethods less sharp.

In selecting a suitable method for the determination of a constituent, several factors should be considered:

1. The number of samples to be run. An occasional sample requires a method which avoids the preparation of special solutions and the setting up and conditioning of special apparatus. For the analysis of many samples, apparatus should be designed which permits series runs to be carried out rapidly and with a minimum of effort on the part of the operator. A new branch of chemistry necessarily has many forms of apparatus and modifications of methods. Out of all these, many of which are personal preferences, are evolved essentials which should be retained. Here, again, judgment on the part of the analyst is required, so that he may chose from the many modifications put forward the ones which will best serve his needs.
2. The degree of precision and accuracy desired should be decided upon and the simplest method to achieve this should be adopted. The available instruments for weighing, measuring volumes, color, etc., for general microanalytical work are more than adequate. More often the errors in manipulation or factors of solubility, sharpness of color, etc., determine the error of the method.
3. Where samples of similar composition are to be run, the method can often be simplified. When interfering elements are many and frequently unknown, the procedure must, of course, be modified, and undue simplification is likely to yield unsatisfactory results.
4. It is good technique in any analytical procedure to hold to a minimum operations which involve the transfer of solutions, precipitates, etc. Microprocedures which are not based on this fundamental idea will usually lack precision, and this casts doubt upon the efficiency of an otherwise chemically sound method.

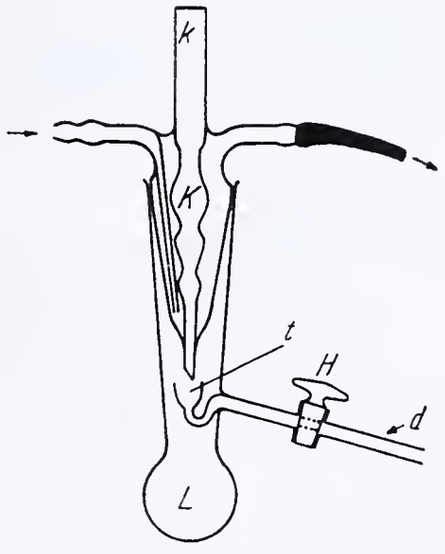


Figure 2. Combination Reflux and Distillation Apparatus

An analyst requires all the tools and techniques that are available. He should be flexible in his outlook, so that the limitations and advantages of both macro- and micromethods will be carefully considered. Speed in routine analytical procedures, which has long been a requirement in industry, has been achieved to a remarkable degree. Here the size of the sample is of little importance and the enthusiastic microchemist must show that his procedure is either faster or more convenient, and not too difficult, before the method is even considered. For the solving of special problems, where only small quantities are available, the microchemist is the last hope, and his services are always greatly appreciated.

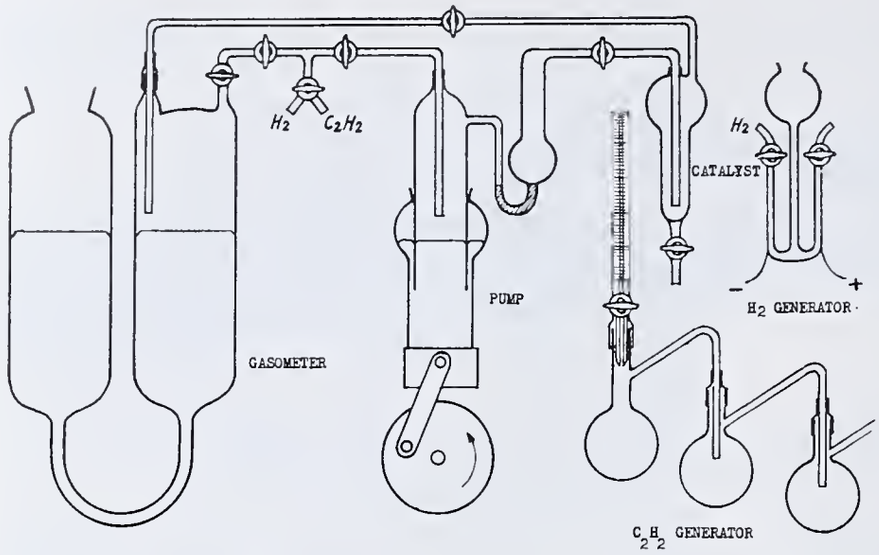


FIGURE 3. CATALYTIC HYDROGENATION APPARATUS

More attention is now being given to a systematic study of the errors in microanalysis. These errors may be grouped as:

1. Weighing
2. Measuring solution volumes (buret errors, etc.)
3. Chemical errors inherent in the procedure
4. Personal

The errors in weighing have been systematically studied (see Microbalance). The errors in volumetric analysis are known and can be controlled by the selection of a solution of suitable normality and measured in a buret so constructed as to permit adequate precision. Titration with solutions 0.001 *N*, with burets reading to 0.001 ml. and error ±1.0 per cent, is now a matter of routine, and for special problems more dilute (0.0002 *N*) solutions and more precise burets (error ±0.1 per cent) can be used. These errors and those of indicators are adequately dealt with by Conway (*70*), Mika (*213*), and Malyarov (*203*).

In a series of determinations on a single sample, most of the values, of course, are close to the mean. Those which appear out of line by inspection are apt to be discarded. This unscientific practice is not justified when mathematical methods are available for calculating when a value exceeds the allowable error of the method and may be classified as an error in manipulation. Likewise, the chances which any one value has of being correct may be determined. In the development of any method, the results should be considered from the standpoint of statistical treatment (*273*). A general text on this subject, of value to the analyst, has been written by Crumpler and Yoe (*73*).

Collaborative studies on the errors involved in the microdetermination of nitrogen, methoxyl, ethoxyl, carbon, and hydrogen and weighing have been completed and are referred to under these determinations in this review. The last three have been subjected to a thorough statistical treatment.

The following example will serve to illustrate the method of expressing precision and accuracy in this review. If the theoretical value for carbon is 50.00 per cent and the found values are 49.7 and 50.3, then the precision and accuracy are ±0.6 per cent. By precision is meant the reproducibility of a method; by accuracy, the deviation from the true or theoretical value.

References to biological micromethods are made only in so far as they illustrate technique and methods which can be applied to general organic microchemistry. Kirk (*149*) has adequately summarized advances in this field.

This review is one of several in a series on analytical topics. In order to keep the present one within reasonable limits, certain methods and instruments are only briefly mentioned as having micromodifications. In such cases, references are given to earlier reviews where sufficient information and illustration will be found.

An attempt has been made, in the description of apparatus and methods, to give a survey of the tools which are at the disposal of the organic microchemist, to show their wide application, to set down their precision and accuracy, and to give a glimpse at advances in design and technique.

SYNTHESIS AND PURIFICATION

ORGANIC SYNTHESIS. Some investigators have found that it is entirely practical to carry out organic syntheses, using micro- or semimicroquantities.

Cheronis (*56*) describes apparatus for refluxing, distilling, extracting, separating, filtering, drying, weighing, and measuring 2 to 4 grams of solids and 2 to 5 ml. of liquid. Detailed directions, using this equipment in teaching organic synthesis, are given for the preparation of nitrobenzene, *m*-dinitrobenzene, cyclohexene, benzoic acid (using Grignard's reagent for introducing the hydroxyl group), ethyl benzene (by the Wurtig-Fittig reaction) aniline (by the reduction of nitrobenzene), and acetylation of aniline. Yields of 0.3 to 1 gram are obtained.

Alber (*4*) synthesized and analyzed organic compounds containing radioactive sulfur, using micromethods.

Erdös and László (*89*) give a summary in English, with nine cuts of apparatus which is useful in carrying synthetic laboratory

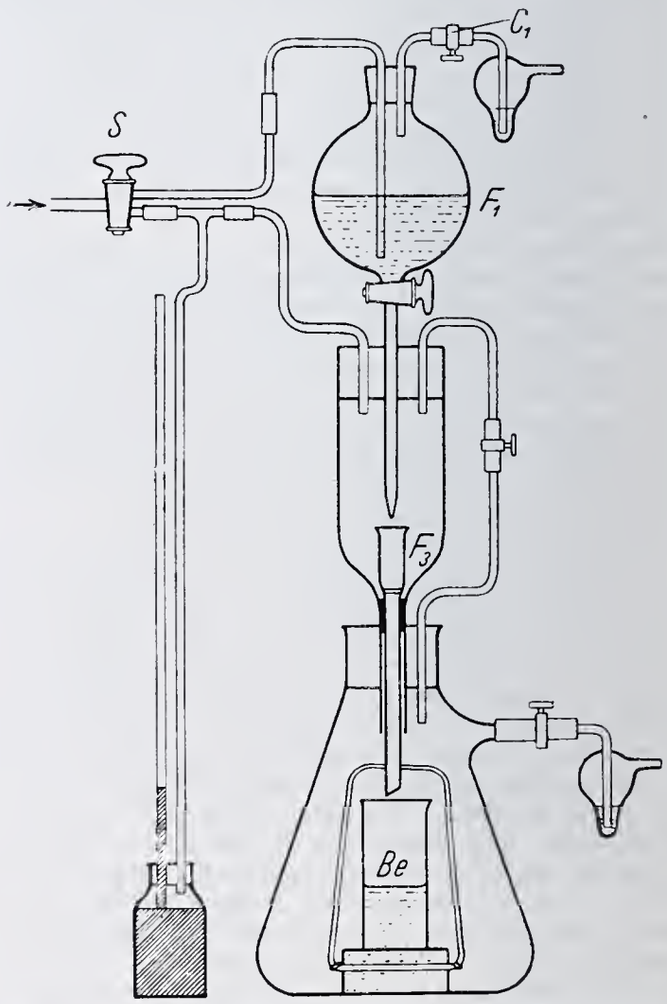


FIGURE 4. APPARATUS FOR REACTIONS OR EXTRACTIONS IN SPECIAL ATMOSPHERES

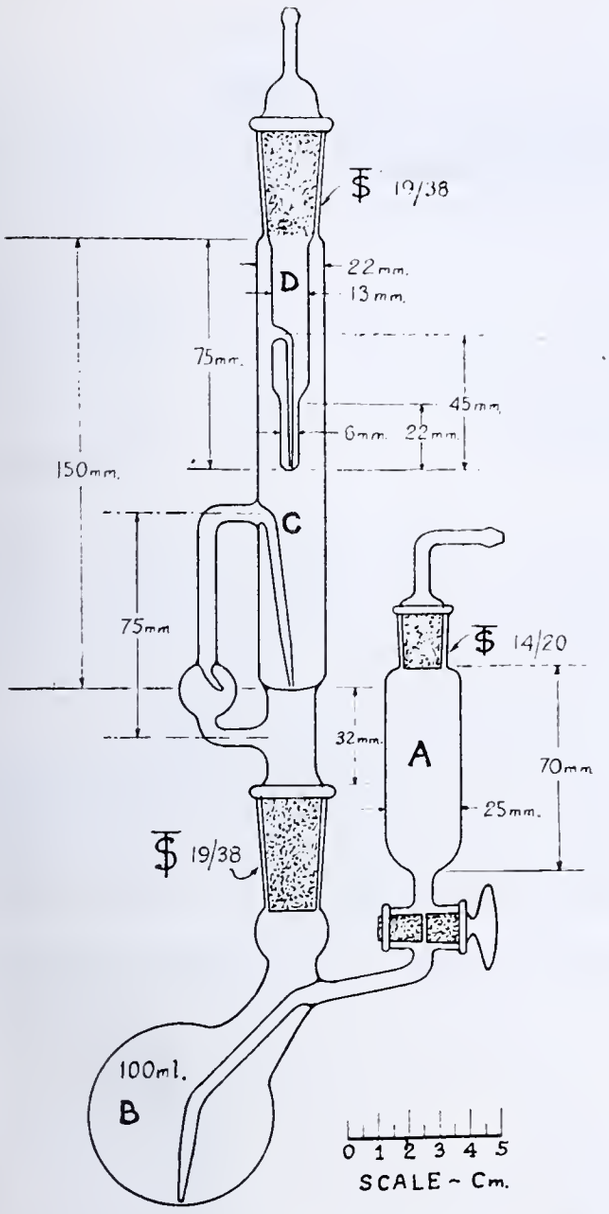


FIGURE 5. APPARATUS FOR QUANTITATIVE LIBERATION AND ABSORPTION OF GASES

work on 50 to 100 mg. or 0.1 ml. of material. An apparatus for distilling under reduced pressure and four types of apparatus for steam-distillation are described. Figure 1 shows one type used for steam-distillation. Steam generated in *A* is led into the sample contained in *B*, and the sample is distilled out through *F* and *K*. A distilling flask, useful for distilling following a reaction involving reflux cooling, is shown in Figure 2. The flask, *L*, 5- to 10-ml. capacity, is the reaction chamber and *K* is a ground-in condenser. The stopcock, *H*, is closed during the reaction. After the reaction is completed by turning *H*, the distilled product is caught in *t* and removed through *d*.

Wright (*371*) summarizes technique with illustrations of apparatus which have been found useful in semimicropreparation and purification of organic substances.

Leĭpunskiĭ and Reĭnov (*183*) describe an apparatus and micromethods for studying chemical reactions of gases, their solubility in liquids, and compressibility at pressures ranging from 10,000 to 12,000 atmospheres and at temperatures up to 450° C.

Dadieu and Kopper (*75*) in a review of micropreparative methods in organic chemistry include a description of apparatus from the unpublished work of O. Kermauner: (1) for the micropreparation by a bomb reaction of $C_2D_2 + DBr \xrightarrow{(AlBr_3\ catalyst)} CD_3CDBr_2$; (2) for reaction of gases in liquids, as, for example, the use of deuteroacetylene and deuterosulfuric acid in the preparation of deuteroacetaldehyde; also (3) an apparatus with a special circulating pump for the catalytic hydrogenation of acetylene to ethylene, using a colloidal palladium solution as the catalyst which is shown in Figure 3. The initial volume of the mixture of acetylene and hydrogen was 500 ml.

A comprehensive summary of microtechnique in synthesis is given by Pfeil (*244*).

Van Straten and Ehret (*352*) propose apparatus (Figure 4) for conducting reactions or extractions in special atmospheres. The reagent or solvent is placed in the 70-ml. separatory funnel, F_1, and the substance that undergoes reaction is placed in the microfilter tube, F_2. The reaction product or leachings are caught in the microbeaker, *Be*. The special gas which fills the apparatus is brought in through *S*.

An all-glass apparatus (Figure 5) for evolving and quantitatively absorbing various gases and vapors is described by Binnington (*34*). The sample is placed in *B*, the reaction mixture in *A*. Air or other gas is drawn through the apparatus and the liberated gas absorbed in the reagent placed in absorbers *C* and *D*. After the reaction is completed, *D* is removed, and its content washed into *C* where the titration is carried out. The apparatus was designed initially for determining 0.5 mg. of bromide by treating it with chromic-sulfuric acid mixture.

RECRYSTALLIZATION. Mechanical loss during crystallization of small quantities must be avoided.

Blount (*38*) describes a simple apparatus, Figure 6 (left), for carrying out this operation. The material is placed in a filter funnel with a G2 porosity sintered-glass plate, which is suspended from the condenser by a platinum wire. The solvent is contained in a flask 3 cm. in diameter and, on refluxing, the solids dissolve. When extraction is complete, the flask is allowed to cool and the substance crystallizes. It may be collected again on the filter and the process repeated as many times as desired.

Craig (*72*) has designed an assembly for the fractional recrystallization, as shown in Figure 6 (center). *H* is the crystallizing vessel and *L* is the filter vessel, which is closed by a glass plug, *M*, not ground in. The fine crystals form the filter mat but, if desired, a sintered-glass plate may be fused in at *L*. *K* and *N* are collars of soft lead or tin. The figure shows the apparatus in the position for the collection of crystals. In the reverse position the material and solvent are contained in the crystallizing vessel, *H*, and for crystallization the apparatus can be heated or cooled by placing in a suitable bath. The apparatus is of such size that it can be readily placed in a small centrifuge. Figure 6 (right) shows a crystallizing assembly which is smaller in size but operates on the same principle. *Q* corresponds to *H*, *R* to *M*, and *S* collects the filtrate. The whole assembly is placed in a stoppered glass tube for centrifuging.

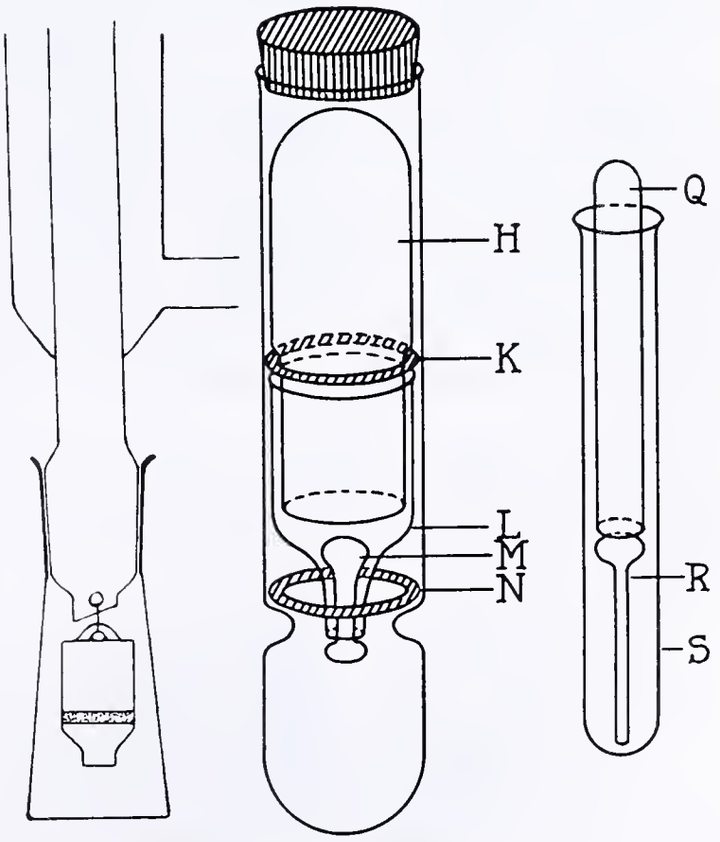


FIGURE 6. RECRYSTALLIZATION APPARATUS

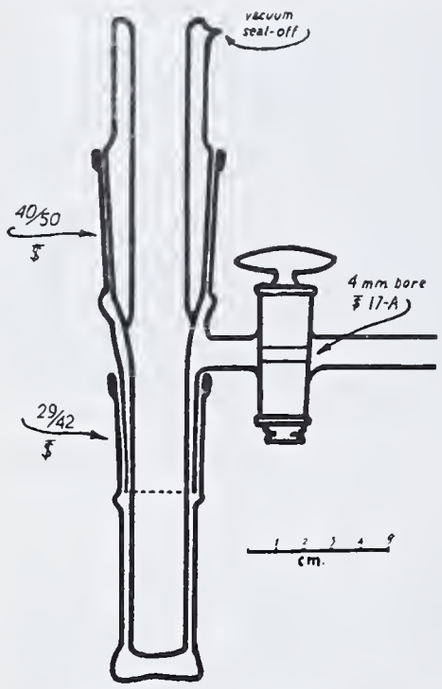


FIGURE 7. HIGH-VACUUM LOW-TEMPERATURE SUBLIMATION APPARATUS

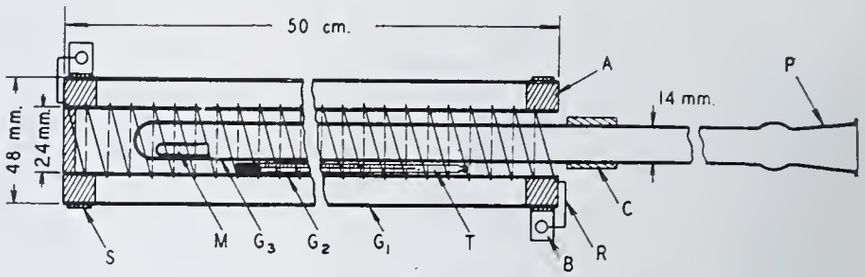


FIGURE 8. FRACTIONAL SUBLIMATION APPARATUS

A. Asbestos tape
B. Binding post and fastener
C. Condenser
G_1, G_2, G_3. Glass tubes 48, 24, and 14 mm. in diameter, respectively
M. Material being sublimed, contained in glass capsule
P. Pump connection
R. Resistance wire
S. Strip of metal to prevent breakage and serve as support for binding posts
T. Thermometer

SUBLIMATION. Purification of solids by sublimation is a simple but effective method, which can be carried out using only a watch glass upon which the material is placed. The watch glass may be heated by a small flame and the sublimate condensed on the under surface of a cool glass plate.

A more elaborate apparatus for quantitative work with traces of material, designed by Clarke and Hermance (*67*), offers advantages. It is electrically heated and thermostatically controlled, and sublimation takes place under a vacuum. Equipment with or without a vacuum has also been designed to fit the microscope stage, so that any significant characteristic of the sublimation process can be more easily followed. Sublimation technique and apparatus, stressing that used with the microscope, are given by Fischer (*100*).

Of interest to those engaged in synthetic work is the high-vacuum semimicrosublimation apparatus of Marberg (*204*). It is constructed of standard-taper joints (Figure 7). The material placed in the lower cup is sublimed by heating and the sublimate collects on the condenser cooled with a low-temperature refrigerant, such as liquid air. The general theory and technique of high-vacuum purification have been described by Hickman and Sanford (*135*), and McDonald (*198*). A novel arrangement (Figure 8) for separating and collecting fractions from a small sample is given by Morton, Mahoney, and Richardson (*218*). After one fraction has been deposited, others may be collected by successively moving the heater jacket back and raising the temperature, whereby a series of bands is formed. The apparatus is also useful in checking the purity of a compound, since the first and last bands should have identical melting points.

EXTRACTION. *Solids.* There are two types of extraction apparatus, siphoning and percolating. Extraction may be employed for removing an active ingredient from a heterogeneous mixture as a crude drug mixture, or removing an undesirable material from an impure product, as in the purification of derivatives.

A comparative study has been made by Batt and Alber (*19*) of methods for extracting solid substances by heated solvents, using macro-, semimicro-, and microapparatus. Where the residue, as well as the extracted material, is to be determined, the less commonly used principle of percolation gives better results than when a paper thimble and siphoning (Soxhlet) apparatus are employed. The best apparatus for this purpose is that developed by Gorbach, which uses a platinum filter dish as a container for the material to be extracted. Six types of apparatus are shown (Titus and Meloche, Gorbach, Colegrave, Wasitzky, Slotta, and Hetterich), and the best operating conditions for each are summarized (*19*). Figure 9 shows the first two, which incorporate features which have been demonstrated to be of value. The accuracy of both micro- and macroprocedures was ±2 per cent. The amount of extractable substance used in the microextractors varied from 1 to 100 mg.

Liquids. An apparatus for the extraction of small quantities of liquid, 1 to 20 ml., with organic solvents such as ether, benzene, etc., has been described by Barrenscheen (*17*).

Fabian (*92*) and later Browning (*48*) designed a microseparatory funnel without a stopcock, which is similar to a pipet. A solution of the material is preferably drawn through the capillary by gentle suction. Both the material to be extracted and the solvent are shaken in the usual manner, and after standing, the two phases are separated by applying pressure, whereby the heavier fraction flows out through the capillary. Figure 10 shows the Browning apparatus.

MICROFRACTIONATION. The fractionation of a single drop of liquid by the Emich method has been improved by Morton

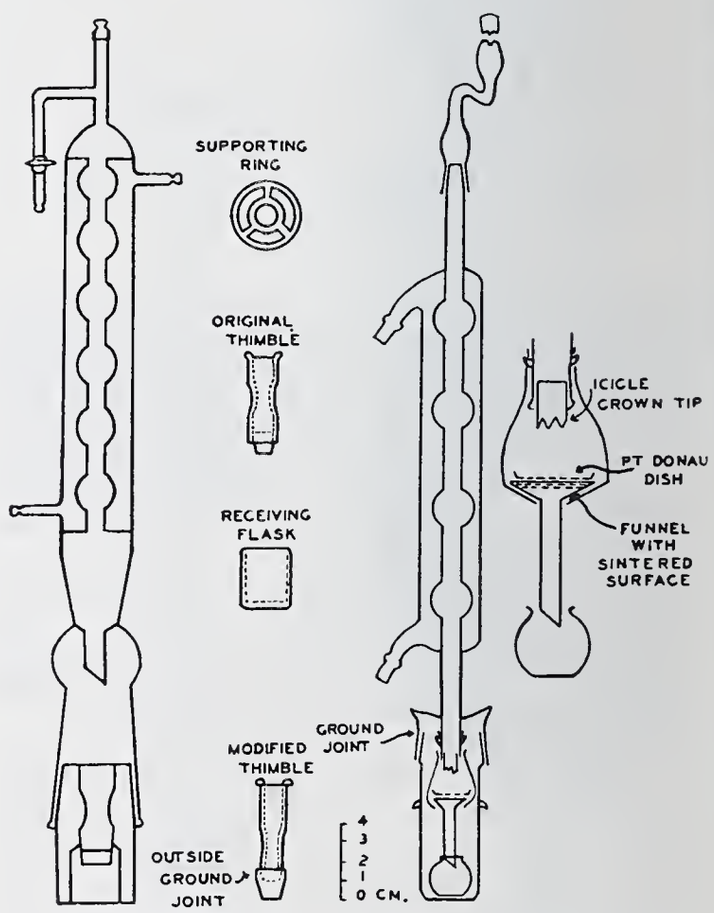


FIGURE 9. EXTRACTION APPARATUS

Left, Titus and Meloche. *Right*, Gorbach

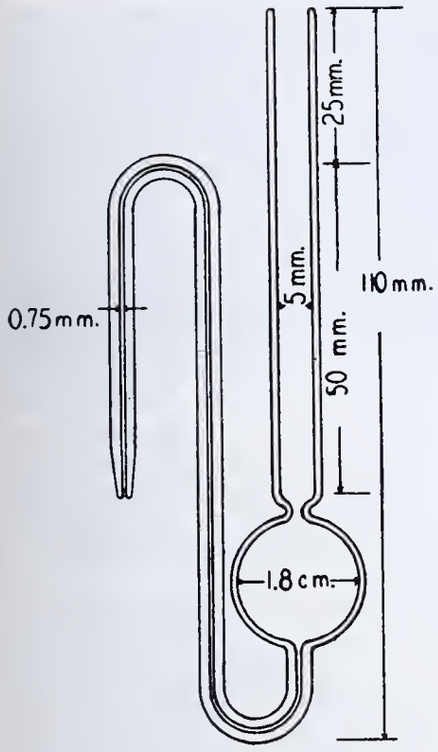


FIGURE 10. APPARATUS FOR EXTRACTION OF LIQUIDS

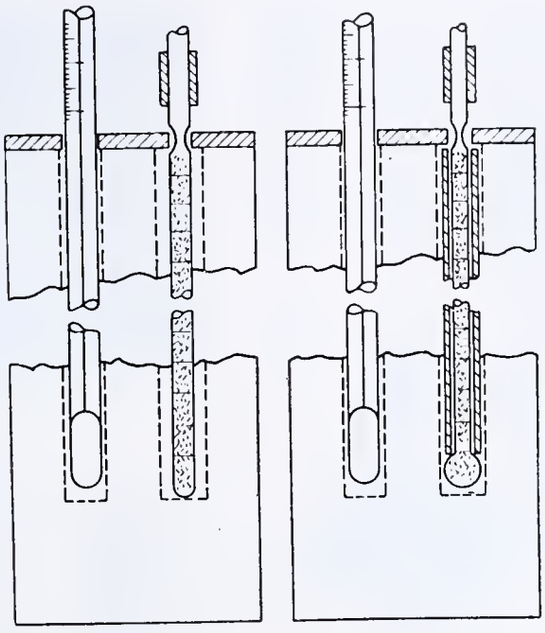


FIGURE 11. FRACTIONATION CAPILLARIES

and Mahoney (*216*), using an electrically heated block (Figure 11).

The capillary is filled with finely ground glass wool instead of being left empty. From 30 to 70 fractions from a single drop (20 to 30 mg.) are possible instead of the usual 6 to 12. It is possible to determine, with reasonable accuracy, the existence of one or more components, as well as the percentage composition.

For the accurate analytical separation of 1 to 10 ml., a spinning metal-band type (*184*) with the band (37.5 cm. long) rotating at 1000 r. p. m. gives a high efficiency (equivalent to 13 plates) with low holdup. It has been shown (*10*) that the holdup on this type of column is 0.12 ml. per theoretical plate, which is approximately 3 times less than any other. A modification by Wyman and Barkenbus (*374*), employing a vacuum, is shown (Figure 12). The analyses reported on samples of methyl esters are accurate within ±5.0 per cent, in the range 10 to 80 per cent. The values for the volatile esters have the greatest error.

An all-glass microdistillation apparatus (Figure 13) for distilling 0.5 to 2.0 grams of liquid into 9 or more receiving cups, each having a capacity of 0.1 ml. under reduced pressure without interruption, has been built by Shrader and Ritzer (*310*). A Vigreux column is used with a holdup of 0.08 to 0.12 gram.

SEPARATION OF MIXTURES OF ORGANIC COMPOUNDS BY CHROMATOGRAPHIC ADSORPTION. Organic chemists have long made use of charcoal and fuller's earth to clarify colored solutions, whereby the colored material is adsorbed and the clear solution remains. It was the botanist, Tswett, who, in 1906, proposed placing the adsorbent in a tube and pouring the colored solution through the packed column. It was demonstrated that some colored organic compounds were selectively adsorbed along the column in bands, depending upon their activity toward the adsorbent medium. This method was first employed to separate plant pigments; hence its name. The amounts of material recovered from the zones by washing out with suitable solvents (eluents) were generally disappointingly small, but with the later development of micromethods of analysis, these small quantities could be more easily analyzed and identified.

About the same time, Goppelsroeder proposed dipping a strip of filter paper in a solution, whereby the components of the solution would rise by capillary attraction and separate out in zones as in the Tswett column. This method, however, has not been found of value in preparatory work for separating organic or inorganic compounds, but for qualitative separations and tests. The filter paper may be treated with reagents which form zones of characteristic color with the components in the solution. The concentration of materials by this method which fluoresce under ultraviolet light is another application in which even some quantitative results have been reported (Germann and Hensley, *112*). The subject of capillary analysis has been reviewed by Rheinboldt (*262*) and by Germann (*111*).

Interest in this subject has revived, and many applications to problems of separating colored and colorless compounds have been made. In certain studies where large quantities of material are not available, or where qualitative runs by the Tswett method are necessary, separations using a small microcolumn may be made. The columns (Figure 14) containing the adsorbent vary from 1 to 5 mm. in bore and are 30 mm. long. At the end of the run, instead of trying to push the

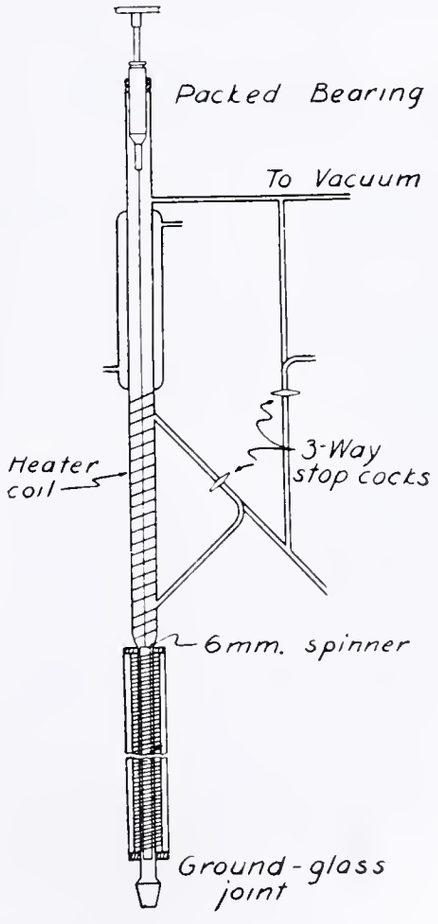


FIGURE 12. SPINNING-BAND VACUUM DISTILLATION APPARATUS

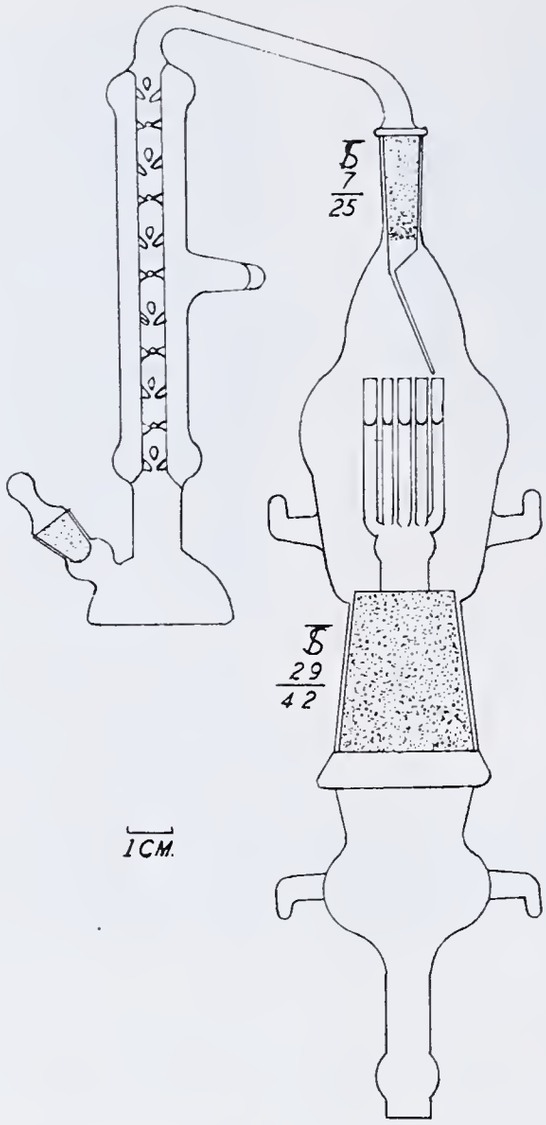


FIGURE 13. FRACTIONAL VACUUM DISTILLATION WITH VIGREUX COLUMN

column of adsorbent out of the tube, the glass may be cut where the zones appear.

Willstaedt and With (*367*) were able to separate semiquantitatively certain carotenoids when the amounts were 30 to 150 micrograms. This method of purification, concentration of traces, and separation of organic compounds has taken its place as one of the useful tools which the organic microchemist has at his disposal for working with relatively small quantities prior to analysis.

Strain (*322*) and Zechmeister and Cholnoky (*375*) give the details of working with this technique, including micromodifications, in its application to the various types of organic compounds. Strain (*323*) has recently summarized the subject from the viewpoint of the analyst. A partial list from Strain will give some idea as to the scope of this method: hydrocarbons, fatty acids, fats, amino acids, phenols, terpenes, benzene derivatives, aromatic-aliphatic compounds, condensed polycyclic compounds, sterols, heterocyclic nitrogen bases, fat-soluble vitamins, water-soluble vitamins, hormones, enzymes, and proteins, anthocyanines and pterins, natural pigments, and coal-tar dyes. Resolution of all mixtures, of course, is not possible, and while some knowledge has been gained as to the best general types of adsorbents, solvents, and eluents, their selection for any particular mixture is largely empirical, and the best conditions must be determined by preliminary tests, using small columns. The best adsorbents found so far are activated aluminum and fuller's earth.

Chromatographic adsorption methods are bound to microanalysis by the fact that, even with improved methods, the amounts obtained in the usual laboratory separations, using a column 6.5 × 42 cm., are rather small—from a few milligrams to a few tenths of a gram. Therefore, for the final identification of these fractions micromethods are essential.

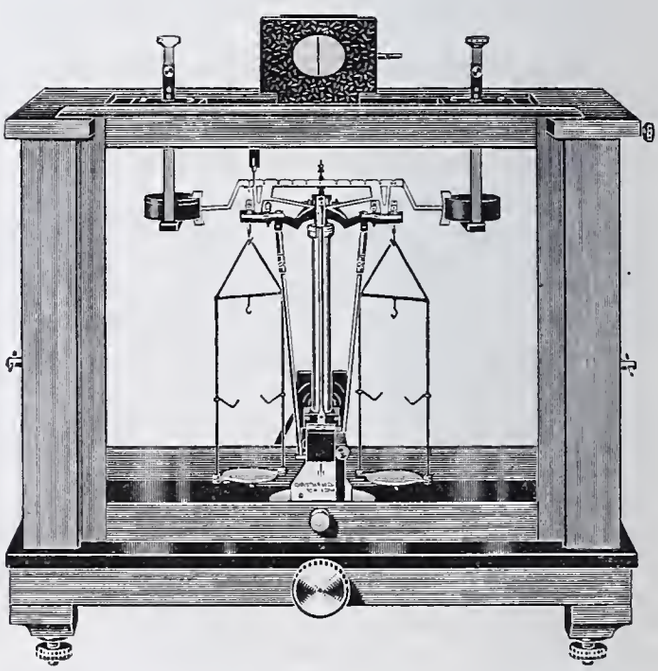
PHYSICAL METHODS

Microbalance

Because of the fundamental importance of the balance in analytical work, a brief summary of later developments and studies on the microbalance and weighing will be given. There are two general classes of balances, one which has a rapid swing period, represented by the Kuhlmann balance, and the other a long-beam balance with a greater swing period which is typical of American-built balances. Where much weighing is done and damping is not used, a short-period balance is to be preferred.

FIGURE 14. MICROCHROMATOGRAPHIC ADSORPTION COLUMN

The precision of the microbalance has been commonly considered in specifications as ±1 microgram. In general practice, however, this precision is not possible. In a recent study (*71*), using six properly working balances, the probable error of any single weighing was found to be ±3 micrograms. In this study, as well as in others (*170*), it has been found that the error in placing the rider accounts for the greater part of this variation. The angle errors cause greater variation than dislocation errors. It has been calculated that a 1° change on the Kuhlmann rider will give a weighing error of 5 micrograms. An attempt was made by Ramberg to lessen the placement error by using a quartz rod rider. However, even with this improvement it was found that an average devia-



Courtesy, Christian Becker

FIGURE 15. MICROBALANCE WITH MAGNETIC DAMPING AND PROJECTION READING

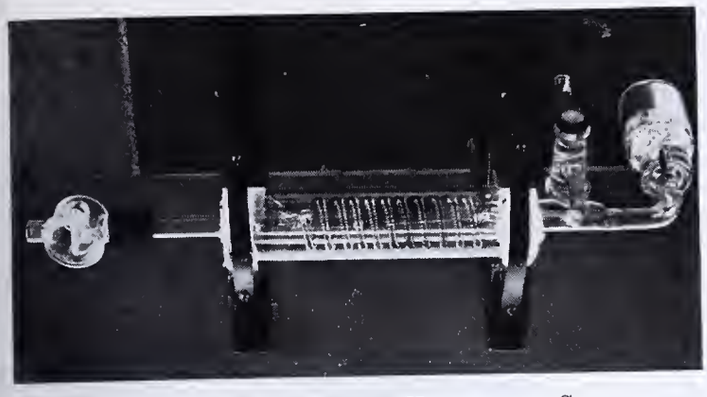


FIGURE 16. DESICCATOR FOR HYGROSCOPIC SAMPLES

tion of 2.6 micrograms was obtained in a series of weighings which is about the same precision as that obtained with other microbalances. For most microprocedures, the error in weighing, 5 to 10 mg., is much smaller than that contributed by chemical manipulation. In this laboratory the weighing variations with standard Kuhlmann balances agree with the above findings. [A cooperative study by several laboratories of microbalance performance has been conducted by the Division of Analytical and Micro Chemistry of the AMERICAN CHEMICAL SOCIETY. A statistical study of the results from twenty-two balances gives the probable error of weighing as 2 to 3 micrograms. A report of these results was made at the meeting in Buffalo, September, 1942 (*269*)].

The improvements which have been made in balances have not increased their sensitivity or precision, but have made them more convenient.

The Ainsworth and Becker balances in this country and the Bunge and Sartorius in Europe use a projection device for easier reading of the scale. Both magnetic and air damping are in use, but must be carefully regulated to prevent overdamping, which will give a premature rest point with a gradual drift to the true rest point. The degree of damping on a magnetically damped balance is easily regulated. Semimicrobalances are also available for the specified sensitivity of ±0.01 mg. Gorbach gives an illustrated review of the different types of European microbalances (*116*). Figure 15 shows a magnetically damped balance. The Ainsworth microbalance may be obtained with an optical lever and an automatic weight carrier (*222*, *223*).

Weighing

Changes in temperature, humidity, and pressure all affect weighing, but practically the changes are usually all so gradual that they cause no serious error. Temperature variations of over 0.5° C. cause a shift in the zero point of balances, for which a correction should be applied. A balance room or laboratory of constant temperature and humidity is the best means of controlling these factors. However, the initial expense and upkeep is too great except for laboratories operating under severe conditions of temperature and humidity change, or where air contains dust or corrosive gases. Static on glassware or samples, which may develop under conditions of humidity below 50 per cent, can be readily discharged in a second or so with an ordinary high-frequency spark coil discharge (*351*).

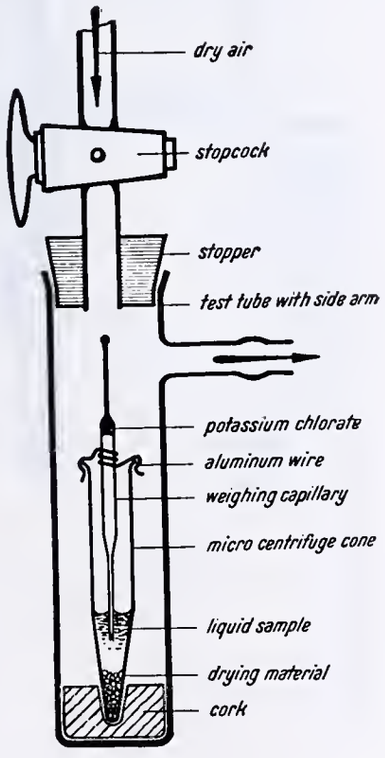
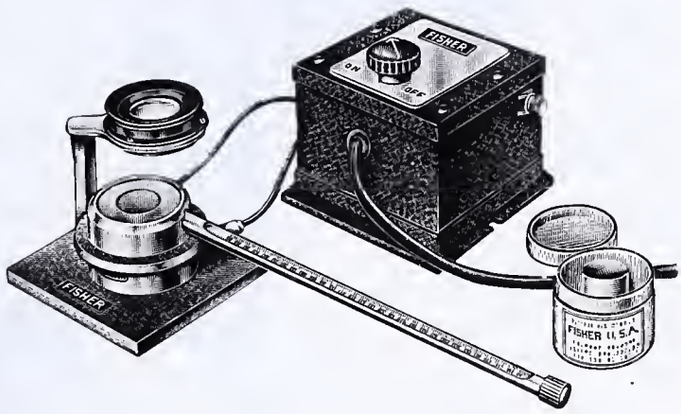


FIGURE 17. APPARATUS FOR FILLING CAPILLARIES WITH LIQUIDS



Courtesy, Fisher Scientific Co.

FIGURE 18. FISHER-JOHNS MELTING POINT APPARATUS

The accurate weighing and handling of hygroscopic substances are difficult and the problem is thoroughly treated by Alber (*5*). The best technique yet developed is that by Rodden (*268*), in which the sample is dried in a boat and transferred to a special weighing stopcock, so arranged that the transfer from the desiccator to the stopcock and then to the combustion tube is accomplished under moisture-free conditions. Figure 16 shows an electrically heated desiccator with the special weighing stopcock attached.

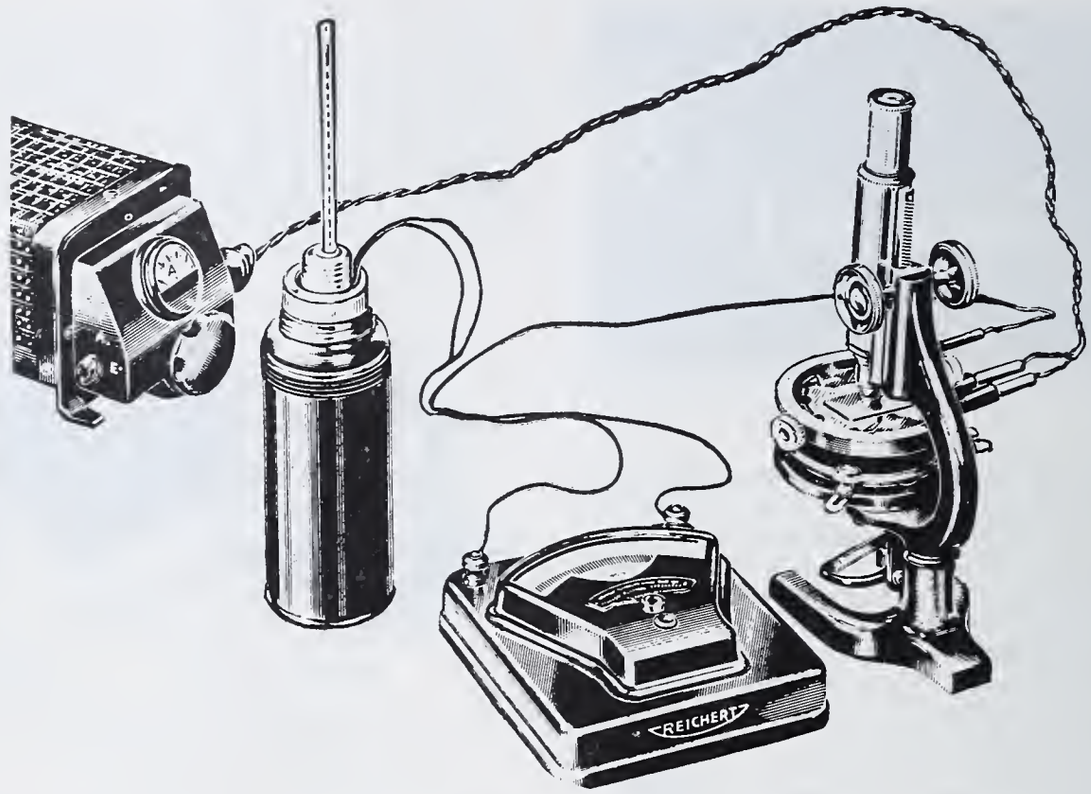
Filling Capillary Tubes

Capillary tubes are best filled by inserting the end of the capillary in the liquid contained in a small vial which is placed in a filter flask (Figure 17) and evacuated momentarily (*5*). This is a great improvement over the usual method of heating and cooling the capillaries.

Melting Point

This determination, even by the standard capillary tube method, does not require more than a few milligrams of sample. For a more rapid and convenient method, an electrically heated copper or aluminum block may be used and the melting point of the sample, placed between cover glasses, is observed through a low-power lens. The commercially available Fisher-Johns apparatus (Figure 18) is a good example of this type. For the determination of the melting point of very small quantities of material down to a single crystal, several special hot stages for microscopes have been designed, some of which allow the melting point to be taken in the presence of an inert atmosphere.

The temperature may be measured with an accurate thermometer or with a sensitive thermocouple. Figure 19 shows a microscope fitted with a Kofler hot stage and thermocouple. The true melting point is most accurately observed under a polarizing microscope for all crystalline compounds except those which are isotropic solids. The temperature at which the color disappears under polarized light is the true melting point, because it represents the point at which the space lattice is ruptured. With such an instrument, Zscheile and White (*378*) obtained a precision of ±0.04° C. on known



Courtesy, Microchemical Service

FIGURE 19. KOFLER-HILBCK HOT STAGE FOR DETERMINING MELTING POINT

compounds. Melting points determined under the microscope are apt to be lower by as much as 1° C. because the point at which melting occurs is more easily seen than by the capillary tube method, which is the one commonly employed for compounds whose melting points are recorded in the organic literature.

A modification by Morton and Mahoney (*217*) of the Emich capillary tube method uses an electrically heated copper block and an optical system to project an image of the capillary on a paper screen (Figure 20). This permits the melting point to be observed more easily than when a liquid heating bath is used. Methods using the electrically heated metal block are not limited in their range of temperature and the rate of heating can be more carefully controlled. While the precise determination of melting point is justified on specially purified compounds, for purposes of identification, a precision of ±0.5° C. is generally satisfactory.

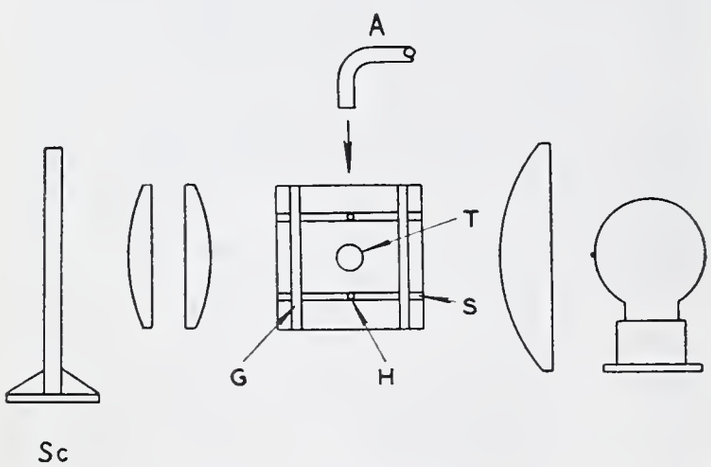


FIGURE 20. APPARATUS FOR OBSERVATION OF MELTING OR BOILING POINT BY PROJECTION

A. Air blast for cooling copper block
G. Pyrex plate
H. Hole for capillary
S. Slit for observation
Sc. Screen
T. Thermometer well

DEPRESSION OF THE MELTING POINT (Determination of Molecular Weight). When a small sample of an organic compound is mixed with camphor or borneol contained in a melting

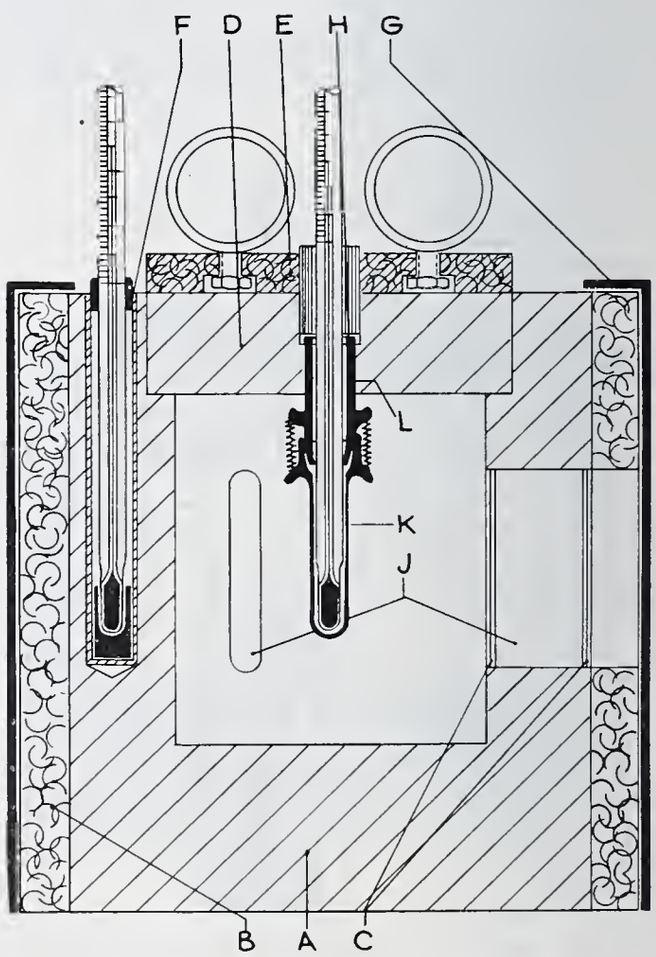


FIGURE 21. COOLING-CURVE APPARATUS

point capillary, a large depression in the melting point is observed. Advantage is taken of this for the determination of the molecular weight of a sample as small as 1 mg. The camphor is first standardized with a pure compound of known molecular weight. The method (*296–305*) is extremely simple and can be carried out by anyone who is familiar with taking a melting point. An ordinary thermometer reading to 0.1° can be used. The method fails with compounds which are not soluble or which decompose.

Cooling Curve. While the determination of a cooling curve is considered the best method of establishing the purity of a solid organic compound, it is seldom used because it requires several grams of the sample. The recent publication of a method (*316*) using only 200 to 300 mg. should make this excellent procedure of more general application. The material contained in a special glass tube is placed in a copper block and heated so that a constant difference in temperature is maintained between the sample and its surroundings. The temperature rise in degrees centigrade is plotted against the time in minutes. The curves which have a characteristic shape when the material is pure are reproducible within 3 per cent. Figure 21 shows the apparatus.

Mixed Melting Point. When two compounds are mixed together and the melting point does not change from that taken separately, it usually indicates that they are identical. This can be quickly determined in a melting point tube with small amounts of material. Constants, such as melting point, cooling curve, and boiling point, which are obtained by heating the compound, have no value for purposes of identification if, during the process, they undergo decomposition. However, modifications formed during heating, which have a definite composition, can be detected by the cooling curve method.

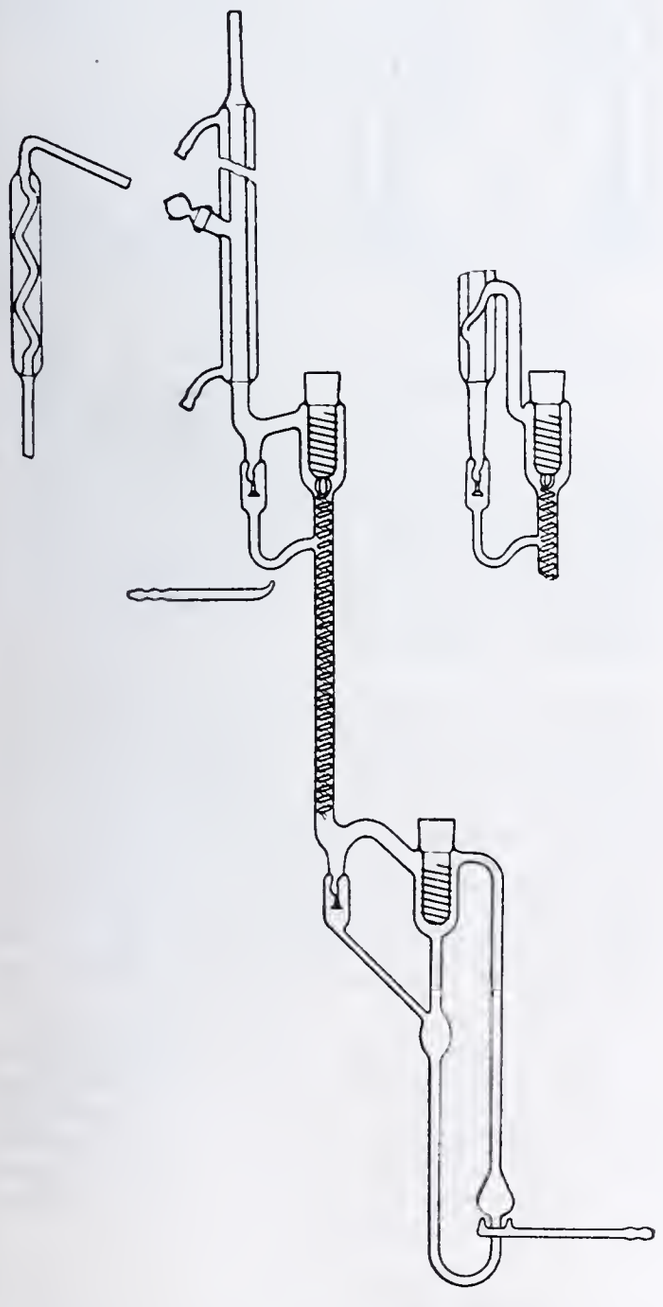


Figure 22. Apparatus for Determining Purity of Liquids

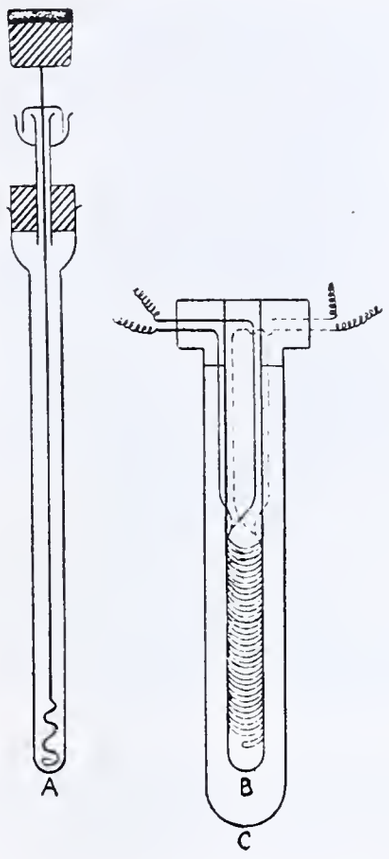


Figure 23. Apparatus for Determining Freezing Point

Boiling Point

The same modification of the Emich method (*217*) described for the melting point (Figure 20) is used to observe the boiling point. The boiling point is reached when the drop of liquid contained in the capillary and heated in a copper block appears at the top of the block. Niederl and Routh (*235*) discuss the determination of the boiling point and the corrections which must be applied.

Purity of Organic Liquids

Swietoslawski (*334*) points out that the purity of an organic liquid can often be determined by the simultaneous measuring of the boiling point and the condensation temperature of the vapor. For this purpose, 25 to 50 ml. are usually required. To allow 8 to 10 ml. of liquid to be used, the design shown in Figure 22, modified from a larger apparatus, has given satisfactory results. While the boiling point of a liquid as normally measured may be off only a few tenths of a degree, on the apparatus shown the difference in temperature between the boiling liquid and the condensation temperature may be as much as 1° C.

An arbitrary scale of purity is proposed. The classification of the liquid according to the exact degree of purity is conditioned by the vapor tension curve of the mixture formed by the impurities and the liquid, and the percentage composition of the impurity present. This method is, therefore, only an aid in the estimation of absolute purity, which must be substantiated by other physical data.

Molecular Weight

By Rise of the Boiling Point. It is very difficult in the Pregl microform of the apparatus, employing a gas burner and a Beckman thermometer immersed in the boiling liquid, to obtain uniform boiling and a constant temperature. This difficulty, of course, is present in some macroforms.

It was Cottrell who first proposed the pumping of liquid and vapor around the thermometer to eliminate superheating.

Riechi (*264*) and later Sucharda and Bobranski (*329*) designed apparatus which incorporate the Cottrell principle and permit constant temperature to be attained more easily. However, these designs do not allow the actual volume of the solvent present to be measured during boiling. To guard against error from changes in pressure, two units should be set up, one with the solvent alone and the other with both solvent and sample. Both apparatus are available commercially.

The micromodification of the Menzies and Wright apparatus, developed by Smith and Milner (*304*), also minimizes the difficulties mentioned above by boiling the liquid with an electrically heated platinum coil placed therein, and using the Cottrell method of pumping liquid and vapor over the bulb of the thermometer, which prevents superheating. The volume of the liquid can be determined accurately by this method. A water-differential thermometer, whose readings are not affected by a change in atmospheric pressure, is used in place of the Beckman. The water differential thermometer, however, limits the choice of solvents to those boiling below 100° C. A sample, 15 to 30 mg. for each addition using 5 ml. of solvent, is required in both methods and the accuracy is within ±5 per cent. The sample, if necessary, can be easily recovered from the solvent.

By Depression of the Freezing Point. The previous methods of determining molecular weight involve heating the material which, in some cases, may decompose the compound or cause it to dissociate. The molecular weight is not commonly determined by measuring the lowering of the freezing point, using microquantities.

A miniature Beckman apparatus (*142*), in which 2 to 3 ml. of solvent and 5 to 10 mg. of sample are used, has given very satisfactory results in this laboratory. A design (*168*) which uses a sensitive thermocouple, instead of the Beckman microthermometer, to measure the lowering of temperature has given good results with 5 to 10 mg. of sample in 1 ml. of solvent (Figure 23). *A* shows the tube with the electromagnetic stirrer for the solvent mixture, which is placed in tube *B*, wound with silver wire for the electro-resistance thermometer. Tube *C* is the usual air jacket which is placed in the freezing mixture.

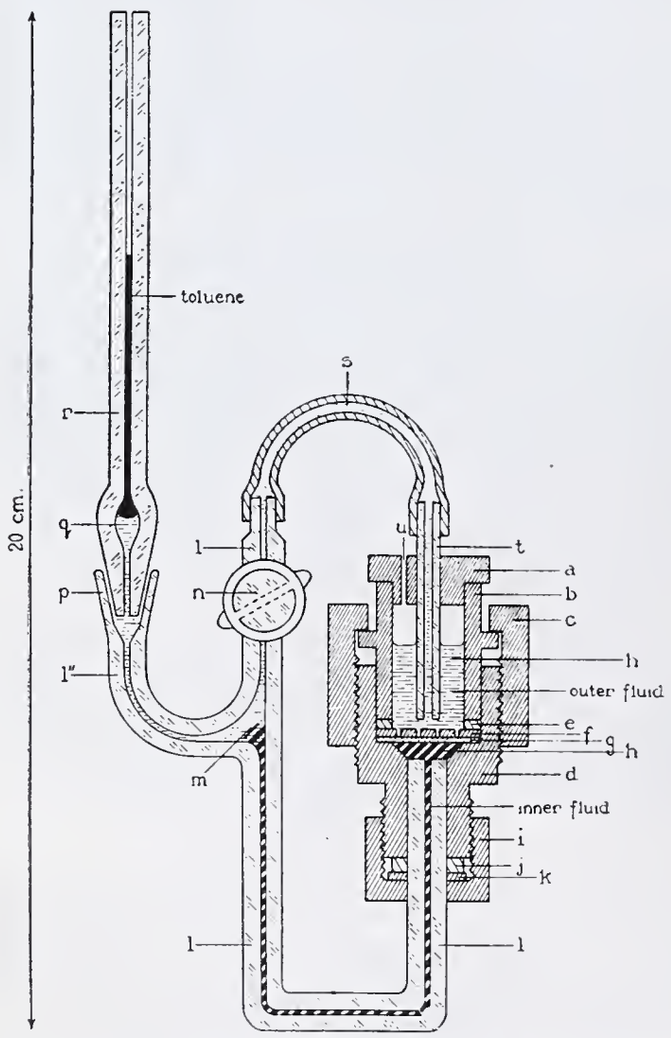


Figure 24. Apparatus for Measuring Osmotic Pressure

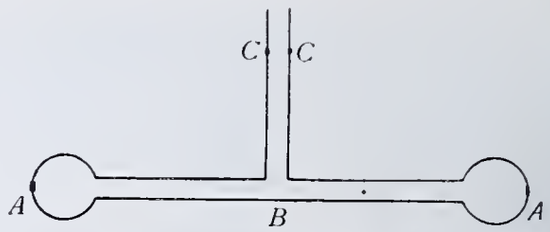


Figure 25. Diagrammatic Sketch of Thermoelectric Method

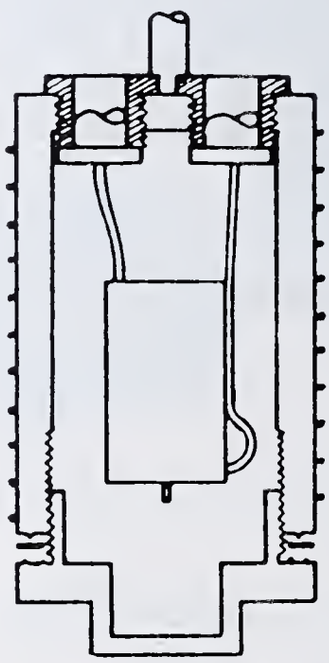


Figure 26. Calorimeter for Heat Capacity

By Isothermal Distillation. The method of determining molecular weights by isothermal distillation, which has received considerable attention, is based upon the fact that if two solutions of different molarity are placed in a common container, solvent from the solution of lower molarity will distill into that of higher molarity until they are equimolar. While the method can yield accurate results, the elapsed time necessary to make a determination requires 1 to 2 weeks. The method is, therefore, suitable only for those investigations in which time is not a factor. It requires about 5 mg. of sample and generally involves the preparation of a series of comparison standards.

Niederl and Levy (*230*, *232*, p. 230) have worked out an improved but conventional technique with which a series of known of varying concentrations is paired with the unknown, contained in capillaries. The molarity can be determined within ±0.01 in the range 0.05 to 0.15 molar. Clark (*62*) prefers Singer's method, which involves preparing only one standard. The standard and a weighed amount of unknown contained in 1.5 ml. of solvent (approximately 0.1 molar) are placed in separate but connected bulbs, each having a graduated side arm for measuring the volume of the solution. The system is evacuated and the solutions are allowed to distill isothermally until the volume readings become constant. From these the molecular weight may be calculated. The results reported are all within 2 per cent of theory.

By Victor Meyer Method. For volatile liquids the best method is a micromodification (*306*) of the Victor Meyer apparatus in which 5 to 10 mg. of sample are vaporized in a container of known volume. The volume of the unknown sample is then determined by measuring with a mercury manometer the change in pressure at constant volume from which the molecular weight can be calculated.

Osmotic Pressure

For the Determination of High Molecular Weights. An example of the type of apparatus used for measuring

osmotic pressure of 0.2 ml. of fluid by the membrane method is shown in the design (Figure 24) taken from the work of Bourdillon (*41*).

The unit holding membrane *g* is made from hard rubber. The inner fluid represents the sample. At room temperature the final reading can be made after 8 hours. There is no correction for specific gravity or capillarity. The precision reported on solutions as low as 0.025 *M* is within 5 per cent.

By the Thermoelectric Method. The calculated theoretical difference in temperature that should exist between two thermally insulated surfaces in moist air at 20°, one wet with water and the other with 0.9 per cent sodium chloride, should be about 0.06°.

Baldes (*12*, *13*) has modified Hill's thermopile method by measuring this difference in temperature from which the osmotic pressure may be calculated. Thermocouples of manganin and constantan wire, 0.1 to 0.05 mm. in diameter, are used. In Figure 25, *A B A* represent constantan wire about 15 mm. in length, soldered to manganin, the junctions being at *A*, which, in turn, are soldered to copper wire at *C*. Loops containing the thermojunction at *A* are made 1 to 2 mm. in diameter. The thermocouple units are insulated with Bakelite varnish. A drop of the known (0.1 to 2 cu. mm.) is placed in one loop and compared with a drop of the unknown sample. The osmotic pressures of aqueous solutions can be determined accurately within a fraction of 1 per cent on quantities as small as 0.1 mg. A critical discussion of the limitations and factors which affect the accuracy of this method is given by Roepke (*270*).

Calorimeters

Heat Capacity of Organic Compounds. Stull (*328*) has designed a semimicrocalorimeter (Figure 26) for determining the specific heats, together with heats of transition and fusion of organic compounds, over the temperature range 100° to 320° K.

The apparatus does not employ a vacuum and requires only 5 to 6 ml. of sample. Measurements were made with a maximum error of 1.25 per cent.

Swietoslawski *et al.* (*18*, *337*) have determined specific heats of solids and liquids, using a microcalorimeter in an apparatus with twin adiabatic calorimeters.

Note. Swietoslawski *et al.* (*336*) report that the smallest calorimeter used for radiological investigations was a silver vessel weighing 0.48 gram and having a thermal capacity of 0.08 calorie, which included the glass containing the sample. This small vessel is placed in a copper block which is evacuated and the whole is placed in a vessel containing water. Temperature is measured by a Beckman thermometer or by means of a thermocouple.

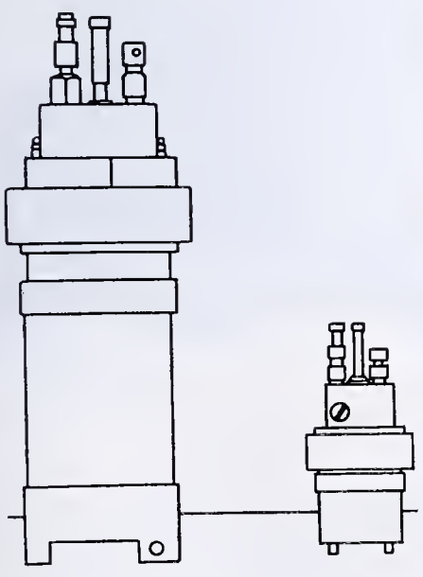


Figure 27. Oxygen Combustion Bomb

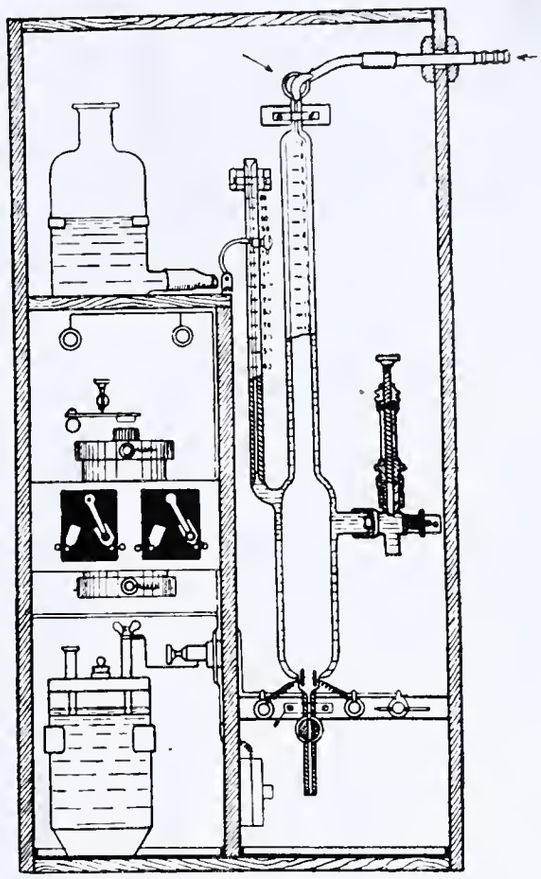


Figure 28. Union Gas Calorimeter

Lipsett, Johnson, and Maas (*192*) designed a calorimeter of the adiabatic type having a heat capacity of 1 calorie and a volume sufficient to hold 4 ml. of solvent. It was employed for measuring heats of solution.

Heats of Adsorption and Vaporization. Swietoslawski and Bartoszewicz (*335*) have designed an adiabatic microcalorimeter requiring only a few centigrams of liquid with which they determined the heats of vaporization of water, benzene, and chloroform with an error not exceeding ±0.03 per cent. The heat of absorption on activated charcoal of benzene, ethyl alcohol, and carbon tetrachloride was measured with an error of 0.6 to 1.7 per cent.

Heat of Combustion. By the Oxygen Bomb. Padoa and Foresti (*241*) developed a microcombustion bomb of 10-ml. capacity for organic material, which was used in a Bunsen ice calorimeter.

When using 6 to 8 mg. of sugar and benzoic acid, they obtained an accuracy of within 1 per cent, and with 30-mg. samples, 0.2 per cent. More recently Papp (*242*), using a similar apparatus, determined the heat of combustion of Acheson graphite accurate to 1 per cent.

Roth, Ginsberg, and Lasse (*278*, *279*) used a bomb of 21.5-ml. capacity and a sample of 100 to 200 mg. The results were accurate to ±0.5 per cent. Figure 27 shows the bomb compared to a bomb of regular size.

Vrijling (*358*), working with the Roth-Hugerschoff calorimetric microbomb mentioned above, obtained satisfactory results for the heats of combustion on benzoic acid and petroleum oils.

Gas Calorimeter. This determination with the conventional apparatus uses a large volume of gas.

Blackie (*36*), working on a problem in which only a few milliliters of gas were obtained from the carbonization of small samples of coal, made a study of the Union gas calorimeter and found that, with careful manipulation and standardization of the procedure, as little as 5 ml. of gas could be used. The instrument (Figure 28) consists essentially of a graduated ex-

plosion pipet into which a few milliliters of the gas to be tested are drawn, the rest of the space being filled with air for combustion. The pipet is surrounded by a jacket containing a liquid of a high coefficient of expansion, which jacket is connected to a graduated capillary tube. On explosion the heat evolved is given up to this liquid, which expands, the rise being proportional to the heat absorbed. For purposes of comparison, the pipet is filled with dilute sulfuric acid and electrolytic gas generated. This volume is measured, diluted with air, and exploded, the rise in the capillary tube being noted. As the heat generated in this explosion is known, the heat given out by the gas, whose calorific value is required, can be calculated from the ratio of the rises in the capillary tube. The capillary rise per milliliter of standard electrolytic gas was 4.28 mm. The error with gases up to 550 B. t. u. should not exceed ±0.5 per cent; with gas up to 900 B. t. u., ±2 per cent.

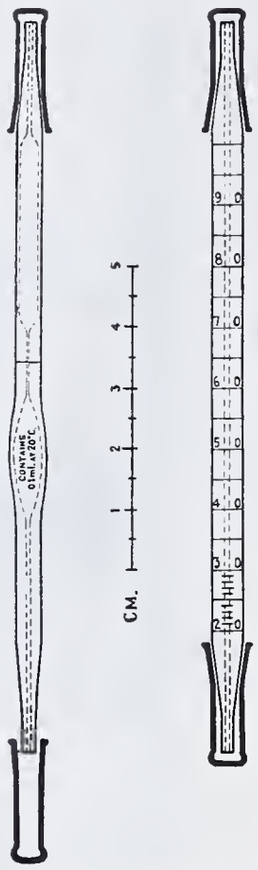


FIGURE 29. PYCNOMETERS

Density

SOLIDS. A general review of the micromethods for the determination of the density of solids and liquids is given by Blank and Willard (*37*) and by Alber (*3*).

Rapid methods for measuring solids using microquantities are not too satisfactory as regards accuracy. Two relatively simple and rapid methods may be used.

1. A capillary tube, 15 to 20 cm. in length and 1 mm. in internal diameter, is calibrated in milliliters and sealed to a larger U-shaped tube. The confining liquid is sealed in the apparatus by mercury. A weighed sample, 10 to 200 mg. (not finely powdered), is placed in a cup on the end of a flexible steel wire. This is inserted under the mercury, so that the sample finally rises to the surface and enters the limb containing the confined liquid. The volume of the sample is read by the rise of the liquid in the capillary tube and from this the density may be calculated. The accuracy is approximately 5 per cent.
2. A well-known method, restricted to a limited range of density, consists in varying the density of a heavy liquid, such as carbon tetrachloride or methylene iodide, with a lighter, miscible solvent until a small particle of the material just floats in the liquid. If the material is finally powdered, the entrapped air carried into the liquid is removed by centrifuging. If a particularly accurate value is required, the final adjustment may be made by controlled cooling or heating of the liquid in a dilatometer, until an exact balance between liquid and solid is obtained. The limiting factor with the method is the accuracy with which the density of the reference liquid is determined. Alber (*3*), measuring the density of the reference liquid with special micropipets, obtained an accuracy of 5 parts per thousand, when using volumes of 0.01 to 0.1 ml. The amount of material required is 50 to 250 micrograms.

DENSITY, USING THE MICROSCOPE. An approximately 1-ml. cell, constructed from glass tubing of known diameter, and mounted on a glass slide, is partially filled with a liquid, such as water, whose height is determined by means of a microscope, the fine adjustment of which has been calibrated. A sample of 10 to 100 mg. is introduced into the cell and the change in height determined, from which the volume and density are calculated. The accuracy is approximately 1 per cent, as determined by the density, using the macropycnometer method (*37*).

LIQUIDS. The determination of the density of liquids, unlike solids, is one of the most important physical constants in organic analysis. The method for this purpose should have an accuracy of 1 per cent or better over a wide range, and it should be possible to recover the liquid for other determinations.

The *Schlieren* method, while it is accurate to 0.0005 and requires little sample, suffers from the fact that the sample is not recoverable under ordinary circumstances, and numerous reference liquids must be available.

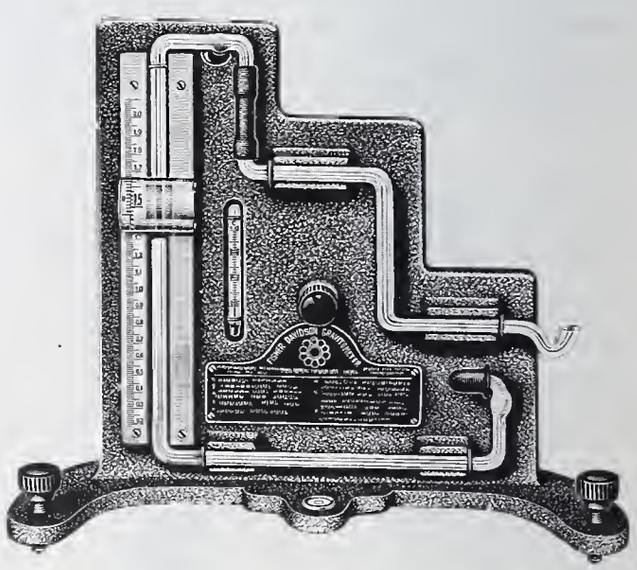
The pycnometer is the most precise method. For microwork the best form is the pipet type (*3*), as shown in Figure 29. The greatest error comes from reading the volume, which is such that weighing to ±0.01 mg. is sufficient when using the 1-mm. pipet. This gives an accuracy within 0.5 part per thousand, when using a volume of 0.1 ml. With the smaller pipet of 1-mm. bore, the accuracy is 10 parts per thousand, and with 0.5-ml. bore, it is within 5 parts per thousand.

A more rapid method for general laboratory work consists in comparing a liquid of known density with that of an unknown, using the balanced-column method. The volume of the sample required is 0.25 to 0.75 ml., depending upon the density of the liquid under test. A known and constant pressure is applied to the unknown liquid in the capillary, and the rise is noted. The point to which water will rise under the same pressure is then determined, and from this the density of the unknown may be calculated. The accuracy is approximately 3 parts per thousand. A commercial apparatus (Figure 30), involving this principle, and using amounts of liquid as low as 0.3 ml. is available. The unknown sample is placed in one column and compared to a standard placed in the other. The accuracy is dependent upon the standard liquid used for comparison. The scale is graduated in 0.01, and one-half a division can be easily estimated.

Fenger-Eriksen, Krogh, and Ussing (*96*) describe a method whereby the specific gravity of liquid is determined by measuring the rate of fall of drops through an immiscible fluid. A precision pipet is used to expel highly uniform drops of any size between 0.01 to 0.1 ml. It has been used for determining the D_2O content, corresponding to density measurements with an accuracy of 1 to 2 in the sixth decimal place, which is about equal to 0.001 per cent D_2O. Figure 31 shows a precision pipet designed by Rosebury and van Heyningen (*272*) for the method just described.

Dielectric Constant

Sufficient data on the dielectric constant of organic liquid compounds are available, so that this measurement can be an aid in the identification and the degree of purity of liquid substances. The contamination of a liquid such as benzene with water can be detected with an accuracy of 0.001 per cent.



Courtesy, Fisher Scientific Co.

FIGURE 30. FISHER-DAVIDSON GRAVITOMETER

Some modifications of the method do not require a large amount of sample, and the cell (*259*) shown in Figure 32 uses about 1 ml. The coatings of the condenser are formed by platinizing the inner tube on the outside and the outer tube on the inside. An excellent review of this subject is given by Pavelka and Kirigin-Mardegani (*243*), who describe a cell and

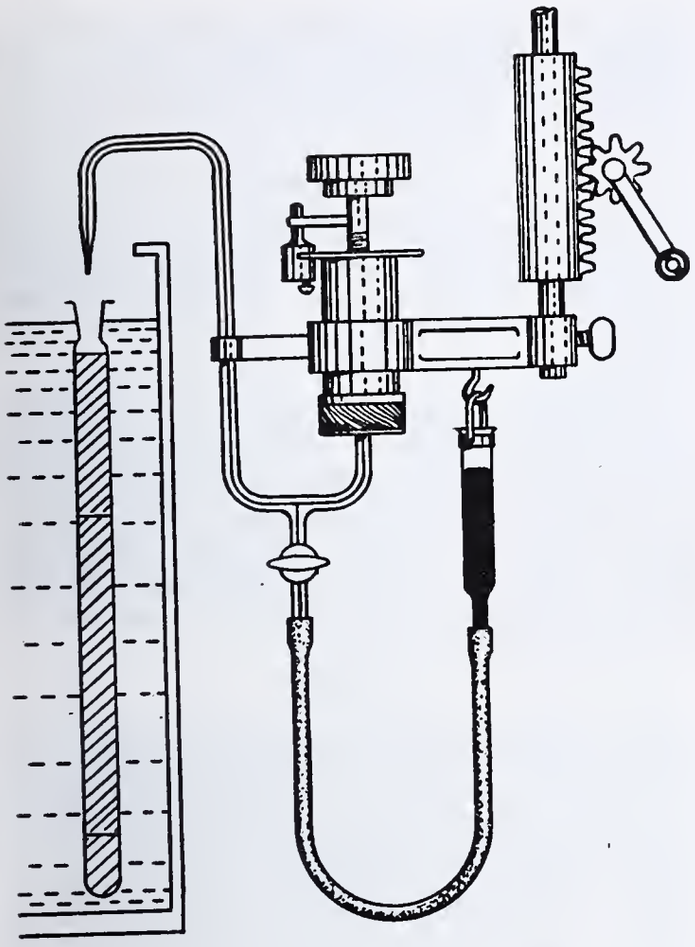


FIGURE 31. APPARATUS FOR DETERMINING DENSITY BY FALLING DROP METHOD

method requiring only a few tenths of a milliliter for carrying out this determination rapidly where the highest accuracy is not required.

Refractive Index

This physical constant is also very useful in the identification of liquids because data are available; but for solids much remains to be done.

The Abbe refractometer, found in most laboratories, requires relatively small amounts of liquid (50 to 100 cu. mm.) and is accurate to ±0.0001. The Zeiss dipping refractometer can be equipped for micro work, which allows a sample of 20 cu. mm. to be measured to within a few units in the fifth decimal place. Methods of less but adequate precision (±0.001) for identification purposes and using less sample (0.1 to 5 cu. mm.) have been developed. Of these, the instrument designed by Jelley (*144*) is rapid and simple, uses only 0.1 cu. mm., and, when properly constructed, is accurate to ±0.001. If the drop of liquid is not homogeneous, this fact may be determined with the microprism, in the case of a mixture of liquids of different volatility, by noting any change in refractive index on standing. In the case of nonvolatile liquids, heating of the prism is necessary and, after cooling, noting any change. Several workers (*6*, *152*, *228*) have reported on a method which correlates the change of depth of focus with a change in refractive index of the liquid. The volume of sample required is approximately 5 cu. mm. and the accuracy is ±0.001. The instrument is calibrated by liquids of known refractive index.

For solids, an immersion method may be applied, using the above apparatus with the same accuracy. However, the accuracy with which the crystal may be orientated is usually of a lower order, such as to give only ±0.003. Here the refractive index of the liquid is varied until the Becke line disappears, which indicates that the refractive index of solid and liquid is the same. Instead of employing a series of standard liquids for this comparison, the refractive index may be changed by varying the temperature of a single liquid. The refractive index of the liquid at that temperature is then determined by any of the regular methods.

Instead of determining the refractive index of the solid, which is time-consuming, the refractive index of the material at its melting or solidification point may quickly be taken by the method proposed by Jelley, using the instrument designed for liquids. If a crystal of the solid is placed in the microprism and heated until the solid melts, its refractive index can be obtained as readily as that for liquids. Frediani (*102*) shows an instrument incorporating these ideas (Figure 33), using the Fisher modification of the Jelley refractometer with an electrically heated prism and thermometer with which the melting point also may be observed, accurate to within 1° to 2° C. The refractive index of compounds which sublime, decompose, or have a high vapor pressure at the melting point cannot be determined by this method. The results are accurate to ±0.002.

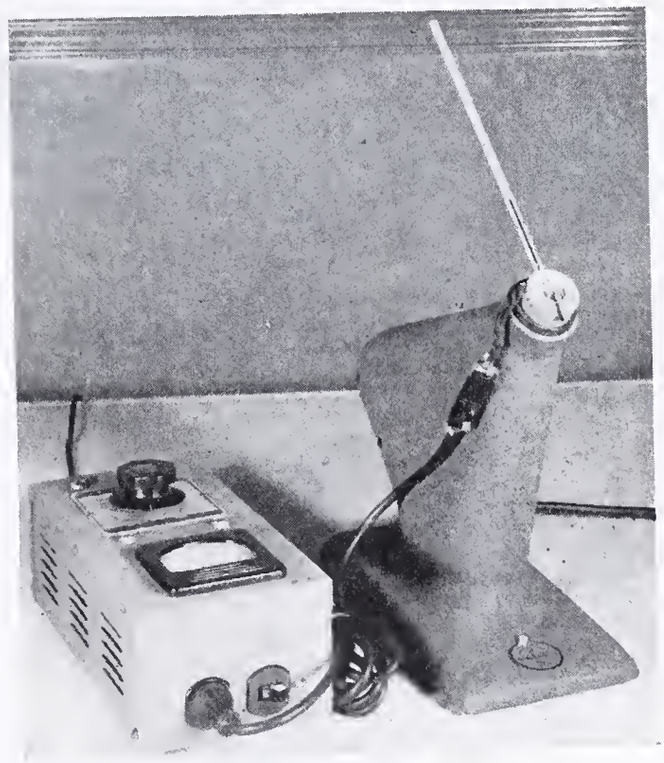
Similar identification methods have been published by Reimers (*260*), using the Kofler method for determining refraction at the melting point.

FIGURE 32. CELL FOR MEASURING DIELECTRIC CONSTANT

Specific Rotation

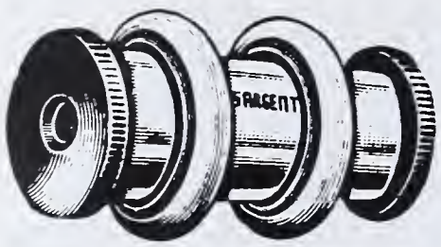
Fischer (*99*) showed that by using capillaries, 5 cm. in length, as polarimeter tubes, results which equaled those obtained with the regular macromethod were possible. Donau (*81*) used tubes of 0.4- to 0.5-mm. bore with satisfactory results. The average of results when compared with capillaries both 5 and 10 cm. long varied approximately ±1.0 from the average of those obtained with the regular-size 10-cm. tube. The variations in successive readings on the same sample are slightly greater than those with the macrotubes.

The Fischer tubes were 1.5 mm. in bore and 5 to 10 cm. in length. The variations on cane sugar, using these tubes, were less (approximately ±0.5 per cent) than the accepted values. It is, therefore, entirely satisfactory (*33*) to measure specific rotation with samples of 5 to 10 mg. and volumes as low as 0.1 ml. with the instruments which are



Courtesy, Fisher Scientific Co.

FIGURE 33. APPARATUS FOR DETERMINING REFRACTIVE INDEX OF SOLIDS AT MELTING POINT



Courtesy, E. H. Sargent & Co.

FIGURE 34. FISCHER POLARIMETER MICROTUBE

used for macropolarimetric measurements. These tubes (Figure 34) are commercially available in 5- to 10-cm. lengths, bore 1.6 to 2.5 mm., and volume 0.1 to 0.5 ml.; semimicrotubes in 10- to 20-cm. lengths, and bore 2 to 4.5 mm. These latter may be had in either metal or glass.

Determination of Solubility

The sample, either liquid (0.1 cu. mm.) or solid (0.1 mg.), is drawn into a capillary tube of 0.5-mm. bore and then about 10 cm. of solvent are added. The end of the capillary is sealed and the two liquids are centrifuged to the closed end. Mixing may be accomplished by means of a thin glass thread with the end fused to form a droplet, or by sealing the other end of the capillary and centrifuging the liquid back and forth. If, after this, the liquids appear clear and homogeneous, they are soluble; if turbid, or if two phases persist, they are insoluble. (For a quantitative study of the phenol-water system, see Smith, *318*.) In the case of solids, it has been found best for qualitative analysis to add the maximum amount of solvent—25 times the weight of the sample.

If a residue remains, the material is termed insoluble. The capillary method of determining solubility is recommended for all semiquantitative work (*291*).

TOEPLER-SCHLIEREN METHOD. This method, which was mentioned under Density, is recommended only for the rapid qualitative detection of the solubility of liquids or solids. The observation was made many years ago by Toepler that when one liquid is added to another, or when a soluble solid is added to a liquid, *Schlieren* or streaks occur, due to regions of changing refraction. These disappear after solution is complete and it again becomes optically homogeneous.

In applying this method to the determination of the solubility of solids and liquids (*291*), the sensitivity must be decreased by using a simple glass cell made from tubing of 4-mm. inside diameter and 60 mm. long, sealed at one end. This holds the static sample, the solvent in this case. The fluid sample whose solubility is under test is added to the static sample from a micropipet with the tip just under the surface of the solvent. The tube is held so that the illumination is obliquely downward. If streaks are observed, the sample is soluble. For solids, a saturated solution is first prepared by adding the solid to the solvent contained in the depression of a spot plate. The clear solution is then used as the test liquid.

Davis and Parke (*76*) describe a nephelometric method capable of measuring solubilities of polycyclic hydrocarbons down to approximately 1 microgram per liter. The method consists in making serial dilutions of a suspension of the hydrocarbon in water and determining nephelometrically the amount of hydrocarbon per unit volume beyond which further dilution causes no reduction in light-scattering, which remains equal to that of the pure solvent. The precision is approximately 5 to 10 per cent.

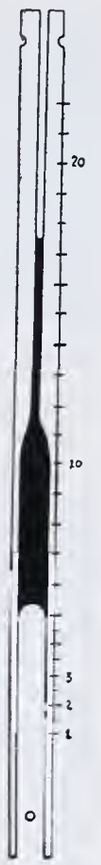


FIGURE 35. SURFACE TENSION BY VERTICAL CAPILLARY METHOD

Surface Tension

The Sugden Parachor, which relates surface tension and density, has proved to be of value in the study of the structure and identification of organic compounds. Hence the determination of surface tension is becoming increasingly more important.

While the du Noüy ring method requires only 1 ml. of liquid, the capillary rise method will give satisfactory results with less than one tenth of this amount. Sutton (*332*) and Natelson and Pearl (*227*) describe modifications which require less than 0.1 ml. The surface tension is determined by the height attained by a liquid in a calibrated tube, consisting of a narrow capillary, 0.2 to 0.3 mm., fused to a wider one. The tube, during a determination, is held vertical, as shown in Figure 35. Two to three minutes are required for the column to come to equilibrium. The density of the liquid must be known with this method. The following formula is used in calculating:

$$S = \frac{hdgRr}{2(R - r)}$$

When the values obtained by the Natelson technique are compared with those obtained by the du Noüy ring method on nine organic liquids, they are all lower by approximately 1.5 per cent. The precision of the method is approximately ±0.5 per cent. Sutton prefers to measure the capillary rise with a manometer. For operating details the original articles must be read.

Ferguson and Kennedy (*97*) describe a method, using a volume of only 1 to 2 cu. mm., in which the density of the liquid need not be known. A short thread of liquid is drawn into a horizontal capillary, shown at the right in Figure 36. By increasing the pressure, through raising the container at the right, the thread of liquid is moved along the capillary until it is plane with the end. This is judged more easily by using a lower-power microscope and a small lamp, as shown at the left of the capillary. The pressure required to bring the liquid to the "plane" position is measured by a manometer filled with aniline. With tubes of less than 1-mm. bore, operating in a horizontal position, the formula for calculating the surface tension is:

$$\gamma = 1/2\ rhpG$$

where h and p refer to the manometer liquid.

Bowden (*42*) modified Sugden's capillary rise method so that reactive or hygroscopic liquids can be run. Sugden's method involves the measurement of the difference in level to which the liquid rises in two capillary tubes of different radii. Figure 37 shows the two capillaries, B and C, sealed to the tube, A, 7- to 10-mm. bore. The liquid, if necessary, may be vacuum-distilled

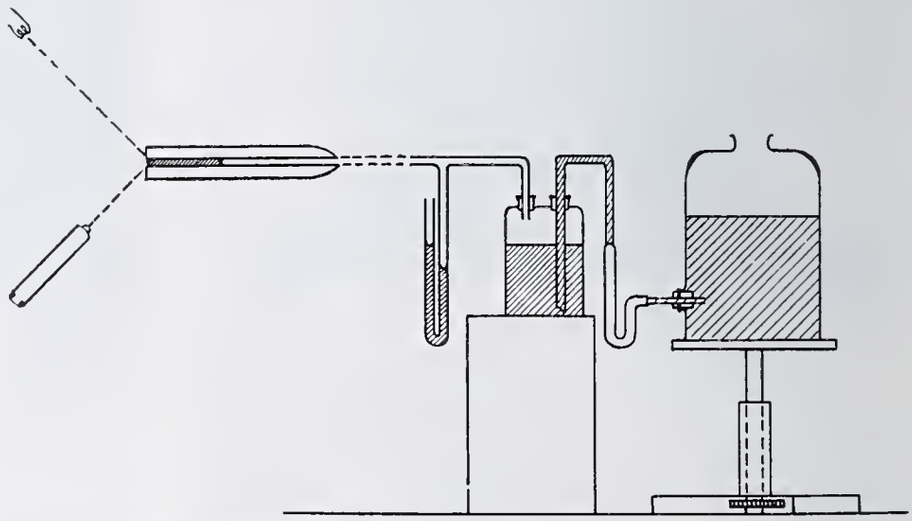


FIGURE 36. SURFACE TENSION MEASUREMENT BY HORIZONTAL CAPILLARY

into A in the absence of air and sealed at S. The surface tension can, therefore, be measured under its own vapor pressure and over moderate ranges of temperature.

The Bowden apparatus, while not developed specifically for micro work, does not require excessive amounts of sample and, since Sugden's apparatus allows working with smaller quantities, the diameter of tube A undoubtedly could be made smaller.

The formula used is:

$$\gamma = \frac{3h + (r_2 - r_1)(D - d)gr_1r_2}{6(r_1 - r_2)}$$

where h = difference in height in the two capillaries
r_1 and r_2 = radii in the two capillaries
D = density of liquid
d = density of vapor
g = acceleration due to gravity

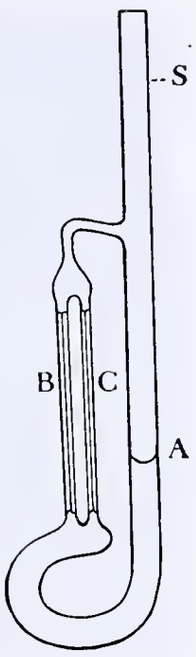


FIGURE 37. SURFACE TENSION BY DOUBLE CAPILLARY METHOD

Sugden's method (*330*), using a cathetometer for measuring the differences in height, is accurate to ±0.3 per cent.

Green (*118*) designed an apparatus which allows the rapid determination of surface tension by the drop-weight method and requires less than 5 drops of liquid. The values are read from a graph scale on the instrument, calibrated in dynes per centimeter.

Viscosity

Since viscosity is associated with structure, constitution, symmetry, polarity, saturation, and molecular size, it can often be an aid in the identification of pure compounds. It has been particularly useful as an aid in the analysis of petroleum fractions.

Cannon and Fenske (*54*) discuss the inherent errors of the capillary-type viscometer. They describe a micromodification (Figure 38, left) which is simple and which, by varying the capillary bore from 0.3 to 2 mm., will cover a range of 0.5 to 800 centistokes. The precision is ±0.2 per cent and the accuracy is approximately ±0.5 per cent.

Bowman (*43*) describes a more complicated apparatus for routine work requiring only 0.03 gram. Its operation, however, is simple and rapid. The method depends on the rate of fall under gravity of a short segment of liquid contained in a longer capillary, which is jacketed as shown in Figure 38 (right). The assembly consists of two jacketed capillaries for different viscosity ranges, together with boiler and controls for maintaining a constant temperature. The precision is 0.1 per cent in the range 2 to 10,000 centistokes, and the absolute accuracy is better than 4 per cent.

MOLECULAR WEIGHT BY VISCOSITY. Through the work of Staudinger (*321*) on the relationship between molecular weight and viscosity, it is possible, by measuring viscosity, to determine the molecular weight of compounds with an accuracy of ±10 per cent in the range 25,000 and higher, using a sample of 5 mg. in 5 ml. of solvent. For making comparisons in a series, this is an excellent method, but for absolute values, careful standardization by osmotic pressure measurements must first be carried out.

The Microscope in Organic Analysis

Inorganic analytical reactions are readily carried out under the microscope, and microscopy, as it is commonly practiced, deals largely with identification by the formation of characteristic crystals on a microscope slide. Most organic reactions, however, are not amenable to this technique. While alkaloid microscopy has developed to a high degree the formation of characteristic crystals, the organic chemist must usually be content to study the crystallographic and optical properties of the crystals of the organic material itself, and of easily formed derivatives. The work of Bryant (*49, 50*) during the past ten years, on the optical crystallography of organic compounds, provides an excellent example of this specialized technique with the polarizing microscope. However, it is not often possible to use microscopical methods as the sole means of identifying organic compounds because optical constants have been determined for relatively few of the enormous number of such known compounds.

The greatest value of microscopy at present consists in comparing the unknown with a selected number of knowns whose properties have been established as similar by chemical tests, melting points, etc. By such means the identity can usually be quickly proved. Then, if necessary, the optical properties and measurement of the crystal angles can be studied carefully and compared with the known, whereby proof of identity often can be more firmly established than by further chemical tests which, even by micromethods, would require considerable sample.

Jelley (*145*) has repeatedly drawn attention to the analytical possibilities of the large influence of wave length on various optical constants of organic compounds. He has described methods of identification by comparing the dispersion of birefringence of different compounds which have some other physical property in common—for example, the same melting point. Although the microspectrographs which he has described are not commercially obtainable at present, and the technique calls for a specialized knowledge of crystal optics, other highly specialized instruments, such as ultraviolet spectrographs and spectrophotometers, have been

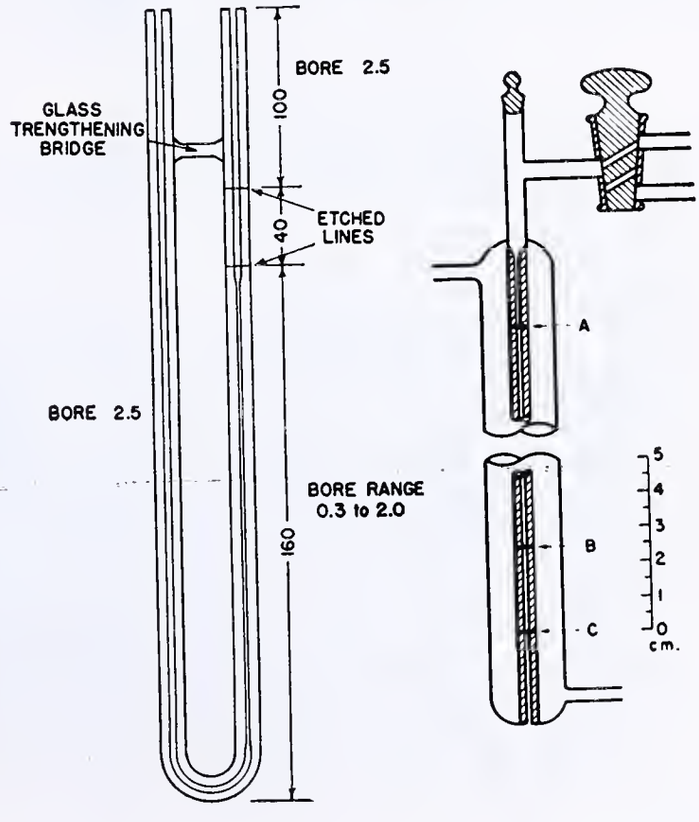
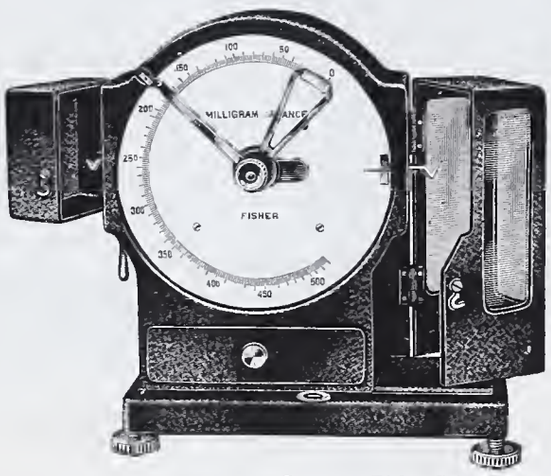


FIGURE 38. VISCOMETERS

developed to the stage where they are commonly used in analytical laboratories.

QUALITATIVE ANALYSIS

A few years ago, if a small amount of an unknown organic material was submitted for identification, unless the analyst was fortunate enough to get an early clue, the material would be used up long before the usual systematic tests had been completed. Organic microchemists until recently have paid little attention to the technique to be applied to the systematic identification of organic compounds. This is in contrast with the wide use in colleges of micro- and semimicrosystems of qualitative inorganic analysis and the publication of numerous books on this subject. The savings in time, material, and reagents, so well established in inorganic microanalysis, have prompted an investigation into the methods which can be applied to organic microanalysis. Most of these tests are adaptations of the standard macroprocedures. Much of the microtechnique used has been published but only recently has any attempt been made to organize it into a systematic, practical scheme of analysis.



Courtesy, Fisher Scientific Co.

FIGURE 39. TORSION BALANCE

The ordinary microscope can be used to advantage in a preliminary examination of the unknown sample as to its homogeneity, crystalline structure, and color. A polarizing microscope can often give additional information on mixtures whose crystals are similar in color and shape, but whose behavior is different under polarized light. If, from this preliminary examination, the substance appears nonhomogeneous, it must be purified before the physical constants can be run. Solids may be recrystallized or sublimed, liquids fractionally distilled. The determination of the cooling curve has long been recognized as the most accurate criterion of purity (see Physical Methods).

Included in the preliminary examination is an ignition test on a few milligrams of the material on a platinum spatula to determine the approximate melting point, the odor given off, the character of burning, and the nature of any residue which, if present, may be further tested for the metals present.

After the preliminary examination, the pure material may be identified by the determination of its physical constants, the optical and crystallographic properties of its crystals, the preparation of derivatives, and the qualitative and quantitative determination of its elements and groups.

In qualitative and preparative work a balance which permits rapid weighing without great accuracy is useful. A torsion-type balance meets these requirements, one of which is shown in Figure 39.

The following section is a summary of a series of papers by Schneider and Foulke (*290*) dealing with micromodifications of methods for qualitative organic analysis based mainly on the scheme of Mulliken and Huntress (*226*).

After the preliminary examination, the compound is tested for the elements most often present other than carbon and hydrogen. These tests are all carried out with not more than 1 mg. of material. The sample is first decomposed by a magnesium powder–potassium carbonate mixture, according to a micromodification of the Barkenbus and Baker (*16*) method. The fusion mixture is leached out with a drop of hot water. Portions of the clear liquid are spot-tested for nitrogen by adding ferrous sulfate and forming Prussian blue, for halogen by adding nitric acid and silver nitrate, and for sulfur by adding acetic acid and a drop of lead acetate.

CARBON, HYDROGEN, AND OXYGEN. Classification reactions are next carried out for compounds of carbon, hydrogen, and oxygen. Micromodifications of the reaction have been developed for:

The fuchsin test for aldehydes
The Molisch test for carbohydrates
The titration of acids
The ferric chloride test for phenols
The saponification of esters, using a diethylene glycol solution of the alkali as proposed by Redeman and Lucas (*257*)
The tests for acid anhydrides and lactones
The phenylhydrazine test for ketones
The alcohol test with sodium

ETHERS AND HYDROCARBONS. If the substance does not give a positive reaction in any of the preceding tests, it is placed in the group of ethers and hydrocarbons. A microtechnique based on the scheme found in the sectional and numbered tests of Mulliken and Huntress has also been worked out for additional tests which limit the field further:

The Fehling and osazone tests for sugar
The acetyl chloride and zinc chloride–hydrochloric acid tests for alcohols
The bromine and phthaline tests for phenols
The bisulfite tests for aldehydes
The Tollens silver mirror test
The bromine addition, iodoform, aluminum chloride, solubility and specific gravity tests for hydrocarbons. (The exact specific gravity need not be known, so that the *Schlieren* procedure with standards chosen for the different ranges can be used to advantage.) For quantitative analysis of hydrocarbons, see Gas Analysis.
The oxidation of side chains
The saponification equivalent of esters (this quantitative determination is run by the Redeman-Lucas method, *257*)

NITROGEN. Garcia and Schneider (*108*), again using the scheme of Mulliken and Huntress, call compounds containing nitrogen those of the second order. These are divided into colored or colorless compounds. The former are small in number and need not be divided further. As the result of microtitrations, the second group is divided into three genera: (1) acid species, (2) basic species, and (3) neutral species. The microtechnique, which involves no special apparatus, has been worked out for the following tests:

Tests for Genus 1. Millon's test, Mulder's reaction, Murexide reaction, Adamkiewicz-Hopkins-Cole reaction.

Tests for Genus 2. Specific tests for ammonia, tests for the salts of the ammonium type, detection of primary amines with nitrous acid, Rimini test for primary aliphatic amines, Simon test for secondary aliphatic amines, and acylation of amines and hydroxyl compounds by: direct acylation, Schotten-Baumann reaction, acylation in pyridine, and formation of picramides.

Separation of amines by formation of aryl sulfonyl derivatives.

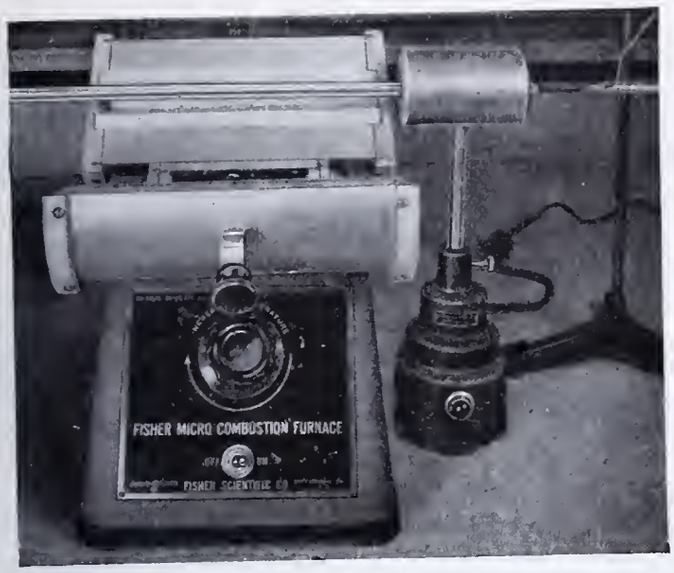
Formation of salts of amines: picrates, salts of 3,5-dinitrobenzoic acid, and salts of *p*-toluene sulfonic acid.

Tests for Genus 3. Tests for nitro group; diphenylamine reactions for nitrates, nitrites, aliphatic nitro compounds, and nitrosoamines; biuret reaction; hydrolysis of species of order 2 with hydrochloric acid, sulfuric acid, and potassium hydroxide; alkali decomposition test; silver nitrate treatment of the original substance.

Most of these tests require about 3 to 6 mg. but some only a few micrograms.

ELEMENTARY ANALYSIS

Elementary analysis has received more detailed study than other branches of microchemistry because it serves organic chemistry—a very large and important field—and also be-



Courtesy, Fisher Scientific Co.

FIGURE 40. THERMOSTATIC SLEEVE WITH COMBUSTION FURNACE

cause the methods were taught and popularized by Pregl. This influence is revealed in the many books and monographs on organic elementary microanalysis published both in Europe and in this country. Of those in English, the one by Niederl and Niederl (*232*) is the best for student use. Detailed directions are given for the common determinations by standard procedures and an up-to-date bibliography on the determinations covered is included.

Combustion Furnaces

About 1900 the method of heating combustion tubes with charcoal was being replaced with the far more satisfactory gas-fired unit. Today, electrically heated macrocombustion units are preferred in most laboratories because the heating is more uniform and the furnace temperature can be more easily controlled. For micro laboratories, electric heating has the added advantage that sharp fluctuations in room temperature are avoided, which permits more rapid and precise weighing.

Since, until recently, no electric furnaces for micro work could be purchased, many designs of units built by individual investigators have been published.

Several commercial units are now available, with or without thermocouples. They are of the split type, so that when required they can be pushed away and the combustion tube allowed to cool. The same unit can be used for other determinations, such as halogen, sulfur, and Dumas nitrogen. The gas-heated cymene bath for heating the lead dioxide in the carbon and hydrogen determination is being replaced by an electrically heated, thermostatically controlled unit, which is a great improvement. Figure 40 shows such a thermostatic sleeve with the combustion furnace unit. The sample, however, continues to be heated with a gas burner in all units except one (Figure 41), in which a small movable electric furnace is used.

Sargent (*223*) has designed an apparatus for the determination of carbon and hydrogen, consisting of an electric furnace and a thermostatic sleeve, together with an electrically heated preheater. These, with the rest of the familiar carbon-hydrogen setup, are mounted on a base so that the apparatus is a convenient and integrally designed unit.

While these units are very convenient for laboratories where a few samples are analyzed, some study has been made of designs for laboratories with many samples, so that a minimum of attention is required during the combustion. In contrast to the all-purpose units which are now manufactured, such apparatus must be designed for maximum convenience for the particular determination. The design must be relatively simple, or more time will be spent in keeping the apparatus in working order than is saved by speedier analyses.

One operation that takes up much time in all combustion determinations is the burning of the sample by moving the furnace or gas burner manually along the combustion tube. Consequently, various devices for moving such a furnace have been designed.

Figure 42 shows Reihlen's device (*253*), in which a clockwork arrangement moves the gas burner along the combustion tube. Ten carbon and hydrogen determinations can be run in an 8-hour day.

The author has found satisfactory a design (*123*) in which an electrically heated split-type furnace is moved by means of a screw turned by an electric motor whose speed is varied as the furnace passes over insulated segments. The speed is made slower at the point where the furnace approaches the sample, so that decomposition will proceed smoothly. Figure 43 shows the carbon and hydrogen apparatus with automatic combustion.

Royer (*282*) uses the same principle but prefers to move the furnace at one fixed speed.

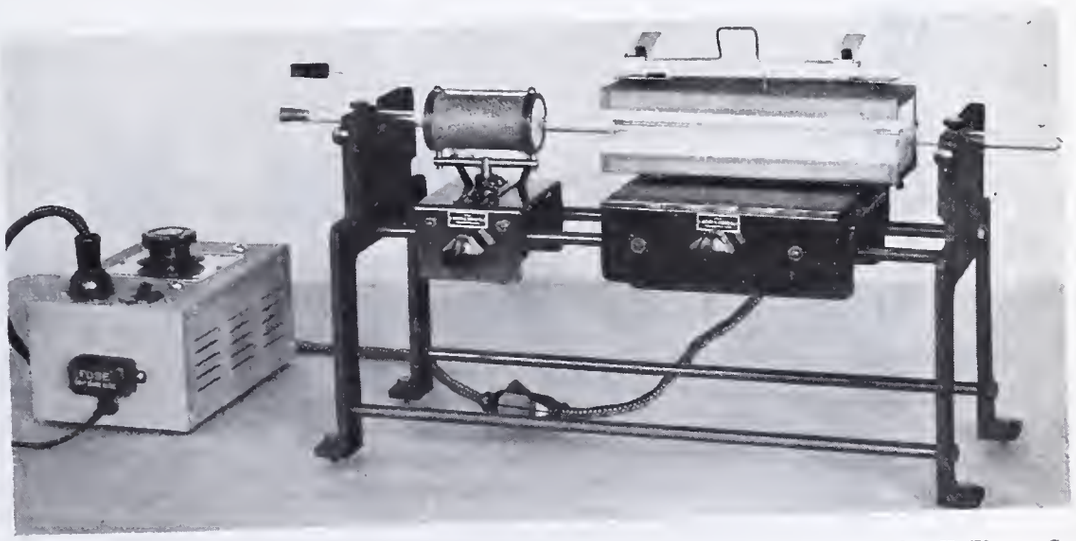
Electric furnaces for heating the sample should be of the split type with lateral movement, so that they need not be turned off at the end of the run to allow cooling of the combustion tube. If they are not so designed, much time is lost in waiting for the furnace to cool or heat.

COMBUSTION TUBES. Side-arm quartz or the new Corning Vycor tubes are much preferred over hard glass because of their freedom from bending under high temperature. Their high cost should be considered prohibitive only by those who have an occasional analysis to run.

Determination of the Elements

By Oxidation Methods

The system commonly employed for the determination of the elements depends upon decomposing the organic com-



Courtesy, Arthur H. Thomas Co.

FIGURE 41. ELECTRIC COMBUSTION UNIT WITH ELECTRIC BURNING FURNACE

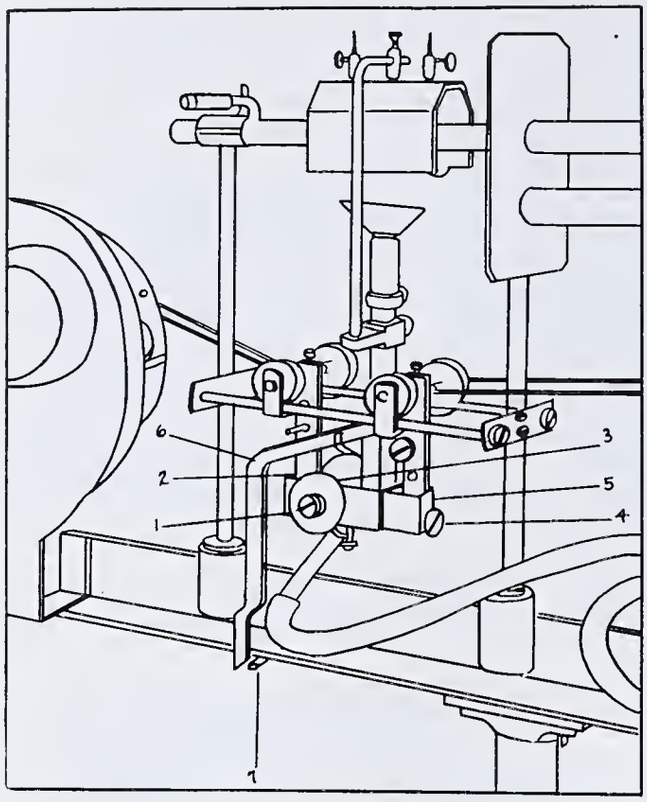


FIGURE 42. REIHLEN AUTOMATIC COMBUSTION FURNACE WITH GAS BURNER

pound by heat in the presence of an oxidizing agent, such as (1) elementary oxygen and a catalyst, (2) wet oxidation, or (3) a dry reagent, such as sodium peroxide or copper oxide. The desired element is retained and determined by appropriate methods. Burning in oxygen gas is preferable because no residue remains and the determination can be carried out with a minimum of interfering ions.

CARBON AND HYDROGEN. *Combustion Tube Filling.* Analysts are divided into two groups, as to the best method of filling combustion tubes for the determination of carbon and hydrogen.

1. Liebig-Pregl. Referring to Figure 44, section *C* is filled with lead dioxide pellets, *B* with silver wool, and *A* with a copper oxide–lead chromate mixture. The fact that metallic silver will take out both halogens and sulfur when heated (preferably by a separate furnace at 450° to 500° C.) has resulted in eliminating the lead chromate from the copper oxide filling (*234*). This results in a much longer life for the combustion tube and the modification should be generally adopted. Substitution of platinized asbestos and platinum foil for part of the copper oxide section has long been recognized as a particularly efficient catalytic mass for substances difficult to burn. Smoother burning, especially of volatile compounds, is obtained if the platinum foil projects 1 cm. beyond the furnace heating section, *A*.

2. Dennstedt-Friedrich (*103, 105*). This method relies entirely on catalytic combustion over platinum, which is placed in *A* (Figure 44). Lead dioxide contained in two boats is placed in *C* to remove all interfering gases. Some workers prefer to place additional boats containing silver and red lead in *B*, if compounds high in sulfur and halogen are run.

The results obtained by the two methods are the same. Method 1 requires more time in filling the tube. The presence of copper oxide ensures an excess of oxygen if the sample decomposes rapidly. When many samples of varying and unknown composition are analyzed with this filling, less care is necessary in decomposing the sample. The filling will last for 300 to 400 analyses.

Method 2 appeals to those who occasionally analyze only a few compounds. The tube may be rapidly assembled and the boats easily changed at any time to fit the type of compound analyzed. There is no need to add and condition the lead peroxide boat if the compound does not contain nitrogen. Sulfur and halogen can be removed by silver which need not be conditioned. The sample should be heated with a little more care, since there is no secondary source of oxygen.

Absorption Tubes. Absorption tubes, in fulfilling their function as a holder of the absorbent, have been found to cause errors in three ways: (1) the diffusion of oxygen gas and a decrease in weight due to the infusion of air, (2) infusion of moist air and an increase in weight, and (3) static charges accumulating on the glass surfaces. This diffusion and infusion are intensified, of course, when a tube is taken into a balance room of different temperature from that of the combustion room.

This can be prevented by designing a suitable tube or by cutting the diffusion to a minimum by inserting fine wires into the capillaries of the Pregl tube (Figure 45, upper). The sealable type employs mercury (*266*) or a steel ball (*146*) to close the

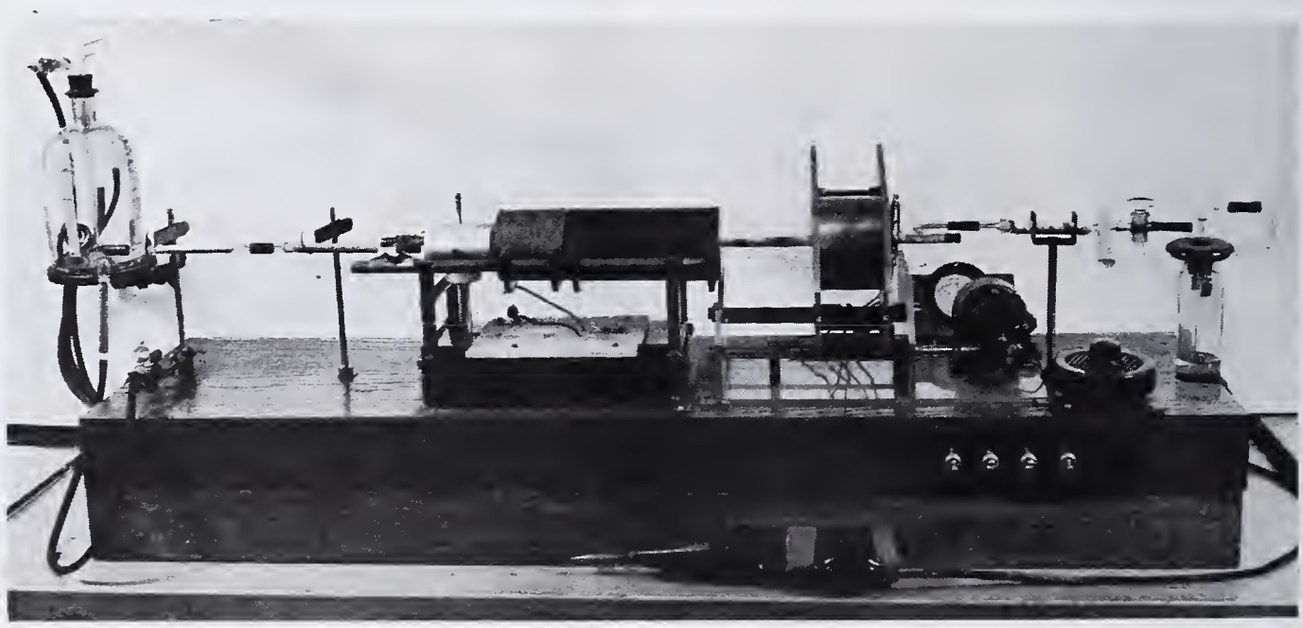


FIGURE 43. HALLETT AUTOMATIC COMBUSTION UNIT WITH ELECTRIC FURNACE

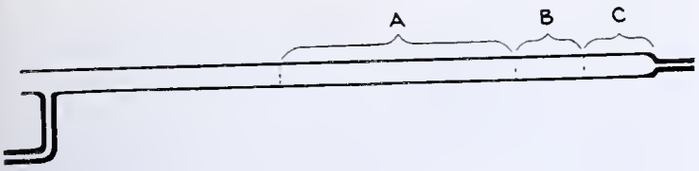


FIGURE 44. COMBUSTION TUBE

capillary opening. The fundamental weakness of the mercury-sealed tube is the possible loss of mercury if the tube is handled carelessly. Another type uses a ground joint (Figure 46) which may be turned to open or close the tube. Many modifications of these sealable tubes have been designed. Still others weigh oxygen-filled tubes with unsealed capillaries and report that satisfactory results are obtained (*282*). The diffusion is so slow that the weighings are not materially affected if the same weighing schedule is adhered to from run to run. In the author's laboratory this method is used with satisfaction. Practically all analysts both in this country and in Europe agree that the best filling for absorbing carbon dioxide is Ascarite. One filling will absorb 500 or 600 mg. Royer (*282*) has found that the drying agent after the Ascarite is not necessary. Of the water-absorbents, indicating-Drierite (anhydrous calcium sulfate) is the most convenient. Other water-absorbents which are as satisfactory but are not indicating are magnesium perchlorate trihydrate and phosphorus pentoxide.

Time of Combustion. The average time taken for burning and sweeping is approximately 45 to 60 minutes, and microanalysts have regarded as critical the rate of combustion and sweeping published in earlier procedures. Laboratory workers with many samples to analyze sometimes wonder how rapidly an analysis could be carried out and still produce acceptable results.

By using a larger combustion tube and packing the filling tightly, Brodie (*46*) cut this time to 25 minutes for 15- to 50-mg. samples. Titov (*344*), using a similar technique, reports 30 minutes for 50- to 60-mg. samples. It has been the experience in this laboratory that a 4- to 6-mg. sample can be burned and swept through with 200 to 300 ml. of oxygen in 15 minutes, using the regular-size microcombustion tube and an automatic combustion furnace (Figure 43). The weighing of both absorption tubes is completed in 8 minutes. As many as twenty samples per 8 hours have been run. (This includes one run over the noon hour unattended.) Belcher (*24*) also uses a high rate of oxygen flow, 12 ml. per minute, whereby a determination requires 35 minutes; using a higher temperature (1350° C.), the combustion time is only 10 minutes.

While the usual weight of sample taken for analysis is 4 to 6 mg., acceptable results with an amount as low as 1 mg. have been reported (*231*).

Procedures have been published for the volumetric determination of water (*187*) and carbon dioxide (*187, 289*) but they are cumbersome when compared to the speed with which the gravimetric determination can be carried out.

Accuracy and Precision. Special studies by Power (*245*) and reports of individual analysts indicate that a skillful operator may expect a precision and accuracy of 2 parts per thousand for carbon and hydrogen, while the average operator will have a variation of 3 parts per thousand. In precision work where a large macrosample (0.2 to 0.5 gram) is used, a precision and accuracy of 2 to 3 parts per ten thousand have been demonstrated (*98, 261*).

Methods which employ wet oxidation are limited to the determination of carbon only and the precision and accuracy are generally not equal to those obtained with combustion in oxygen nor as rapid (*155*). A reliable oxidizing agent has not yet been found. However, recent work by McCready and Hassid (*197*) points to a more satisfactory procedure by using the Van Slyke–Folch combustion mixture, consisting of chromium trioxide, potassium iodate, phosphoric acid, and fuming sulfuric acid. A single determination takes 30 minutes. The carbon dioxide is absorbed and weighed.

NITROGEN. *Dumas* (range 1 to 0.03 mg.). This method depends upon a reliable source of pure carbon dioxide for sweeping the elementary nitrogen after the sample has been decomposed by heat in the presence of copper oxide. Much of the study of this determination has been on methods for the elimination of contamination of the carbon dioxide from the nitrogen of the air. Such contamination will, of course, give a variable blank, which makes it impossible to obtain reliable results.

The common source of carbon dioxide is calcium carbonate (marble) and hydrochloric acid contained in a Kipp generator, so arranged as to prevent contamination from the air. Another method consists in preparing it from a sodium acid carbonate solution and sulfuric acid in a special generator. These methods, once the apparatus is free from air, normally give a satisfactory supply of carbon dioxide. Dry ice contained in a Dewar flask is now being widely used as a source in those laboratories where dry ice is customarily stored. Six pounds placed in a 2-liter flask will last for 3 weeks. The generator is usually freed from air by standing overnight. Carbon dioxide obtained from cylinders (*281*) has been used successfully, but a relatively high pressure of carbon dioxide in the apparatus is necessary.

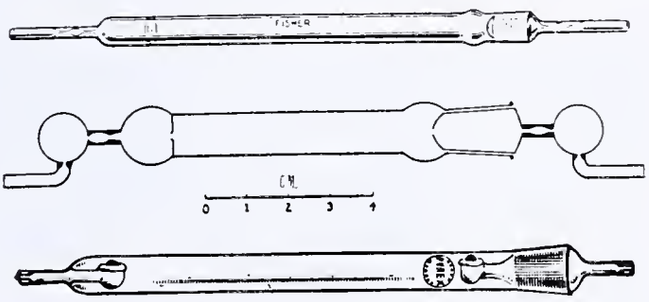


FIGURE 45. ABSORPTION TUBES

Upper. Pregl type
Center. Hallett mercury seal type
Lower. Johns steel ball type

The Dumas method has been generally considered reliable for all forms of nitrogen. However, several reports of compounds giving low results are recorded (*271*). They are usually compounds which form tars or coke on burning—for example, derivatives of pyrimidines, purine, and chlorophyll. However, the nitrogen Kjeldahl values of these compounds were acceptable. Such compounds may often be detected by a slow but continuous flow of bubbles into the nitrometer long after the time a normal compound would have finished burning. The correct nitrogen value for these compounds has been obtained by mixing the sample with copper acetate before adding it to the copper oxide and increasing the temperature (900° to 1000° C.) at which the sample is burned. Passing oxygen generated from potassium chlorate over the heated sample has given good results in some cases. Extra copper must be employed in the combustion tube to take care of the unused oxygen. These modifications are not at present developed to the point where they are reliable and convenient as a general method for such compounds. When it is imperative to determine the nitrogen, these modifications should be tried, but they should be checked by the Kjeldahl method as well as by a carbon and hydrogen determination.

For a more permanent and convenient apparatus the author has found that the capsule method of Clark (*63*) can be used even for microsamples. It allows the combustion tube to remain in place and to be kept at the proper temperature. By means of a special four-way stopcock constructed without dead air spaces, the nitrometers are permanently

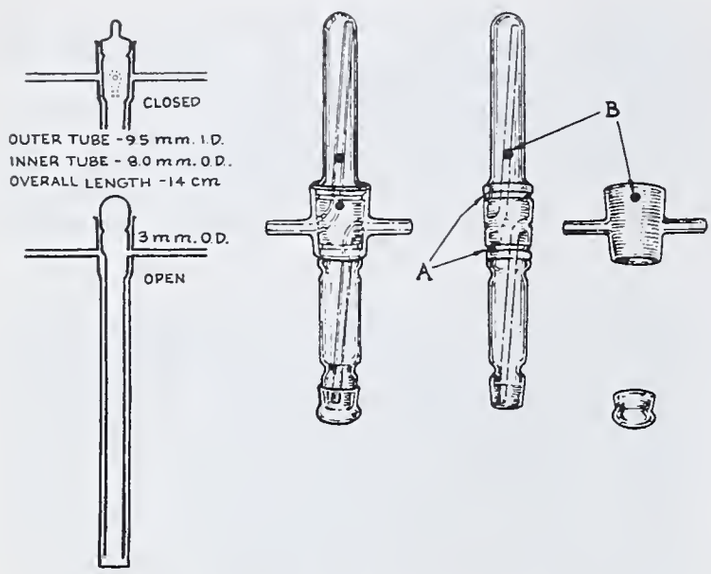


FIGURE 46. ABRAHAMCZIK-TYPE ABSORPTION TUBES
Left, Prater (*246*). *Right*, Clark and Stillson (*66*)

connected to the combustion tube. The section of the combustion tube where the capsule is placed is larger, consisting of a tube 11 mm. in inside diameter. Figure 47 shows the automatic combustion unit with the modification just described.

Because of the variation in operating conditions and in the purity of the carbon dioxide, an increasing number of operators favor considering the vapor pressure of the potassium hydroxide, the film correction of the wall of the nitrometer, and blank separately, in calculating the final nitrogen volume. When operating conditions are uniform, these factors, once determined, are, of course, constant.

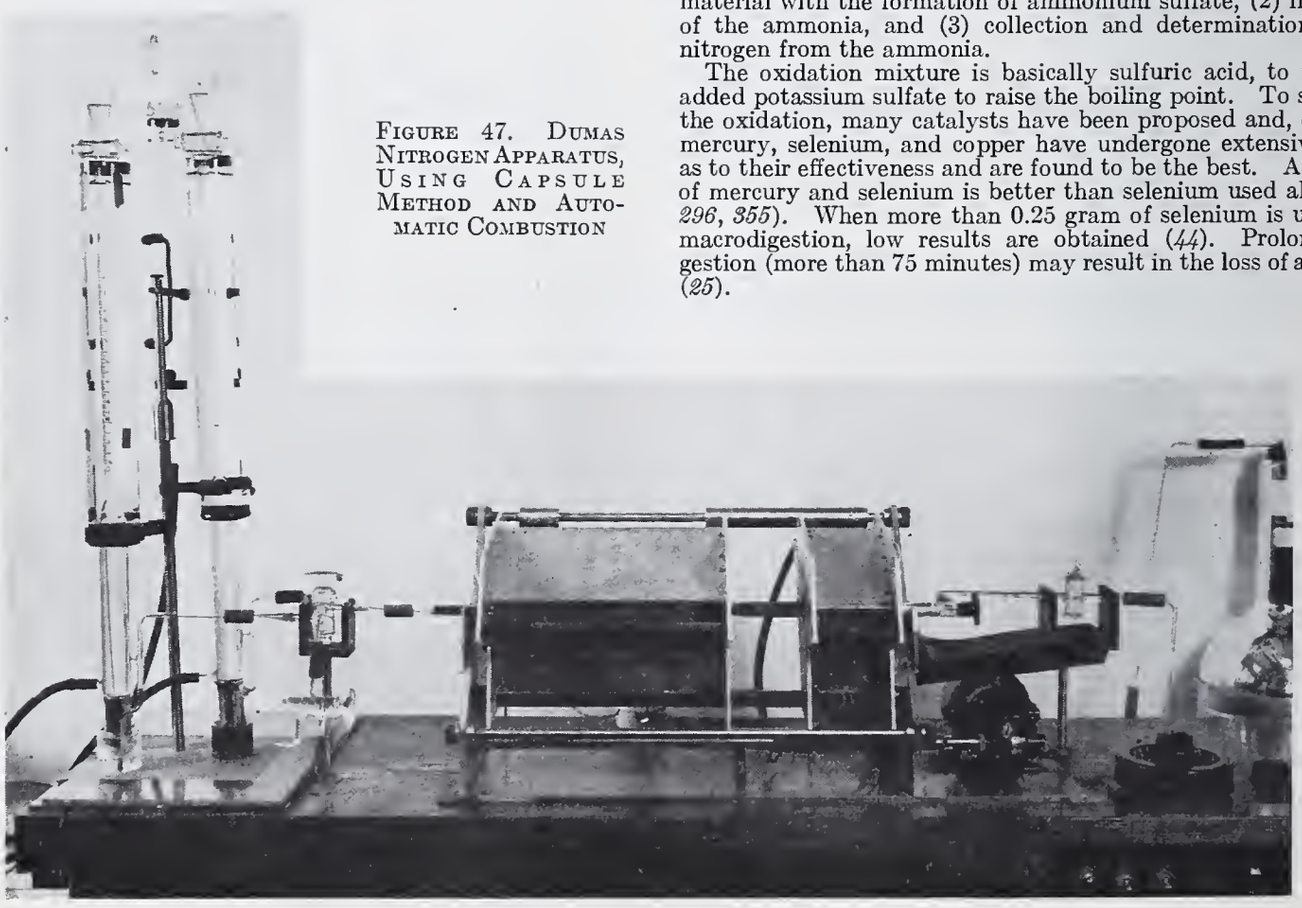


FIGURE 47. DUMAS NITROGEN APPARATUS, USING CAPSULE METHOD AND AUTOMATIC COMBUSTION

Placing the sample in the Pregl shaking tube has been abandoned and the sample is now weighed in a porcelain boat which is added directly to the tube. Static charges developed in a shaking tube make it impossible in many cases to rinse the sample quantitatively into the combustion tube.

Very few references (*166*, *339*, *357*) appear in the literature on the determination of nitrogen below 1 per cent by the Dumas method.

It has been found that samples up to 50 mg. can be burned successfully and the nitrogen collected in a special nitrometer graduated in 0.001 ml. with a total capacity of 2 ml. Using the capsule method, samples as large as 50 mg. have been burned and nitrogen percentages as low as 0.2 per cent determined with an accuracy of ±10 per cent or a variation of ±0.02 in the hundredths place, and using gas volumes as low as 0.03 ml. Vetter (*357*) reports that samples as low as 0.01 per cent can be analyzed, but the author has not had occasion to determine such low percentages. When the regular nitrometer graduated to 0.01 ml. is used, an unknown sample yielding only a few hundredths of a milliliter of nitrogen cannot be measured (*120*). By constructing a nitrometer graduated in thousandths of a milliliter, with a small inverted funnel at the top, the nitrogen can be transferred readily. The small inverted funnel is lowered below the surface of the potassium hydroxide in the funnel of the large nitrometer, and, after the entrapped air is removed by a syringe pipet, the nitrogen is released and caught by the inverted funnel, from whence it is pulled into the nitrometer for measurement. The corrections to be applied appear to be the same as those for the nitrometer of larger bore (3.6-mm.).

As in the carbon and hydrogen determination, it has been found (*21*) that the burning and sweeping time can be decreased, so that only 20 to 30 minutes are required. The time, using the automatic combustion unit (Figure 47), is 25 minutes. It is not necessary to heat the sample twice.

Figure 48 shows the automatic combustion apparatus of Royer (*281*), in which the standard procedure of removing the tube for filling at the end of each run is followed.

Kjeldahl. (0.1 to 3.0 Mg. of Nitrogen.) This method has much to commend it for speedily carrying out routine multiple determinations on compounds of known structure where the proper modification of the oxidizing mixture can be used.

There are three distinct steps: (1) oxidation of the organic material with the formation of ammonium sulfate, (2) liberation of the ammonia, and (3) collection and determination of the nitrogen from the ammonia.

The oxidation mixture is basically sulfuric acid, to which is added potassium sulfate to raise the boiling point. To speed up the oxidation, many catalysts have been proposed and, of these, mercury, selenium, and copper have undergone extensive study as to their effectiveness and are found to be the best. A mixture of mercury and selenium is better than selenium used alone (*23*, *296*, *355*). When more than 0.25 gram of selenium is used in a macrodigestion, low results are obtained (*44*). Prolonged digestion (more than 75 minutes) may result in the loss of ammonia (*25*).

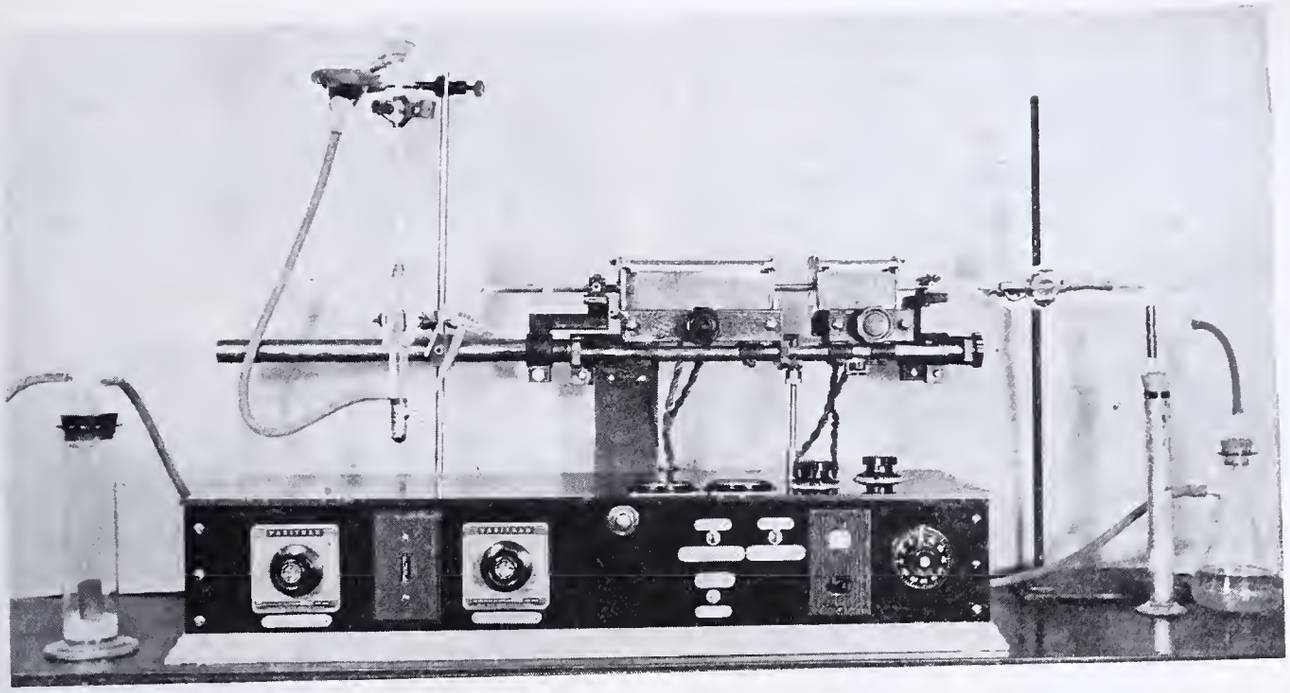


FIGURE 48. ROYER AUTOMATIC DUMAS NITROGEN APPARATUS

Compounds such as hydrazine, nitro, nitroso, and azo must first undergo a preliminary reduction (*287*) prior to digestion, using hydrogen iodide and phosphorus. Diazo compounds should be coupled with phenol to a stable azo derivative before being reduced with hydriodic acid. Volatile compounds must be reduced in sealed tubes at 200° to 300° C. Where a Dumas nitrogen apparatus is available, it will be found more reliable, faster, and convenient to use this method in preference to the modified Kjeldahl procedure just described, for general laboratory analysis of compounds of widely varying composition. For series determinations when the structure is known and the modification has been tested, the Kjeldahl method is much faster. An excellent study of this determination is given by Clark (*65*), using the Gunning-Arnold-Dyer and Friedrich procedures applied to various types of compound. The ammonia is absorbed in boric acid. These methods have been subjected to collaborative study and found satisfactory (*2*).

Many designs of apparatus for the liberation and steam-distillation of ammonia have appeared, the best of which eliminate rubber connections as far as possible, but they are all satisfactory. With the now general use of standard-taper joints, a Kjeldahl digestion flask with a ground-glass joint offers advantages (*349*) in that the sample need not be transferred after digestion to the distillation unit. Figure 49 shows such an apparatus built in this laboratory, using the Nichrome coil heater of Clark (*65*) in the steam generator.

While the collection of the ammonia in standard acid and titration with alkali yields accurate results, considerable work in several laboratories has demonstrated that the alkali may be eliminated and the ammonia collected in boric acid solution, which is then titrated directly with standard acid. The mixed indicator change is not too sharp to an unpracticed eye. However, the method is reliable and simple for routine work when the volume, pH, and end-point color are carefully controlled and standardized (*340*, *359*).

Another widely used method of determining ammonia is by the hypobromite reaction in which nitrogen is evolved. The excess hypobromite is determined by adding potassium iodide and titrating the excess iodine with 0.01 *N* thiosulfate. Here, again, the method to be satisfactory must be carefully standardized (*214*, *253*).

From recent investigations it appears that both the Dumas and the Kjeldahl methods have their limitations but that the latter, with suitable modifications, is reliable for analyzing a much larger variety of compounds than has been considered possible heretofore.

Submicromethods. (10 to 0.3 Mg. of Nitrogen.) In certain biological studies it is necessary to limit the size of the sample and to determine the nitrogen present. The transfer and distillation of such small quantities of nitrogen are not practical if the regular micro-Kjeldahl procedure is used.

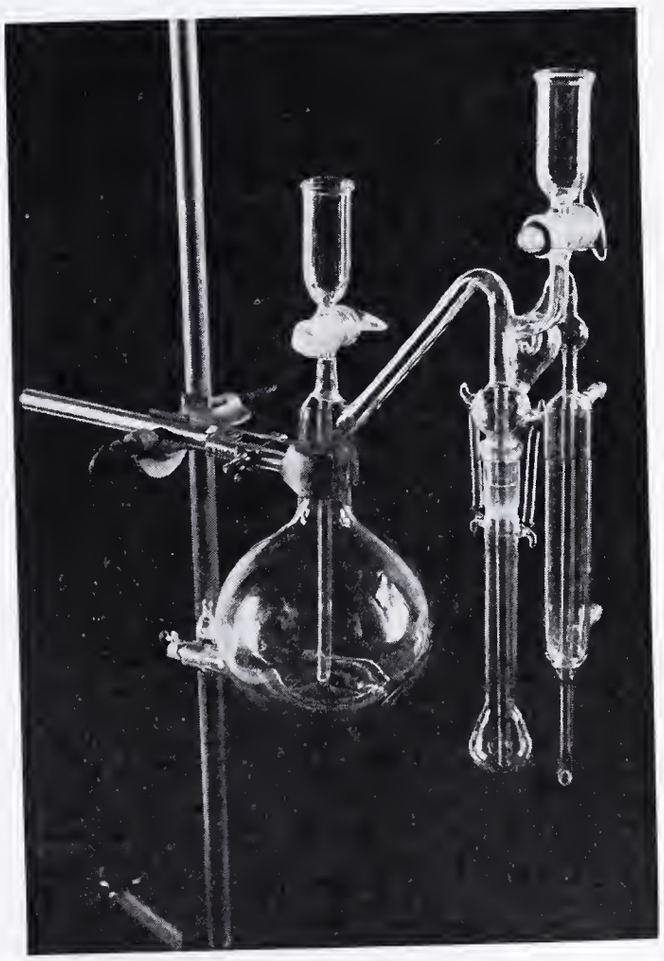


FIGURE 49. KJELDAHL NITROGEN DISTILLATION APPARATUS

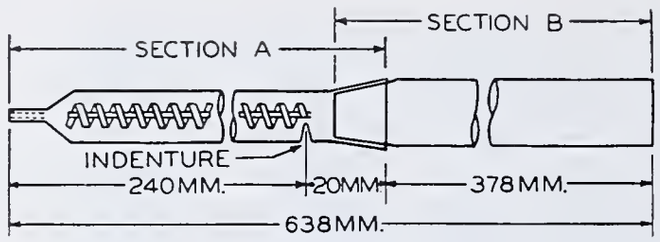


FIGURE 50. REMOVABLE ABSORPTION TUBE

Recently, methods have been developed to determine accurately quantities as low as 0.3 microgram of nitrogen per sample, using the Conway diffusion method for distilling off the ammonia (described under Trace Analysis).

Note. The digestion of the sample by electric heating, as suggested by Clark (*65*), is preferable because bumping is largely eliminated. An electrically heated digestion unit is available from the American Instrument Co.

HALOGENS AND SULFUR. *Oxidation of Organic Matter.*

1. The catalytic combustion method, in which the organic material is burned with oxygen in the presence of platinum catalyst, is reliable for both halogen and sulfur. Two modifications (Figure 50, *22*, and Figure 51, *124*, *125*) of the combustion tube have appeared which permit the absorbed combustion products to be washed from the apparatus without removing the combustion tube from the furnace, at the end of each run. The catalytic combustion method allows titration or precipitation to take place with a minimum of interfering ions. It is not suitable for relatively large samples of volatile compounds, low in halogen, because of the possibility of explosions, nor for volatile compounds, such as carbon tetrachloride, which are high in chlorine (*69*).

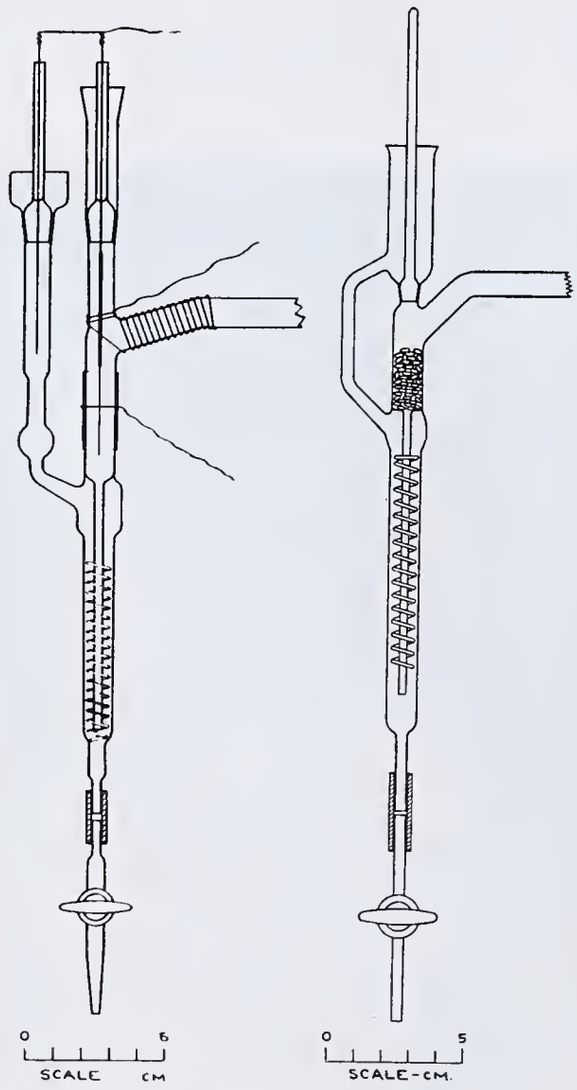


FIGURE 51. ABSORPTION APPARATUS

Left. For sulfur
Right. For halogens

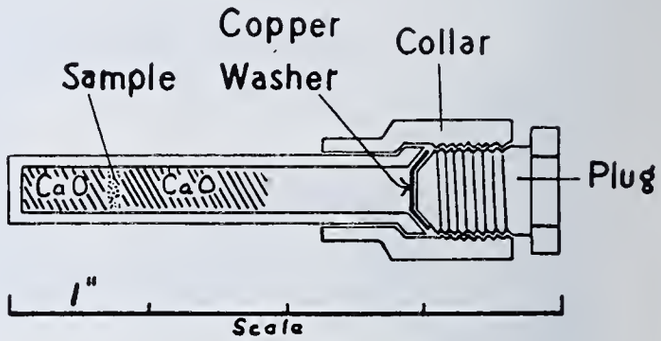


FIGURE 52. LIME FUSION BOMB

2. For such chlorine compounds the well-known lime fusion method is best. MacNevin and Baxley (*199*) use the iron bomb shown in Figure 52, in which to heat the sample. The method has been used in this laboratory for traces of chlorine (0.1 to 0.2 mg.) in volatile solvent compounds (50 to 100 mg.) with satisfactory results. Iodine and bromine cannot be run by this procedure. By using the same method, but placing the mixture in a sealed glass tube and heating to 425°, Weber (*360*) has successfully analyzed chlorine, bromine, and iodine.

3. The Carius method of decomposing a sample in concentrated nitric acid in a sealed tube at 300° C. has been a standard procedure for years. Even in the micromodification it still suffers from the disadvantages of an occasional explosion, the long heating period, and the opening of tubes under pressure with a possible loss of material and contamination from glass splinters. A recent paper (*229*) on these defects shows how they may be minimized. The heating period is reduced to 30 minutes. An electrically heated bomb furnace is available from the American Instrument Co. Details for constructing a furnace of this type are given by Kuck and Griffel (*169*).

4. Procedures using a micro- and semimicro nickel Parr bomb have been carefully studied (*297*). Because of the relatively large quantity of sodium nitrate, sugar, and sodium peroxide, a blank on new reagents is especially necessary. Because of this high salt concentration, the conditions of precipitation in the case of sulfate—e. g., barium sulfate—must be carefully controlled to eliminate coprecipitation. The method requires careful measuring of the reagents and thorough mixing with the sample to ensure proper ignition. The method is usually reliable, and, when several bomb cups and a titration procedure are used, it is rapid for series runs. The average time for a determination is 30 minutes. When salts, such as sodium, are analyzed, there is no danger of loss of sulfur or halogen, due to holding back part of the sulfur as sulfate in the ash—a weakness of the catalytic combustion method.

It is equally satisfactory for chlorine, bromine, or iodine, although Colson (*69*) reports poor results for chlorine in carbon tetrachloride.

A disadvantage of the bomb procedures for gravimetric determination in the case of liquids, which must be weighed in a capillary, is the solution and, later, separation of silica with the precipitate. Modifications (*185*) overcome this but when possible it is more convenient to use a titration procedure.

Estimation of Halogen. The gravimetric determination of halogen as the silver salt is time-consuming and is useful only for the occasional analysis when the preparation of standard solutions is not warranted. The gravimetric factor for silver iodide is so unfavorable that in micro work very careful technique is required on the part of the analyst. While the end point in the Volhard method (*299*) does not appear sharp to those who use it only occasionally, precise results (±0.01 ml.

of 0.02 *N* silver nitrate) are possible when the eye becomes accustomed to it. In the case of chloride, the silver chloride must be removed by filtering or centrifuging to prevent fading of the end point.

The method (*298*) of oxidizing iodide to iodate with bromine water and subsequently liberating six iodines from potassium iodide, which are titrated with sodium thiosulfate, is preferred for iodine because of the excellent factor. The method can also be used for the determination of iodine in the presence of chlorine and bromine (for review see Leipert, *181*).

Similarly, bromine may be oxidized to bromate with chlorine water and the same procedure followed. It is especially useful for small amounts of bromine in the presence of chlorine (*164*). A review of one hundred and fifty papers on the microanalytical determination of bromine is given by Leipert (*182*).

Adsorption indicators are used in the titration of chlorine and bromine with silver nitrate but the end-point color change is not too sharp. The addition of acetone (*51*) or a measured amount of the halogen will give a more satisfactory titration. The color change is easily observed when more silver halide is present.

Volumetric Sulfur. A good indicator for the titration of sulfate with barium chlorides has been sought for some time. Studies in Europe (*1*) and in this country (*126*) on the use of tetrahydroxyquinone have shown that while the end point is not strikingly sharp, any chosen color point is reproducible under standardized conditions with adequate precision. For routine work it is to be preferred over the gravimetric procedure. Its use with the Parr-bomb procedure has been advocated but the end-point change is difficult to detect (*201*).

When the gravimetric procedure is used, time can be saved by adding picric acid to speed up the precipitation of the barium sulfate. It can be safely filtered after one hour (*185*). Ignition of the precipitate is not necessary, and drying at 150° C. gives equally accurate results (*286*).

By Hydrogenation Methods

The precision and accuracy for the determination of halogen and sulfur are within 1 per cent.

Another system introduced by ter Meulen (*174, 209, 210*) for semimicroanalysis is based upon decomposition of the organic material in a combustion tube in the presence of hydrogen and the passing of the gases over a suitable catalyst, whereby:

Nitrogen (thoriated nickel) gives ammonia, which is titrated (*119, 136, 176, 208, 312*). Soda lime is added to remove halogen and sulfur.

Halogen (ammonia and nickel) gives ammonium halide or (oxidized chromite) (*175*) gives halogen acids (*52, 313*).

Sulfur (platinum or platinized asbestos) gives hydrogen sulfide (*80, 110, 117, 195, 342, 364*).

Oxygen [nickel chromite (*114*), nickel (*188*), nickel thoria (*53, 157, 350*), platinum in silica gel (*215*)] gives water.

Note. Zimmermann (*376*), Unterzaucher (*348*), and Korshun (*165*) have successfully used Schutze's method, in which the decomposition products are passed over hot carbon in the presence of nitrogen. The carbon dioxide is oxidized with iodine pentoxide, absorbed, and weighed.

Arsenic, cadmium, and mercury (*210*) have been determined in organic compounds by passing the gases with hydrogen over heated asbestos into a detachable cooled tube in which the metal is deposited and weighed.

Carbon and hydrogen (*119, 153*) are determined by burning the sample in oxygen in a combustion tube and passing the gases first over a manganese dioxide catalyst at 400°, then over heated lead dioxide, where any sulfur or nitrogen oxides and halogen are removed. This simple tube filling, together with a low-temperature catalyst, makes the method an improvement over that of Liebig-Pregl. A modification, whereby the lead dioxide is replaced by potassium chromate, and Pb_3O_4 and platinum are added to the manganese dioxide, is given by Hepner and Pojas (*131*). Overheating may lead to destruction of the catalyst, so that it is advisable to use electric heating.

The results obtained by these procedures are as accurate and rapid as those by other methods. They have been tested by a sufficient number of investigators to warrant further study and more general adoption. The nitrogen and halogen determination by this method is a simple titration. In the case of sulfur, the hydrogen sulfide can be titrated iodometrically, and the author has obtained excellent results with this method. Greater interest has centered around the determination of oxygen by hydrogenation because this element has always been determined by difference in the oxidation system. Most of the factors which contribute to successful results have been studied, so that the method should soon take its place as a reliable procedure.

Because the oxidation methods are the ones commonly taught and used, they have been exhaustively studied with all types of compounds; this is not true, of course, of the ter Meulen system. When decomposition takes place in hydrogen, the coke and hydrogenation products must be burned out periodically with oxygen. If this is not done, low results may be obtained, owing to retention of part of the constituent by the carbon. Most of the references to the ter Meulen system are recent and present the latest developments as well as past studies.

Less Common Elements in Organic Compounds. Organic compounds containing elements, other than those ordinarily present, are often prepared and a determination for such constituents may be necessary. The determination of metals by burning to the sulfate, oxide, or the metal itself, requires careful manipulation and considerable sample because of the low factor. It is always assumed that other interfering constituents are not present.

Much study has been given, particularly in biochemistry, to the estimation of traces, but the microdetermination of such constituents of organic compounds, present in amounts of several per cent, has not received as much attention. The following outline summarizes methods which can be applied to these determinations.

Meyer and Hoehne (*211*) report on methods for the estimation of metals in complex organic salts by combustion in oxygen of a 5- to 30-mg. sample for 30 minutes and weighing the metal or its oxide. Hydrogen is used for reduction when required. From 1 to 8 mg. of residue is weighed and the results are accurate to approximately ±1 per cent. Cobalt and nickel are weighed as the metal; iron, chromium, and vanadium, as the oxide. Calcium, barium, sodium, and potassium are usually determined as the sulfate by adding sulfuric acid to the sample in a platinum boat, placed in a platinum cylinder to prevent loss by creeping. Roth (*276*) has determined rubidium and cesium in organic compounds by this method.

Tabern and Shelberg (*338*) discuss the general problem of the decomposition of organometal compounds for macrosamples and prefer a sulfuric–hydrogen peroxide digestion.

Arsenic. Sloviter, McNabb, and Wagner (*315*) have obtained satisfactory results in determining arsenic in organic compounds by decomposing with nitric-sulfuric acid and precipitating the arsenic with hypophosphorous acid. The arsenic is collected on a filter and dissolved in a measured excess of bromine (bromate-bromide solution). The excess bromine is determined iodometrically by titrating with 0.1 *N* sodium arsenite. The precision of the titration and the accuracy of the method with compounds containing 30 per cent arsenic, when using samples of 30 to 50 mg., are ±1 per cent. Halogens do not interfere. The time required to carry out a single determination is 2 hours.

Kolthoff and Amdur (*163*) reduce the combined arsenic to elementary arsenic with hypophosphite reagent, dissolve in an excess of standard ceric sulfate, and back-titrate the excess with arsenic trioxide with an accuracy of 0.5 per cent for 1 mg. and within 2 per cent for 0.1 mg.

Wintersteiner (*301*) uses a sample of 5 to 10 mg., which is decomposed with sulfuric acid and hydrogen peroxide. Potassium iodide is added and the liberated iodide titrated with 0.01 *N* sodium thiosulfate. The sample may be decomposed with

sodium peroxide in a Parr microbomb and the arsenic titrated, as just described (*20*). Bromide and iodide interfere and must be removed.

Satisfactory results may also be obtained by precipitating the arsenic as magnesium ammonium pyroarsenate.

For quantities of arsenic up to 1 mg., Cassil (*55*) prefers to take the digested sample and evolve the arsenic as arsine, which is absorbed in mercuric chloride–gum arabic solution. It is then titrated with 0.05 *N* iodine solution. The accuracy is ±1 per cent.

Jacobs and Nagler (*143*) and How (*139*) present excellent summaries with references to the analytical microdetermination of arsenic.

Boron. Roth (*275*) fuses the organic material with sodium carbonate in a platinum crucible and titrates the boric acid with mannitol; or the boric acid may be distilled as the methyl ester absorbed in alkali and titrated. The results reported are accurate to within 1 per cent.

Calcium. Calcium is usually precipitated as the oxalate and may be weighed in this form (*255*) or the calcium oxalate may be titrated, using potassium permanganate (*293*). Ceric sulfate, added in excess and back-titrated with thiosulfate, has given satisfactory results. Lindner and Kirk (*189*) have found that, with small quantities of calcium, the potassium permanganate method is accurate, owing to compensating errors, and they, therefore, prefer to titrate directly with ceric sulfate, using phenanthroline–ferrous sulfate as the indicator.

Cobalt. Cobalt may be precipitated with anthranilic acid as $Co(C_{17}H_6O_2N)_2$ (*361*). Nickel and copper interfere.

Copper. For amounts of copper above 1 mg. the electrolytic method with direct weighing is satisfactory. MacNevin and Bournïque (*200*) have shown that weighing errors are greater than those of manipulation. Hecht and Reissner (*130*) have obtained accurate results with amounts of 1 mg. or less by precipitating with the following organic reagents: (1) 5,7-dibromo-8-hydroxyquinoline, $Cu(C_9H_4ONBr_2)_2$; (2) benzoinoxime (*178*), $Cu(C_{14}H_{11}O_2N)$; and (3) salicylaldoxime, $Cu(C_7H_6O_2N)_2$.

Fluorine. The decomposition of organic fluorine compounds is sometimes attended with great difficulty. Hubbard and Henne (*140*) used a microcombustion procedure of burning the sample in oxygen and passing the gases over silicon dioxide at 900° C. to form silicon fluoride, which was absorbed in water and titrated with cerous nitrate. However, some compounds, especially those with high fluorine content, are not decomposed by this treatment. Methods using an alkali-metal fusion have given satisfactory results on a variety of such compounds. Elving and Ligett (*86*) have made a study of the alkali-metal fusion method for decomposition of macrosamples and found it satisfactory for all types of fluorine compounds—solid, liquid, and gas. Vaugh and Nieuwland (*353*) used metallic sodium in ammonia for decomposing organic halogen, including fluorine compounds. In connection with this work, they showed that a micromodification could be used. It seems likely, therefore, that a microprocedure, as suggested by Elving and Ligett, using the alkali fusion method, could be worked out employing thorium nitrate. Considerable work on this titration for amounts below 50 micrograms has been done, but for satisfactory results, rigid standardization is necessary. For larger quantities of 1 mg. or more, the method should not present difficulties (*68*).

Iron. 1. For 0.5 to 5 mg., reduction and titration with dichromate, using diphenylamine, have given good results (*303*). Titration with potassium permanganate may also be used (*160*).

2. By reduction with silver (*236*), Fe^{++} is formed, which can be titrated with ceric sulfate (0.002 to 0.015 *N*) but with results low by approximately 1 per cent of the amount present. For drop analysis, Kirk and Bentley (*151*) use a cadmium-mercury liquid amalgam for reduction. An excess of ceric sulfate is added, which is back-titrated with standard ferrous ammonium sulfate solution, using phenanthroline–ferrous sulfate indicator.

3. Straub (*324*) and Rappaport and Hohenberg (*254*) have used the iodometric method for the determination of iron. Results accurate to within 1 to 2 per cent may be expected.

Lead. The separation of lead from impurities by electrolytic deposition is practical but, unless great precaution is taken, the lead deposit may be of variable composition. Therefore, some workers prefer to titrate the lead iodometrically (*302*), or the lead after electrolysis may be changed to lead sulfate and weighed. By this method, Brantner and Hecht (*45*), with 0.5 to 5.0 mg. of lead, succeeded in obtaining results accurate to within ±1 per cent.

Magnesium. For 0.5 to 1 mg. of magnesium, precipitation and weighing as magnesium ammonium phosphate ($MgNH_4PO_4 \cdot 6H_2O$) is satisfactory. There is no need to ignite to the pyrophosphate (*368*). Benedetti-Pichler and Schneider (*27*) used the method and obtained results on 0.5 mg. of magnesium accurate to ±0.5 per cent.

Strebinger and Reif (*326*) precipitate the magnesium with 8-hydroxyquinoline [$Mg(C_9H_6ON)_2.2H_2O$]. The error is approximately ±1 per cent. Calcium interferes when it is present in concentrations greater than 0.04 mg. with 5 mg. of magnesium.

Mercury. The organic material containing 2 to 5 mg. of mercury is destroyed with concentrated nitric acid by the Carius method and the metal is electrolytically deposited on a gold cathode. The accuracy and precision of the method as shown by the results of eight compounds, reported by Verdino (*356*), are within ±0.4 per cent.

Rutgers (*283*) prefers to decompose the organic compound in a tube with oxygen, charged with the vapors of aqua regia. The mercuric chloride formed is electrolyzed as above.

Boetius (*39*) burns the organic substance in oxygen and passes the gases over heated lead oxide and silver to remove the halogen. With compounds containing nitrogen the material is burned in the presence of carbon dioxide, with a tube filling of lead chromate, copper, and silver-covered pumice. The mercury is caught in a cooled tube filled with gold, which is weighed after the combustion.

Decomposition in a flask of the organic material, using concentrated nitric acid, gives satisfactory results but a reflux condenser must be attached to prevent loss of mercury during digestion.

For organic salts the method of Rauscher (*256*) is both accurate and rapid. The sample containing 2 to 50 mg. of mercury is reduced by ethanolamine in 5 to 15 minutes and the metallic mercury centrifuged (*311*) and weighed or dissolved in nitric acid and titrated with thiocyanate. Results are accurate to ±0.2 per cent. Where the mercury is directly bound to carbon, this method is not generally applicable, but refluxing with sodium in ethanolamine and dioxane has been used with some success for such compounds.

Sloviter, McNabb, and Wagner (*314*) have modified Rupp's volumetric method for determining semimicroquantities of mercury in organic compounds. The sample is decomposed with potassium persulfate and concentrated sulfuric acid. The mercury is precipitated as the metal by reduction with hydrazine sulfate of the double iodide. The metal is dissolved in 0.1 *N* potassium bromate–potassium bromide solution, potassium iodide is added, and the liberated iodine is titrated with 0.05 *N* sodium thiosulfate. A single analysis requires 3 hours and halogens do not interfere.

Phosphorus. The well-known Lorenz (*193, 300*) method of precipitating the phosphorus as ammonium phosphomolybdate has given uniformly good results in microprocedures. The precipitate may be weighed or titrated. The organic material is best decomposed by nitric acid and hydrogen peroxide or by fusion with sodium peroxide in a Parr microbomb. The Carius method (*239*) may give low results, through combination of the phosphoric acid with the glass.

Potassium. 1. The determination of potassium by precipitating as potassium sodium cobaltinitrite has received much study (*267*) and the conclusions drawn point to a lack of reproducibility by this method among different analysts, owing to the variable composition of the precipitate. If conditions are carefully standardized, however, satisfactory results accurate to within 1 per cent are obtained.

2. The most accurate and reliable method for amounts of 0.5 to 1 mg. consists in precipitating as potassium chloroplatinate and weighing (*60*). Sodium up to four times that of the potassium present does not interfere. Results accurate to 0.5 per cent are obtained. Bullock and Kirk (*51*) prefer to reduce the potassium chloroplatinate in a neutral water solution with metallic magnesium and titrate the chloride with silver nitrate. The results reported for this procedure on 0.5 to 1 mg. of potassium are accurate to 0.5 per cent.

A volumetric method based upon the equations:

$$K_2PtCl_6 + 6KI = K_2PtI_6 + 6KCl$$

$$K_2PtI_6 + 2Na_2S_2O_3 = K_2PtI_4 + 2NaI + Na_2S_4O_6$$

has given good results according to Shohl and Bennett (*309*). Results on amounts of potassium, 0.4 mg. and above, are accurate to ±2 per cent.

A bibliography on the determination of potassium is given by Cimerman and Rzymowska (*57*).

Selenium. The compound containing 0.5 to 2 mg. of selenium is burned in oxygen and the gases are passed over platinum contacts. The selenium dioxide formed is absorbed in water. It can be estimated by reducing from metallic selenium with sulfur dioxide and weighing as the metal if the amount is 2 mg. or above (*7*). Smaller amounts (0.5 to 1.0 mg.) can be determined accurately by treating the selenious acid with bromide–hydrobromic acid mixture and titrating the selenium iodometrically

(*362*) with 0.02 *N* thiosulfate. The accuracy and precision are ±1 per cent.

Silica. For amounts below 5 per cent, the well-known colorimetric method (*79*) of forming yellow silicomolybdic acid with permanent standards made from picric acid is best. Phosphorus and iron interfere. For larger amounts, ashing and determining the silica by loss of silicon tetrafluoride must be used. When the residue is treated with hydrogen fluoride satisfactory results with samples containing 1 mg. or more of silica and accurate to ±1 per cent are reported (*292, 343*).

Silver. For 0.5 to 5 mg. of silver, the Volhard method may be used, in which the silver is dissolved in nitric acid and titrated with 0.01 to 0.02 *N* ammonium thiocyanate, using ferric alum as the indicator.

Schulek (*294*) proposes a method which can be used in the presence of chlorides, bromides, and cyanides. The organic matter is first destroyed by sulfuric acid and hydrogen peroxide. To the neutral solution is added excess potassium cyanide. The excess is then destroyed by formic acid; 2 per cent phosphoric acid and an excess of bromine are added. Phenol is added to destroy the excess bromine and the cyanogen bromide is determined by adding potassium iodide and titrating with 0.01 *N* sodium thiosulfate. Results agree with the Volhard microprocedure:

$$AgCN + HCN + 2Br_2 = AgBr + HBr + 2CNBr$$
$$CNBr + 2HI = HCN + HBr + I_2$$

Sodium. The organic material is ashed and the sodium, 0.5 to 1 mg., is determined as sodium zinc uranyl acetate by the method of Barber and Kolthoff (*15*). In a drop-scale method, Holmes and Kirk (*137*) have succeeded in reducing the uranium salt with cadmium and titrating with ceric sulfate. Results accurate to ±1 per cent are reported when using 0.13 to 4.13 micrograms of sodium. Phosphorus interferes and must be removed.

Strontium. Strontium, 4 to 5 mg., has been successfully determined as the carbonate or sulfate (*325, 377*).

Tin. One milligram or less may be determined iodometrically with results accurate to ±0.005 mg. (*138*).

Zinc. 1. The organic material containing 1 mg. of zinc is ashed and then determined iodometrically by a modified Lang procedure (*134, 177*) according to the equation

$$2K_3Fe(CN)_6 + 2KI + 3ZnSO_4 = K_2Zn_3[Fe(CN)_6]_2 + 3K_2SO_4 + I_2$$

2. If the zinc is precipitated as the sulfide, it may be determined by liberating the sulfur as hydrogen sulfide and titrating iodometrically.

3. It may be precipitated with 8-hydroxyquinoline and weighed. The error is ±0.005 mg (*58*).

4. It may be precipitated by oxine [$Zn(C_9H_6ON)_2$] and titrated with a measured excess of potassium bromate mixture:

$$C_9H_7ON + 2Br_2 = C_9H_5ONBr_2 + 2HBr$$

Four moles of bromine react with the oxine, which is equivalent to one of zinc. The excess bromine is titrated with sodium thiosulfate. The error is ±0.005 mg (*59*).

DETERMINATION OF GROUPS

ACETYL. The determination of acetyl has been carefully studied The procedures can be classified in two groups:

1. Those in which aqueous acid hydrolysis is followed by distillation and estimation of the acetic acid formed. The method of Elek and Harte (*84*) is an example in which aqueous *p*-toluene sulfonic acid is used. The acetic acid is vacuum-distilled and determined iodometrically.

2. Those employing alcoholic acid hydrolysis which is followed by distillation of the resulting ethyl acetate. The acetyl in the acetate is estimated by hydrolysis with an excess of standard alkali and by titrating the excess. Strong alkali must be employed and for this reason it has been difficult to obtain accurate results on titrating alkali of this concentration with a microprocedure. Clark (*61*) has modified this method for semimicroquantities by steam-distillation of the acetic acid, followed by titrating with 0.02 *N* alkali. Matchett and Levine (*206*), in a method which can be modified for semimicroquantities, fractionally distill the ethyl acetate into 0.1 *N* alkali using a packed

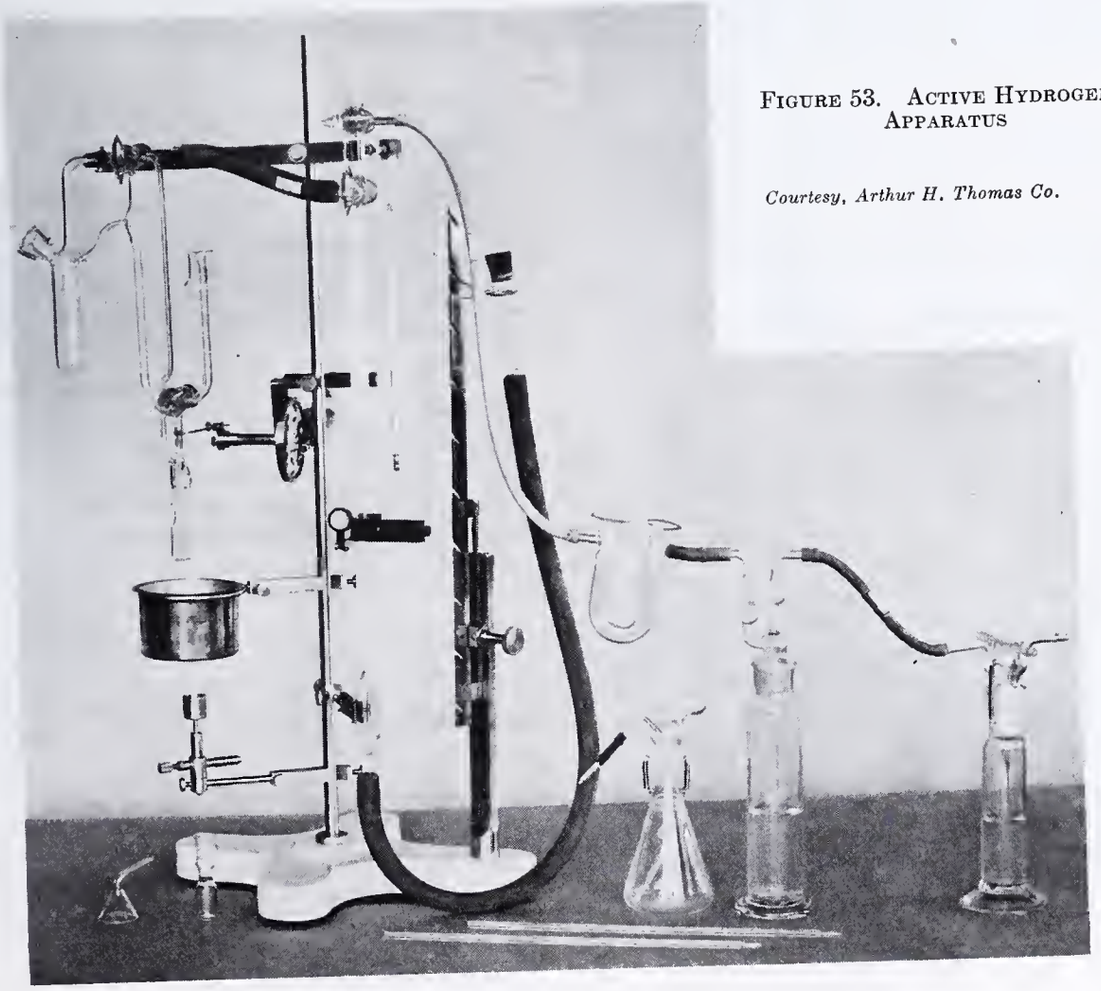


FIGURE 53. ACTIVE HYDROGEN APPARATUS

Courtesy, Arthur H. Thomas Co.

column. The column is arranged for operation under total reflux with intermittent, small-volume take-offs.

Carboxyl. In addition to the usual method of determining carboxyl by titration with alkali (*233*), Tsurumi and Sasaki (*346*) have developed a micromodification of the method of Fuchs (*104*) and Hunter and Edwards (*141*), which depends upon the fact that even weak organic acids will immediately liberate hydrogen sulfide from solutions of potassium hydrosulfide, saturated with hydrogen sulfide, one molecule of hydrogen sulfide being evolved for each carboxyl group. The method possesses the advantage over the titration procedure that, since the reagent is a solution, acid with hydrogen sulfide, with few exceptions lactones and alcohols or phenol hydroxyl have no effect.

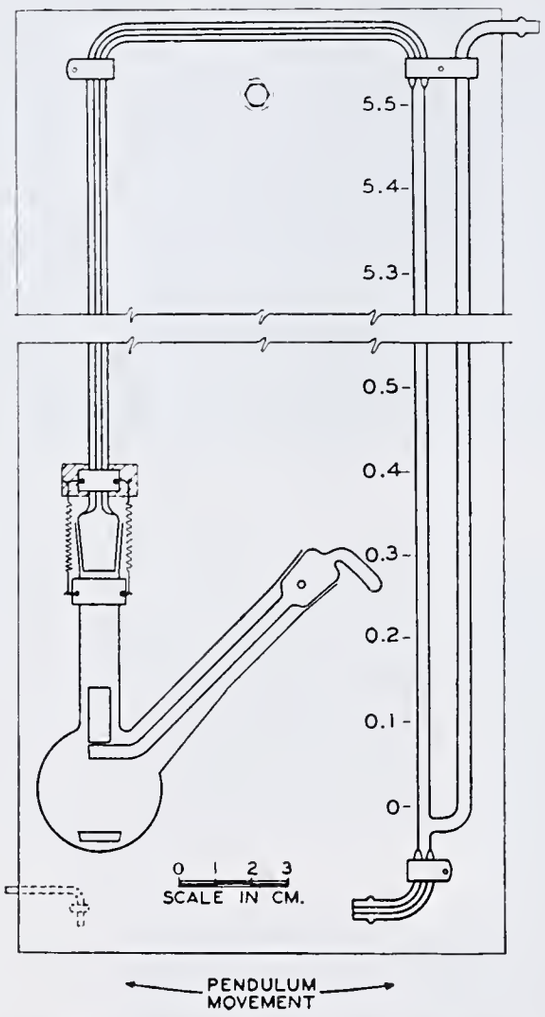


Figure 54. Single-Unit Noncompensating-Type Hydrogenation Apparatus

Carbonyl. Falkenhausen (*93*) has successfully used a micromodification of the method of Strache, in which the compound is treated with a measured quantity of phenylhydrazine and sodium acetate. The excess phenylhydrazine is determined by adding Fehling solution and the evolved nitrogen is measured.

SH Groups. In glacial acetic acid, compounds containing SH groups can be dehydrogenated to disulfide with iodine in stoichiometric proportions (*172*).

Double Bonds. Unsaturation is measured by (1) quantitative hydrogenation (see under Microhydrogenation), and (2) bromine addition by the Becker gravimetric method, using a 1- to 10-mg. sample (*274*).

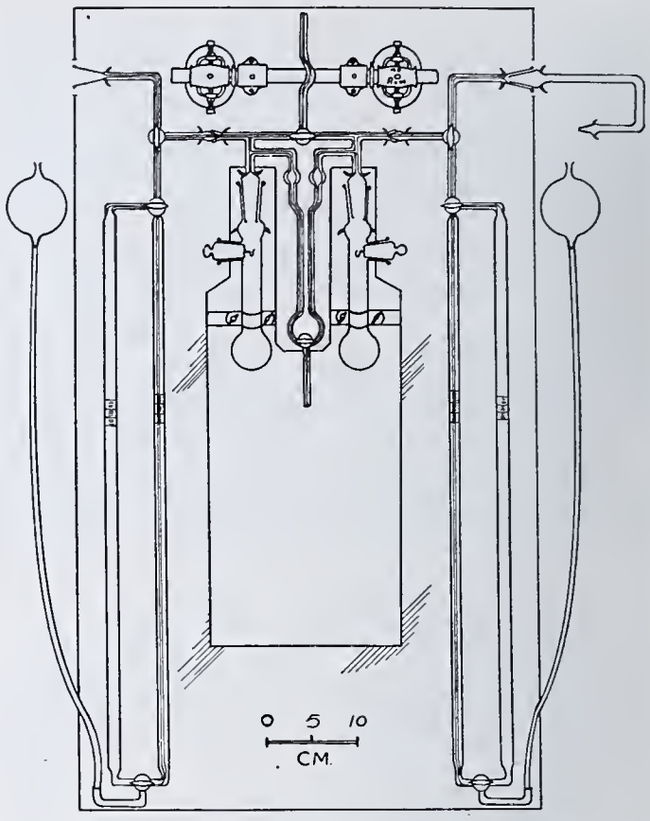


Figure 55. Double-Unit Compensating-Type Hydrogenation Apparatus

Exchangeable or Active Hydrogen. By the micromodification of the method of Chugaev and Zerevitinov, a known amount of Grignard reagent is added to the sample and methane is formed, which is measured (*319*) or burned (*91*). Figure 53 shows the apparatus used for the volumetric method.

Special Methods Limited in Application. The substance is dissolved in deuterium oxide, evaporated to dryness, and the increase in weight caused by replacement of active hydrogen by deuterium (*366*) is determined.

A modification of the above method has been developed by Hamill (*127*), which is much slower but serves to distinguish between active hydrogen and labile, or slowly exchanging, hydrogen, as in the methylene group of malonic acid. The determination depends upon the decrease in density of 15 to 100 mg. of 98 per cent deuterium oxide due to exchange, which is measured by a small quartz float. The method is accurate to ±3 per cent.

Methoxyl-Ethoxyl. This determination has become standard and reliable among microprocedures. Occasionally compounds which are not soluble in the digestion mixture give difficulty. The method has been subjected to collaborative study by the Association of Official Agricultural Chemists, and the results obtained were satisfactory (*64*). The iodometric titration of the methyl iodide or ethyl iodide is preferred to the gravimetric determination.

Elek (*83*) discusses the different modifications and the best conditions for carrying out this determination. The precision and accuracy are ±1 per cent.

Kolka and Vogt (*162*) report low results on the macro scale for pentamethyl anisole because of distillation of the compound out of the reaction mixture, with solidification in the condenser. This was overcome by sealing the sample with the reaction mixture in a tube and digesting for 2 hours at 135° C. The tube was cooled, opened, and quickly placed in the Zeisel apparatus, and the determination was completed in the usual manner.

The factors affecting the accurate determination of alkoxyl groups in cellulose ethers have been studied over a period of several years by Samsel and McHard (*284*). By using a slight modification of the usual method and apparatus, more consistent results were obtained.

Microhydrogenation

During recent years it has been found that by the quantitative determination of the amount of hydrogen absorbed by certain unsaturated compounds, information as to their structure may be obtained.

The sample, 5 to 10 mg., placed in 5 ml. of a solvent such as alcohol or acetic acid, together with a catalyst of platinum black, platinic oxide, or palladium black is shaken in an apparatus in the presence of hydrogen. The volume of hydrogen consumed is measured, and from this the degree of unsaturation can be calculated. While the time required to carry out a determination depends on the type of compound, the actual time required for hydrogenation is usually not over an hour. The accuracy is 1 to 2 per cent.

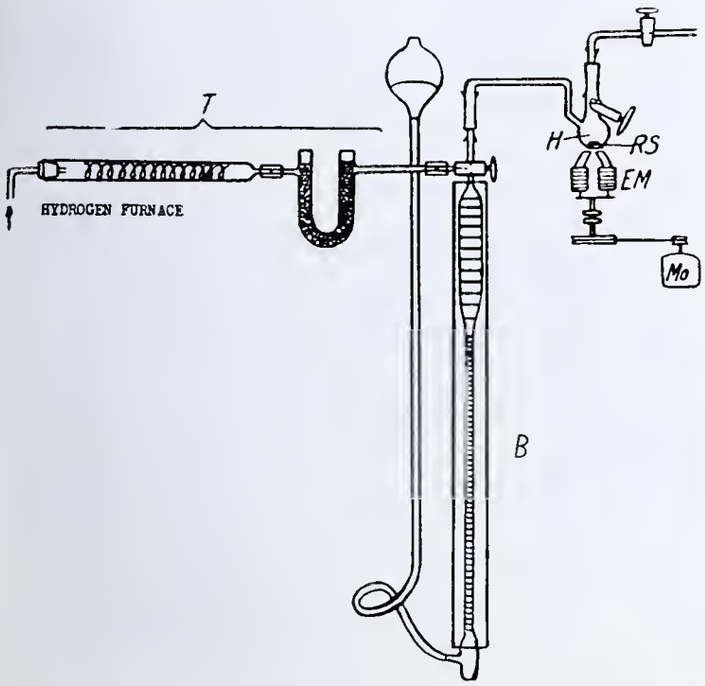


FIGURE 56. HYDROGENATION APPARATUS WITH MAGNETIC STIRRER

Inherent difficulties of this procedure are the need for shaking the apparatus and for accurate measurement of the hydrogen, which involves corrections or compensations for changes in pressure and temperature. Many designs have been published to accomplish this. Figure 54 shows a relatively simple form of apparatus by Johns and Sieferle (*147*), who give the method of calculating corrections for changes in temperature and pressure. The accuracy with this unit is ±2 per cent.

Prater and Haagen-Smit (*248*) have designed (Figure 55) a more complicated apparatus, typical of its class, in which two identical hydrogenation units are assembled, one of which may be used when temperature compensation is required; where this is not necessary, two determinations may be run. Both units carry a 4-ml. and a 50-ml. buret, which allows the apparatus to be used for small-scale preparative work. The maximum error on standards reported was 2 per cent when using 10-mg. samples. This apparatus may be purchased from the National Technical Laboratories, South Pasadena, Calif.

The two forms of apparatus just described are rigidly mounted and the shaking of the contents of the flask is accomplished by oscillating the complete unit with an electric motor.

Shaking a relatively large complicated and fragile unit, in order to agitate the contents of a small flask, leaves room for much improvement in design. To overcome this weakness so that only the flask need be shaken, a glass coil or oscillation around a ground joint has been employed.

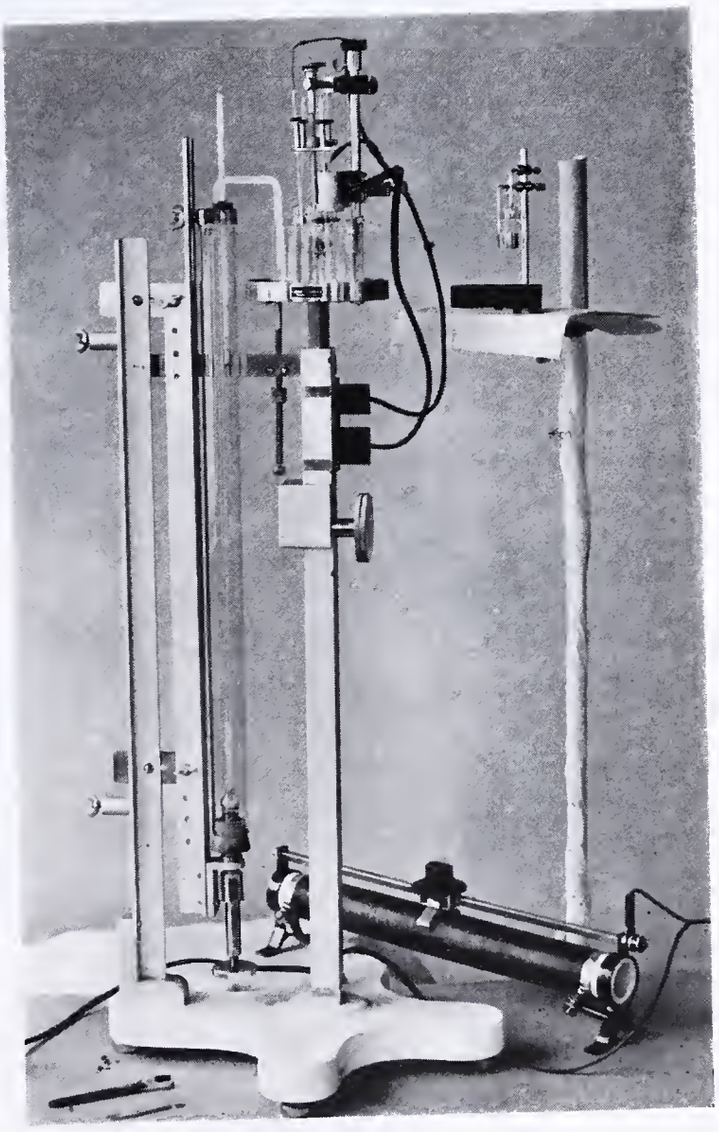
The novel arrangement of Weygand and Werner (*363*), where the contents of the flask are agitated with an electromagnetic stirrer, seems to be more logical and permits a more stable and convenient setup of the apparatus. Figure 56 shows the hydrogen purification train, *T*, the jacketed measuring buret, *B*, the hydrogenation flask, *H*, with the glass-covered iron stirrer, *RS*, which is rotated by the motor, *MO*, and the electromagnet, *EM*. The authors report an accuracy of ±1 per cent when using 3 to 5 mg. of sample.

Gas Analysis

CONSTANT-PRESSURE, VARIABLE-VOLUME METHOD. The technique for the analysis of a gas mixture, 25 to 100 cu. mm., consisting of constituents such as the carbon gases, water, hydrogen, nitrogen, and oxygen, has been studied in some detail. Krogh (*167*) obtained satisfactory results using the liquid absorbing agents commonly employed in standard gas analysis. The method employing liquid reagents has not found favor in precision work involving several components because it is difficult to prevent and eliminate errors of absorption of gases other than the one sought, and the rate at which the bubble rises or falls in the capillary affects the volume reading. While it is true that only differences are measured in gas analysis, it is more difficult with the wet method to keep these factors of vapor pressure and wall-film thickness constant in passing from one solution to another.

Krogh used a capillary of 0.25-mm. bore and volumes of 0.01 to 20 cu. mm. Schmit-Jensen (*288*) extended the system to combustible gases and perfected technique to the point where accuracy of ±0.1 per cent was possible, which was chiefly caused by the error in reading the buret to ±0.1 mm.

Blacet *et al.* (*35*, *307*, *317*) prefer dry reagents which are applied to the sample in a small container. The gas is trans-



Courtesy, Arthur H. Thomas Co.

FIGURE 57. BLACET-LEIGHTON GAS ANALYSIS APPARATUS

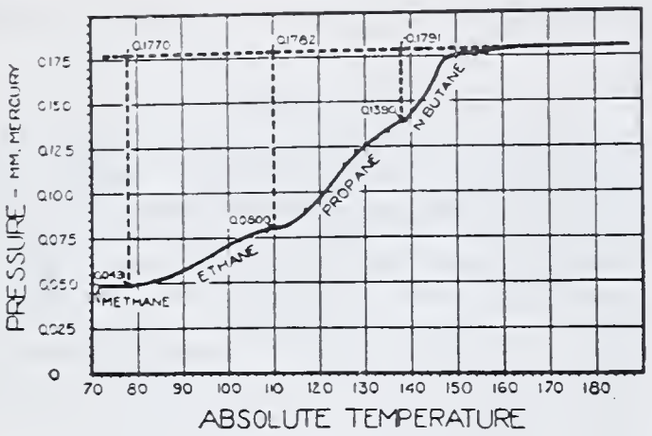


FIGURE 58

ferred and measured in a vertical gas buret of 0.5-mm. bore over mercury, as required. A sample of 50 to 100 cu. mm. is generally used in this method. Figure 57 shows a new assembly of this apparatus which has just been completed.

Specific reagents for such compounds as acetylene, benzene, and ethylene are available, which, with Blacet's combustion technique, allow the separation and determination of simple hydrocarbon mixtures. Complex mixtures must be first separated by other methods, described below, after which the combustion method can be applied for final identification and determination.

Sutton (*331*) uses the same method but prefers the buret in the horizontal position and adds a compensating buret which allows a change in temperature and pressure to be measured and the corresponding change made in the sample-measuring buret. A larger sample is used, 0.1 to 0.3 ml. at standard temperature and pressure, for easier manipulation in routine work. A precision of 0.2 per cent is obtained when using a 0.2-ml. sample, which is that commonly obtained in macro work. Swearingen, Gerbes, and Ellis (*333*) also prefer the horizontal buret.

CONSTANT-VOLUME, VARIABLE-PRESSURE METHOD. Many designs of this type of apparatus, first developed by Langmuir (*179*), have been made and this method was widely used before measurement in capillary tubes was considered accurate. They are usually elaborate, are built for a specific problem, and require some skill to operate and maintain. The gases are handled and passed over solid reagents at low pressures with the conventional vacuum technique, and the volume is usually measured by differences in pressure with some form of a McLeod gage.

Prescott and Morrison (*249*) have demonstrated that by this method it is possible to analyze samples of 5 to 25 cu. mm. at standard temperature and pressure with an accuracy of 2 per cent of the total sample; 5 per cent, with 1 cu. mm.; 0.025 cu. mm. may be detected.

Spence (*320*) and Haden and Luttropp (*121*) use the same principle with modifications. Along with the dry reagents and combustion, cooling may be employed, together with the high-vacuum technique for separation and estimation of the components of gaseous mixtures. Of particular interest for the organic chemist is the application of this technique developed in the methods mentioned above for the analysis of mixtures of hydrocarbon gases.

Sebastian and Howard (*308*) extended Campbell's method to the analysis of hydrocarbon mixtures, using 1 ml. of sample (S. T. P.), which relies upon the characteristic form of the vapor-pressure–temperature curve for the identification and estimation of gaseous hydrocarbon. For every gaseous compound there is a range of temperature over which the change of pressure with temperature is relatively rapid (Figure 58). The limitation of the method lies in the impossibility of determining saturated and unsaturated hydrocarbons of the same number of carbon atoms in a mixture. The method must be calibrated with mixtures of known composition. The sample, of course, is not destroyed in the analysis. The time required for an analysis is 3 to 5 hours, and an accuracy of ±1 per cent may be expected. The method has proved particularly useful in the study of thermodecomposition products of coal, where small laboratory samples are usually obtained. Figure 59 shows the apparatus.

The method of Euchen and Knick (*90*) uses fractional desorption for separating mixtures of methane, ethylene, ethane, and propane. The gas is absorbed on active carbon contained in one arm of a U-tube cooled to −103° C. A clockwork device

A. Dewar flask with vacuum-jacketed tube connection
B. Copper-constantan thermocouple in direct contact with freezing bulb
C. Copper-jacketed freezing tube placed in copper shield 2 mm. thick
D. Lower Dewar flask containing liquid nitrogen
E. Electric heating coil in series with rheostat and ammeter
F. McLeod gage
G, H, I. Mercury reservoirs
J, K, O. Mercury cut-off tubes with mark provided on *J*
L. Liquid nitrogen trap
M. Mercury vapor pump backed by Cenco Hyvac oil pump
N. Electric heater
P. Connecting U-tube for introduction of sample
Q, S. Mercury-filled U-tubes in connection with leveling bottles
R. Expansion bulb
T. Sampling reservoir
U, V, Z. Two-way stopcocks for connection with atmosphere or suction
X. Screw attachment connected with stopper for closing communication between reservoir *G* and McLeod gage
Y. Ground-glass stopper provided with cup for holding weights

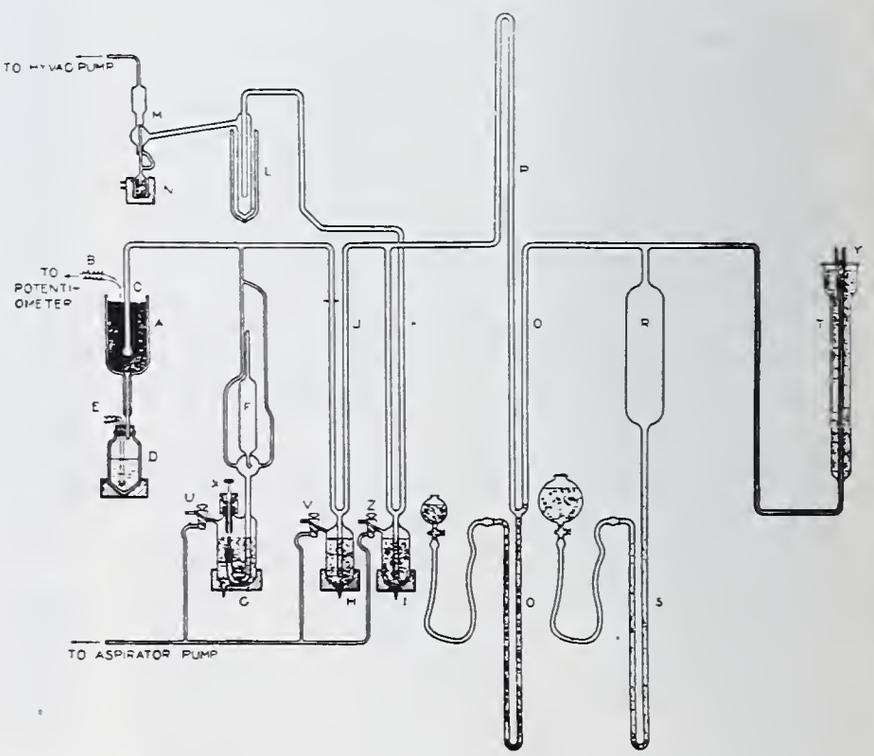


FIGURE 59. APPARATUS FOR MICROANALYSIS OF GASEOUS MIXTURES BY PRESSURE-TEMPERATURE CURVES

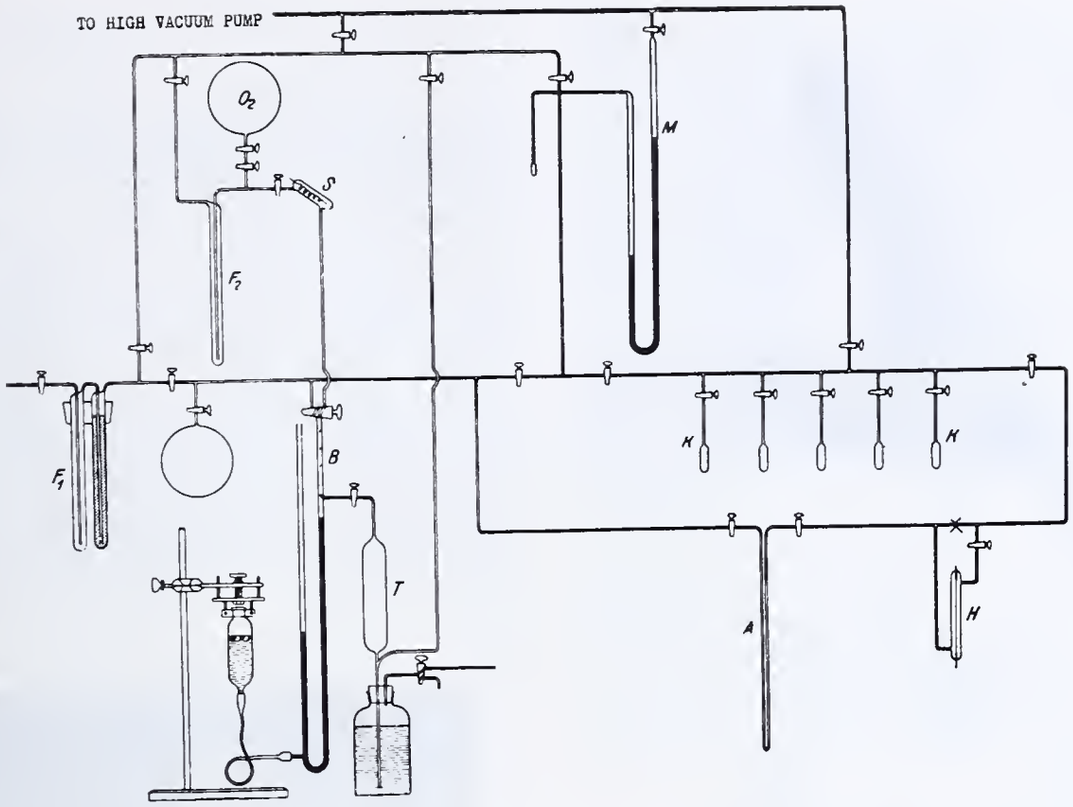


FIGURE 60. APPARATUS FOR ANALYSIS OF HYDROCARBONS BY FRACTIONAL DESORPTION

A. Fractional desorption tube
B. Buret
F_1. Liquid air trap
F_2. Tube for CO_2, H_2O, and O_2 after combustion
H. Hot-wire manometer
K. Receiver
M. Manometer
O_2. Oxygen
S. Platinum wire combustion tube
T. Toepler pump

slowly raises the U-tube into an electrically heated furnace at 250° C. and the liberated gases are delivered by a mercury vapor pump to a collecting vessel. The pressures, p in the U-tube and P in the vessel, are recorded as functions of time. Minimum values of p are found at the end of the desorption of each constituent and the proportions of the constituents are determined from the corresponding values of P.

Küchler and Weller (*171*) have modified the method of Euchen and Knick for mixtures of saturated and unsaturated hydrocarbon. The gases are first passed through a liquid air trap (Figure 60, F_1) and the uncondensable hydrogen and methane are measured and analyzed. The remaining hydrocarbons are fractionally desorbed from active carbon, each fraction is measured, and the pressure is plotted against time, as before. The final identification of each fraction is made by a microcombustion in oxygen over a heated platinum coil, *S*. The products of combustion are collected in F_2, separated by cooling, and measured. When these values are compared with calculated values, the hydrocarbon may be finally identified. A binary mixture such as ethylene and ethane may also be identified and its composition established by this combustion method. About 1 ml. of gas at normal temperature and pressure is required for an analysis, and the accuracy is approximately 1 per cent.

Benson (*28*) has designed an apparatus for condensing and separating volatile hydrocarbons by fractional distillation (Figure 61). The vapor pressure, heats of vaporization, density, and molecular weight are determined on the separated fractions and, from these physical constants, together with the gas analysis micromethods of Blacet *et al.*, amounts of hydrocarbons as small as 5 mg. can be identified. The physical constants can be measured with an error of approximately 1 per cent. Later refinements allow vapor pressure measurements of smaller samples (3 ml. at normal temperature and pressure) and at temperatures up to the boiling point. As an aid in the identification of liquids, an adaptation of the bromination titration method of Uhrig and Levin (*347*) to determine the olefin content, has proved satisfactory.

Aristarkhova (*9*) also uses a fractionation procedure and then analyzes each fraction to obtain a carbon or hydrogen number by which the gas is finally identified. Gas mixtures of 0.03 to 5 ml. are used.

Special Techniques

VOLUMETRIC ANALYSIS USING SMALL VOLUMES. When dealing with quantities below 1 mg., gravimetric procedures are not applicable because a balance of sufficient precision is not available. (Balances weighing 0.01 microgram, or less,

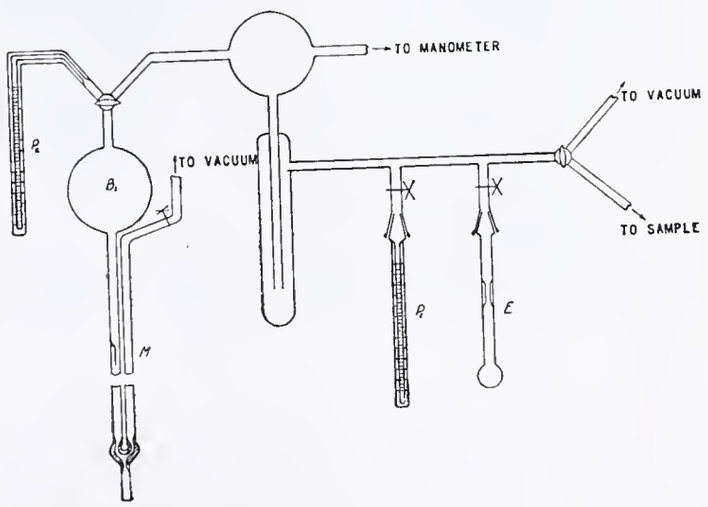


FIGURE 61. APPARATUS FOR ANALYSIS OF HYDROCARBON BY FRACTIONAL DISTILLATION

B_1. Toepler pump
E. Weighed bulb
M. Manometer
P_1, P_2. Micropycnometers

FIGURE 62. KIRK BURET

have been constructed, but their low capacity, need for careful manipulation, and rigid control of atmospheric conditions prohibit their use except for special research problems.) For such work, therefore, volumetric or colorimetric methods are best suited. In recent years the accuracy and precision of these methods have been improved, so that they are now a useful analytical tool. While they have been developed principally for biological studies, they are mentioned here for their application in the solution of special analytical problems.

The disadvantages of dealing with a trace of constituent in a large volume are many, among which factors of solubility, lack of sharpness in indicator change, and faintness of color make even ordinary microprocedures of gravimetric, volumetric, and colorimetric analysis impossible.

Kirk and co-workers (*150*) and Linderstrøm-Lang and Holter (*186*) in a series of papers deal with the technique, apparatus, and methods for determinations using volumes of 0.025 to 0.1 ml. Here the solutions are relatively more concentrated, and by using the standard volumetric methods with special precision pipets (0.01 to 0.2 ml.) and burets (0.5-mm. bore, accurate to ±0.02 cu. mm.), it has been found possible to achieve an accuracy of 1 to 2 per cent in determinations involving a few micrograms of constituent.

Figure 62 shows the buret used by Kirk. It is of the air-interface type, in which the solution does not come into contact with the actuating mercury thread.

Figure 63 shows the Rehberg buret with mercury plunger-type valve and reservoir at the top for filling. These burets and others with modifications, such as interchangeable tips, etc., are now available commercially.

Two examples will serve to show the concentration of solutions used:

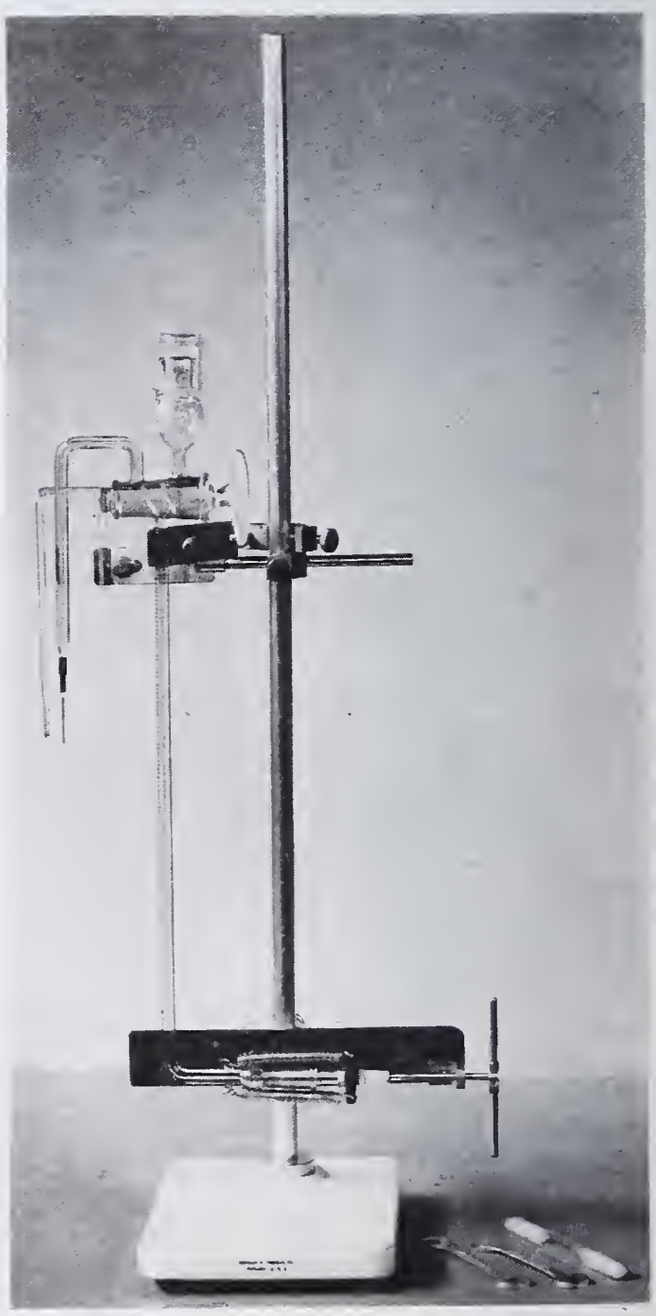
1. Calcium (0.5 to 12 micrograms) may be precipitated as the oxalate and determined by adding a measured amount of 0.01 *N* ceric sulfate in excess of the oxalate. The excess oxalate is titrated with 0.01 *N* ferrous ammonium sulfate, using phenanthroline–ferrous sulfate, as indicator (*154*, *189*).

2. Phosphorus (0.5 to 9 micrograms) may be precipitated as ammonium phosphomolybdate and a measured excess of 0.1 *N* sodium hydroxide added and the mixture heated. Then a volume of 0.1 *N* hydrochloric acid is added and the excess acid is titrated with 0.1 *N* sodium hydroxide to the pink phenolphthalein end point (*190*).

Drop-scale procedures have also been developed for chloride, using a potentiometric titration (*74*, *295*), iron (*151*), nitrogen (*345*), and sodium (*191*). (For a review of potentiometric titrations see Furman, *106*.)

MICRODIFFUSION METHODS. Another system of small-volume analysis is the diffusion method (*70*).

If, for example, a substance is placed in a vessel with a reagent which liberates a gas phase, and in another vessel is placed a reagent which continually removes this gas phase, the reaction, contrary to expectation, goes to completion (Figure 64) under suitable conditions in a surprisingly short time (0.5 to 2 hours). The errors in all these determinations can be kept within ±0.5 to 1 per cent. The simplest diffusion unit is shown in Figure 65. The liquid sample for the determination of ammonia (0.1 to 2 ml.) is placed in the outer chamber and 2 ml. of standard acid are run into the inner chamber. The greased glass lid is put in place over the cell, the lid is pushed back, and 1 ml. of potassium carbonate is added. The chamber is now sealed with the glass plate and, after a suitable time for absorption, the lid is detached and



Courtesy, Arthur H. Thomas Co.

FIGURE 63. REHBERG BURET

the contents of the inner chamber are titrated. To speed up the determination, cells have been made which may be evacuated, heated, or shaken. Nitrogen, 0.1 to 100 micrograms, may be determined with an accuracy of ±0.5 per cent. Nitrogen may be satisfactorily determined from material which liberates ammonia on treating with alkali, as well as from Kjeldahl digests. Prater, Cowles, and Straka (*247*) employ an ordinary Petri dish and absorb the liberated ammonia in drops of glycerol–boric acid mixture, suspended from the glass cover. Excellent results were obtained with amounts of ammonia nitrogen varying from 0.1 to 9 mg.

Other determinations by this technique include volatile amines, such as trimethylamine and halogens—these are oxidized in the outer chamber by acid permanganate or dichromate, and absorbed in 1 ml. of 20 per cent potassium iodide. This liberates an equivalent of free iodine which is titrated with sodium thiosulfate when the amount is above 35 micrograms. For 7 to 35 micrograms of chlorine, the liberated iodine is run directly into a microcolorimeter. Below 7 micrograms, starch is added and the color measured. The chlorine procedure may also be used for the determination of bromine. By this method carbon dioxide from carbonates may be run on samples liberating as little as 0.2 to 1.0 mg. with acid. The gas is absorbed in barium hydroxide, which is titrated with acid using an indicator of thymolphthalein.

Kirk (*345*) has modified these procedures for 1 to 20 micrograms of nitrogen for the drop-scale technique. Figure 66 shows the diffusion cups for Kjeldahl nitrogen designed so that the digestion and diffusion can be carried out in the same unit. The central cell in this apparatus is the small cup, and contains the measured amount of acid to absorb the ammonia which is titrated with 0.025 *N* sodium hydroxide. The probable error is 1 per cent with a precision of 0.3 per cent. Unit *B* has a ground-glass joint, so that the diffusion may be carried out in a vacuum.

In a method for nitrogen developed by Borsook and Dubnoff (*40*) the sample is digested by the Kjeldahl method using special digestion tubes holding 0.5 ml., and an aliquot of the digest is added to the regular diffusion cell.

The final titration on 0.1 ml. containing 0.3 to 10 micrograms of nitrogen is carried out electrometrically, using a glass electrode (Figure 67) and a Beckman pH meter. The precision is ±2.0 per cent and the accuracy within 1 per cent. Figure 68 shows the assembled titration apparatus, in which *g* is a precision microburet and *h* is standard sodium hydroxide.

Determination of alcohol in blood by diffusion has been proposed by Winnick (*369*). This is an adaptation of the method of Widmark. The alcohol diffuses from the sample in the outer chamber of the unit into the central chamber where it is oxidized by a solution of potassium dichromate. The excess dichromate is determined iodometrically. The precision is ±2 per cent and the accuracy ±3 per cent.

The same author (*370*) determines acetone by diffusion into sodium acid sulfite, which forms a complex with the acetone. This is later dissociated and the acetone determined by titrating the liberated sulfite with iodine.

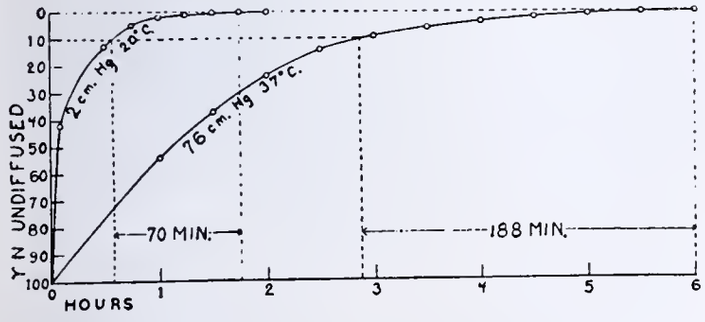


FIGURE 64

Diffusion methods in general require a minimum of mechanical manipulation and the transfer of the end product for the final operation is avoided. The disadvantages are possible interferences from the sample which diffuse and react similarly to the constituent desired. While diffusion methods have been developed primarily for biological chemistry, the method is described here because such procedures can, by modification, be applied to many types of problems in general microanalytical chemistry.

TRACE ANALYSIS. The determination of traces of constituents usually involves the separation and concentration of the trace from a large bulk of material in sufficient quantity for estimation. When macroprocedures are employed the size of the initial sample is often discouragingly large. By employing sensitive micromethods the sample size can usually be held within reasonable limits. The problem may be divided into two parts:

1. The constituent is present in relatively high concentration but the quantity of the sample is small. The technique for these determinations has been worked out by the drop-scale or ultramicromethods already mentioned.
2. The constituents are present in a large volume of material. If the system is a simple one where the trace material is the only one present which varies in quantity, a physical method can often be applied.

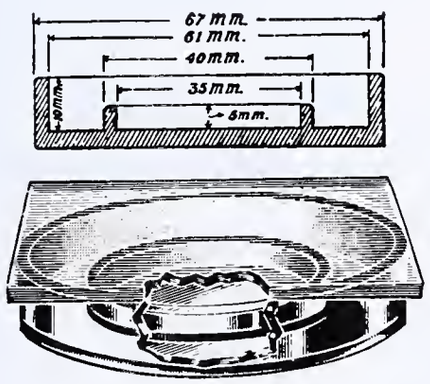


FIGURE 65. DIFFUSION CELL

Methods used include the following:

1. Ultraviolet absorption may be used with gases, liquids, and solids to measure the concentration of the impurity. The method of detecting and estimating trace impurities by their fluorescence under ultraviolet light is widely used, although it is limited to those materials which fluoresce or can be made to fluoresce by combination with reagents. Traces below the range for fluorescence can often be concentrated by capillary or chromatographic methods. Haitinger (*122*) reviews the application of fluorescence to microanalysis and Radley and Grant (*251, 252*) cover the general subject.
2. Infrared absorption may be used for the detection of traces of organic constituents in an organic liquid. The detection and estimation of small quantities of impurities in organic gases, liquids, and solids by infrared spectroscopy have been made practical (*372*). The method requires standardization with knowns for quantitative work or it may be used to compare one or more samples as to their similarity. A sample of only 0.1 ml. is required and this is not destroyed by the analysis. The method is sensitive, 0.1 to 0.05 per cent by volume.
3. The difference in refractive index may be measured by an interference-interferometer (*224*). This method may be applied to liquids or gases. It is very accurate and sensitive—e. g., an accuracy for benzene vapors in air of 0.0008 per cent is reported by Brodskiĭ using a large Haber and Lowe instrument (*47*).
4. Determination of the dielectric constant of an organic liquid—for example, the determination of traces of water in benzene.
5. The detection, by the polarograph, of traces of certain organic compounds which undergo oxidation or reduction at the dropping mercury electrode. The polarograph is becoming a very useful instrument in organic analysis for determining mixtures of organic compounds, as well as for impurities present in trace amounts. While the method normally requires only 10 to 15 ml., special microcells (*202*) holding only a few drops of liquid are available. An excellent review on polarography as applied to organic chemistry is given by Müller (*219*).
6. The development of color on the addition of a suitable reagent and determination of the quantity of impurity by means

of a simple color comparison colorimeter or spectrophotometer. The measurement of color by spectrophotometric absorption is becoming a preferred method because, once standardized with knowns, the calibration requires only periodic checking.

A more important reason is that by the use of color filters or a diffraction grating a desired constituent may be selectively absorbed from a mixture of colors, and its concentration accurately measured. This new development is of great importance to those interested in trace analysis where the development of a desired color without interference is sometimes a difficult problem. Vaughan (*354*) has made a detailed study of the method as applied to metallurgical analysis, Knudson, Meloche, and Juday (*161*) to the determination of aluminum in the presence of iron with the aluminum-iron-hematoxylin system, and Owens (*240*) to organic compounds.

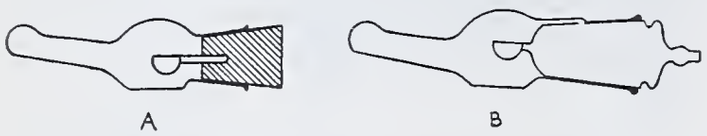


FIGURE 66. KIRK MICRODIFFUSION CELLS

Microspectrophotometry is discussed by Conway (*70*) and Müller (*220*), and a general review of photoelectric colorimetry is given by the latter (*221*). Kul'berg (*173*) describes various microcolorimeters and micronephelometers. Most macroinstruments (*225*) can now be adapted to micro work with cells of volumes as low as 1 ml.

7. The titration of extremely dilute solutions (0.001 *N*) by observing the color change photoelectrically is an interesting application (*194, 221*). Titration of such solutions by the dead-stop electrometric method, while limited in application, can give very precise results in the case of the iodine thiosulfate titration (*362*). The method is ten times as sensitive as the starch method and the concentration of iodine required is 0.06 to 0.09 microgram per ml.

8. Flagg and Wiig (*101*) summarize the application of tracer isotopes to analytical problems. For the organic chemist the stable isotopes can be of service in the analysis of mixtures of closely related compounds. When using N_{15}, a change of 3 to 5

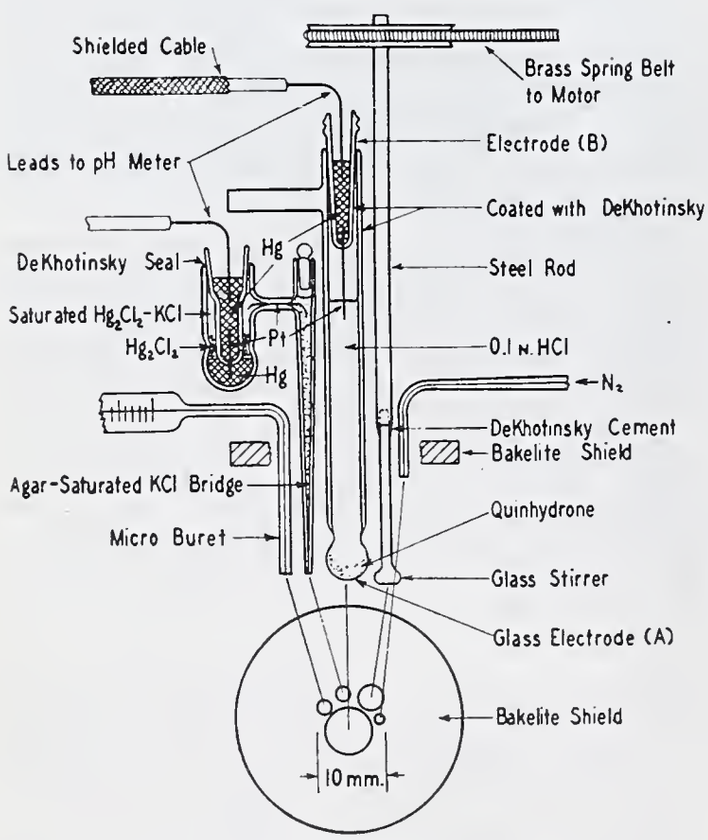


FIGURE 67. GLASS ELECTRODE SETUP

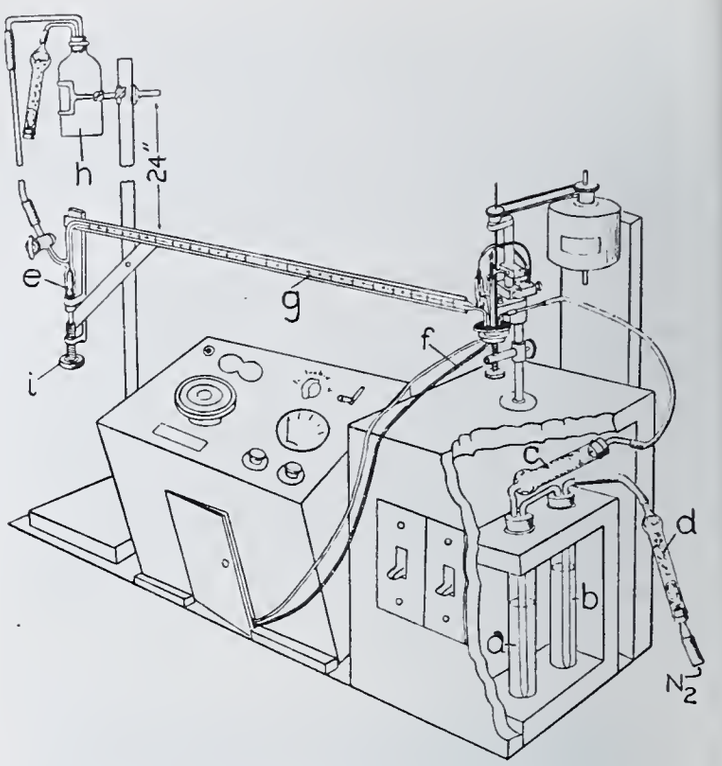


FIGURE 68. ASSEMBLED TITRATION APPARATUS

per cent of the amount normally present can be readily detected. The determination (accurate to 1.5 per cent) of the isotope by the mass spectrometer requires 2 to 25 mg. of the element which has been "enriched". The technique employed for the analysis of mixtures of amino acids is given by Rittenberg and Foster (*265*). The method is limited for most workers to deuterium, which has been available for some time, and to N_{15} which can now be supplied by the Eastman Kodak Company.

Where the trace amount is too low to be determined by the foregoing methods, some form of concentration can be applied until an amount is collected which is sufficient for a determination by micromethods.

For Gases. By freezing out the trace constituent at a suitable temperature; by passing the gas through a reagent, such as activated charcoal, whereby the trace is absorbed; and by concentration of the trace by passing the gas through liquid reagents.

For Traces in Liquids or Solids. By selective adsorption (chromatographic and capillary methods).

Mercury from organic solvents may be concentrated by percolating the solution through asbestos containing cadmium sulfide, or the organic material may be destroyed by digestion and the mercury collected by passing the digested solution through the cadmium sulfide. The mercury is then liberated from the cadmium sulfide–asbestos by heat in a closed chamber and the amount of mercury determined by ultraviolet absorption (*14*).

By combustion in oxygen of the organic material—for example, the concentration of traces of iodine (*196*).

After the organic material is destroyed by wet- or dry-ashing, traces of metals may be concentrated by electrolysis and then determined by microprocedures, or the ash may be run spectrographically (*132*).

For the determination of traces of ash in organic compounds, Norton, Royer, and Koegel (*238*) have shown that results, as accurate as those obtained by the macroashing method, are possible by burning in a combustion tube a 150-mg. sample with oxygen at 800° C. A special automatic combustion furnace (Figure 69) is used and the total elapsed time required is 30 minutes, as compared to 7 hours required for large samples burned in a muffle furnace. The amount of ash weighed was 0.03 to 0.6 mg.

Applications in Organic Fields

The application of microanalysis to certain organic fields is noteworthy and a very brief summary of the more important applications follows.

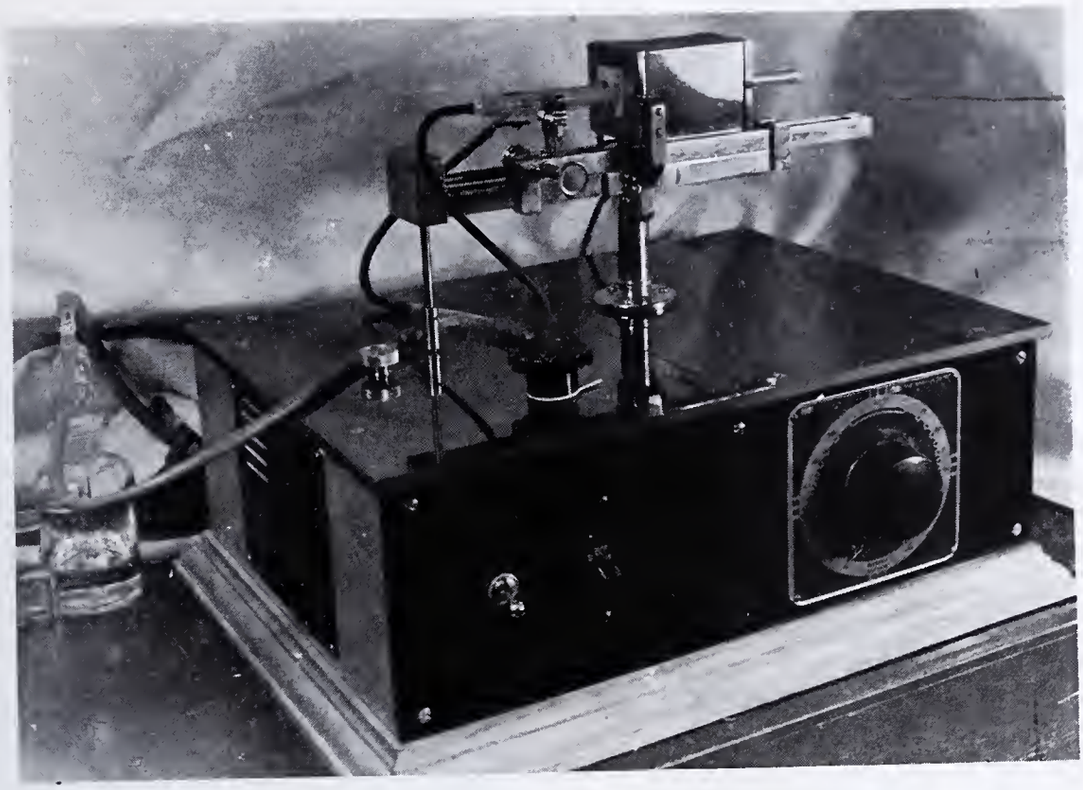


FIGURE 69. ROYER AUTOMATIC ASHING FURNACE

COAL AND SOLID FUELS. Kirner (*156*) has successfully applied micromethods to the determination in coal of carbon and hydrogen, nitrogen (by Kjeldahl or Dumas), sulfur (by Carius), ash, molecular weight, and methoxyl. The determination of moisture, using small amounts, while reproducible, is not representative of a mine-run sample, so that a large macrosample must be used. A critical discussion of the methods is given. The analysis of coal, whether by micromethods or by macroprocedures, shows the same inherent difficulties.

Belcher and Spooner (*26*) prefer semimicromethods, using 50- to 100-mg. samples, in order to eliminate possible sampling errors. Determination of nitrogen in coke, by the Kjeldahl procedure, is relatively slow but may be speeded up by mixing the sample with soda lime and passing steam through the mixture, whereby ammonia is freed and absorbed in dilute sulfuric acid. This ammonia is then liberated by sodium hydroxide, steam-distilled, and determined in the usual manner. The total time for one determination is 25 minutes. Carbon and hydrogen, using the Friedrich modification with platinum contacts and lead dioxide in a boat, has been found more satisfactory than the Liebig-Pregl method. A 50-mg. sample is used, over which oxygen flows at the rate of 12 ml. per minute. The time for one determination is 35 minutes.

It has been shown that the determination of nitrogen in certain coals by the Dumas or Kjeldahl method can yield values which are incorrect, owing to the incomplete destruction of the organic material or to loss of nitrogen from the digest. High results may be obtained in the Dumas procedure, because of the formation of methane.

Sulfur and chlorine are determined simultaneously by burning a 0.5- to 1-gram sample in oxygen at 1350° C. and absorbing the gases in hydrogen peroxide. At this elevated temperature no oxides of nitrogen are formed, so that a mixture of hydrochloric acid and sulfuric acid is obtained. The total acidity is determined by titration with alkali and the chloride is then run on this same solution by adding mercuric oxycyanide which reacts according to the equation:

$$Hg(OH)CN + NaCl \longrightarrow NaOH + HgCl.CN$$

This liberated sodium hydroxide represents the chloride, and the sulfate is obtained by difference. The combustion time is 10 minutes. Using this high-temperature, rapid-combustion technique, Belcher and Spooner (*26*) have succeeded in determining simultaneously carbon, hydrogen, sulfur, and chlorine. In this case, however, the sulfur and chlorine are collected on a weighed and heated silver gauze. The silver sulfate is dissolved off with water and from the loss in weight the sulfur is determined. The remaining silver chloride is dissolved in ammonium hydroxide and by again determining the weight loss the chlorine is estimated.

Gillet, Grandry, and Delaude (*113*) describe a modified Endell-Berl microfurnace for observing, in an inert atmosphere with a microscope, the melting point and decomposition characteristics of coal.

For studying the hydrogenation products of coal, Berl and Koerber (*30*) describe a semimicromethod using 2.5 to 5 ml. of sample for the determination of saturated hydrocarbons by nitrating with sulfuric-nitric acid mixture by which the aromatic hydrocarbons are made soluble. The original mixture is then catalytically dehydrogenated into aromatic compounds which are subsequently nitrated, giving a measure of the sum of the two types of compounds. The material unattacked in the second nitration represents the aliphatic compounds present in the original mixture. Results are accurate to ±1.0 per cent.

EXPLOSIVES. The advantages of using small samples of explosives for tests are obvious. The analysis of explosives for carbon and hydrogen has been successfully accomplished by mixing the sample with an inert material, such as fine sand or ignited kieselguhr (*8*, *107*).

Michel-Levy and Muraour (*212*) have used the microscope in the study of detonation.

Roth (*277*) has studied the same problem, using columns of explosives 15 to 30 mm. long, with an electric method for determining a time interval as small as 3 to 7×10^{-6} second. Results are accurate to ±3 per cent.

Berl and Kunze (*31*) modified the apparatus of Will so that the stability of cellulose nitrate may be determined with a 0.5-gram sample. Later, Berl, Rueff, and Carpenter (*32*) improved the test by keeping the products of decomposition in contact with the sample in a sealed tube and measuring changes of pressure within the tube by means of a "feather manometer". The size of the sample required is 5 to 50 mg. The method gives very reproducible results.

Klemenc and Hayek (*159*) describe a Lunge-type nitrometer for the determination of nitrate nitrogen, using 6 ml. of gas which is measured to ±0.02 ml.

Berl, Hoffmann, and Bremmann (*29*) also used a Lunge-type nitrometer with which they analyzed samples of 30 to 100 mg. to within 1 per cent of the truth.

Elving (*87*) has built a mechanically shaken compensating-type du Pont semimicronitrometer, (Figure 70) one tenth the capacity of the macromodel, requiring only 5 pounds of mercury and 100-mg. samples. The precision with samples of 10 to 13 per cent nitrogen is ±0.2 per cent. The results on cellulose nitrate when compared to those by the standard nitrometer are lower by approximately 1 per cent.

Hydrocarbon Analysis (for analysis of hydrocarbon mixtures see Gas Analysis). *Aniline Points.* Wilkinson (*365*) used a semimicroapparatus for the determination of aniline points employing 1 ml. of olefin.

In the Mellon Institute (*207*) the fellowship of the Gulf Research and Development Co. has worked out methods for the aniline procedure, using 0.5 ml., as well as the titration for organic acidity where 0.05 ml. of sample can be examined. The final estimation is accurate to ±10 per cent, using a photoelectric colorimeter.

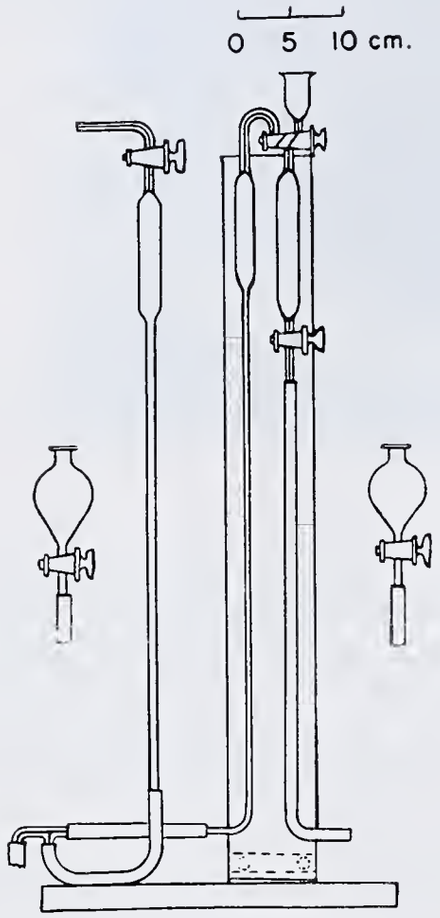


Figure 70. Du Pont Semimicronitrometer

Marion and Ledingham (*205*) have designed a convenient apparatus for measuring and introducing hydrocarbon gases into the combustion tube for the determination of carbon and hydrogen by the regular combustion procedure.

Milk. Several papers have been published on the analysis of milk, employing small samples. Typical among these is Elser's work (*85*) on semimicromethods for the analysis of condensed milk. Eight to 9 grams of milk are used to determine total solids, lactose, sucrose, protein and albumin globulin by the micro-Kjeldahl method, fat, chloride, and ash. The ash is used to determine calcium, potassium, iron, and phosphorus.

Rubber. Wyatt (*373*) has analyzed soft vulcanized rubber compounds by microanalysis for the determination of acetone, chloroform, and alcohol–potassium hydroxide extracts, mineral fillers, total and free sulfur, and sulfur in fillers.

Proske (*250*) has applied the polarograph to the determination of organic compounds, such as accelerators and inorganic constituents. The precision is ±2 to 3 per cent.

Foods. Diemair and Herrmann-Tross (*78*) summarize the micromethods which have been applied to the analysis of foods. The following determinations are covered: proteins and amino acids, fats, lipoid phosphorus, cholesterol, carbohydrates, organic acids, sulfurous acid, metals and non-metals, and miscellaneous references, including vitamins.

The concentration and identification of food colors, especially artificial dyes, offer a difficult problem to which new technique has been applied (1) by capillary analysis (*77*); (2) by chromatographic adsorption (*11*, *341*); and (3) by electrocataphoresis (*285*).

The determination of selenium has been the subject of recent investigations (*158*, *362*).

The testing of grain and flour using small samples has been investigated (*88*), and the results in most cases agree with those employing larger quantities (*128*, *129*).

Fats. The analysis of fats by micromethods has received sufficient investigation to establish the procedures as having adequate precision and accuracy. Gorbach (*115*) outlines the methods and apparatus for the estimation of total fats and lipoids by microextraction, unsaponifiable matter, phosphatide phosphorus, sterols, acid number, saponification number, iodine number, hydroxyl number, thiocyanate, and peroxide values.

Faure and Pallu (*95*) have applied capillary analysis for detecting and studying the rancidity of oils and fats.

Dyer, Taylor, and Hamence (*82*) have developed a semimicromodification for the determination of Reichert, Polenske, and Kirschner values in butter fat.

Pulp and Textile Fibers. *Cellulose.* Geiger and Müller (*109*) have studied the reducing values of cellulose, oxycellulose, and hydrocellulose, using the micromodification of the copper, ferricyanide, and iodine number procedures.

Heyes (*133*) has determined the copper number, using a 0.25-gram sample. Kettering and Conrad (*148*) have developed a rapid semimicromethod for the determination of cellulose in raw cotton fiber (*327*).

Larocque (*180*) describes a small single-rod mill for beating 1 gram of pulp.

The solubility number of cellulose has been determined by Nodder (*237*), using a 0.1-gram sample.

Micromethods for raw cotton for the determination of wax have been developed by Fargher and Higginbotham (*94*).

Ridge, Corner, and Cliff (*263*) describe the microdetermination of copper, iron, and zinc in textile fibers.

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